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ORIGINAL ARTICLE



Effects of inhaled corticosteroids on the expression of TNF family molecules in murine model of allergic asthma

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ABSTRACT

Background: The tumor necrosis factor superfamily member LIGHT (the official gene symbol approved by NCBI Gene Database), an inflammatory factor secreted by T cells after allergen exposure, recently discovered to play crucial roles in asthmatic airway remodeling. However, it is unclear whether LIGHT could be controlled by inhaled corticosteroids, a key component of asthma management. This study was to investigate the effects and potential mechanisms of inhaled budesonide on the expressions of LIGHT and its receptors (LT β R and HVEM) of lung tissues in ovalbumin-sensitized mice. **Materials and Methods:** Thirty-three BALB/c mice were randomly divided into the control, asthma model, and budesonide treatment groups (11 in each group). Mice were sensitized and challenged by OVA to develop mouse model of chronic asthma, and treated with aerosolized budesonide before OVA challenge. Bronchoalveolar lavage fluid (BALF) and lungs were obtained after the final OVA challenge. Protein and mRNA Levels of LIGHT, LT β R, and HVEM in the lungs were investigated by immunohistochemistry, image analysis, and real-time PCR. Expressions of IL-6 and IFN- γ in BALF were measured by ELISA. **Results:** Inhaled budesonide significantly reduced protein and mRNA levels of lung LIGHT, LT β R, and HVEM in asthmatic mice. Correspondingly, the number of eosinophils and neutrophils and IL-6 levels in BALF after budesonide treatment were found to be decreased, whereas the IFN- γ levels in BALF were increased. Moreover, the expressions of LIGHT and HVEM mRNA showed positive correlation with IL-6 levels in the treatment group. **Conclusions:** Inhaled budesonide can down-regulate the expressions of LIGHT, LT β R, and HVEM in the lungs of asthmatic mice, and LIGHT/LT β R/HVEM interactions may be a potentially key target for asthma treatment.

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asthma; LIGHT/LT β R/HVEM interactions; inhaled corticosteroids; airway inflammation; airway remodeling

Introduction

Allergic asthma is a complex and heterogeneous disorder of the airways, characterized by recurrent episodes of airway inflammation composed of infiltrating eosinophils, lymphocytes, mast cells, macrophages, neutrophils, dendritic cells, and myofibroblasts.^[1,2] In this progressive disease, airway remodeling may result from the underlying chronic inflammatory process.^[3] Such remodeling process may increase the irreversible component of airway obstruction, accelerate the decline in pulmonary function, and facilitate the persistence of airway hyperresponsiveness.^[4] It has been established that airway remodeling is associated with persistent airway obstruction, disease severity, and poor long-term clinical outcome.^[5] However, the

mechanisms involved in the airway remodeling are not completely understood. It has been suggested the interplay between airway smooth muscle (ASM) cells and T lymphocytes may be driven by many mediators, and have a particular role in the remodeling process of asthma.^[6,7] Thus, identifying the specific mediators involved in airway remodeling has been a major focus in asthma studies.

LIGHT (the official NCBI gene symbol) is also known as TNFSF14. It is a new member of the tumor necrosis factor superfamily (TNFSF, the official NCBI gene symbol), recently discovered to have a potential pathogenic role in many inflammatory diseases.^[8,9] LIGHT is an inflammatory factor expressed by T lymphocytes in various tissues, with the greatest

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expression found in the lung, spleen and thymus, and binds to both lymphotoxin β receptor (LT β R, the official NCBI gene symbol) and herpes virus entry mediator (HVEM, the official NCBI gene symbol). It has been reported that LIGHT could increase the severity of various T lymphocytes-mediated diseases.^[10] In addition, it has also been found that blockade or absence of LIGHT reduced subepithelial fibrosis, smooth muscle hypertrophy and hyperplasia, and airway hyper-responsiveness after allergen challenge.^[11] Along these lines of investigation, it is hypothesized that LIGHT might be a crucial mediator in the regulation of airway inflammation and remodeling in asthma.

Treatment with inhaled corticosteroids improves lung function and reduces airway hyper-responsiveness in asthmatic patients.^[12] Furthermore, inhaled corticosteroids are effective in suppressing airway remodeling in chronic asthma model through anti-inflammatory and anti-fibrotic pathways.^[13] However, whether these inhibitory effects of inhaled corticosteroids involve LIGHT expression is unclear. In the present study, we examined the effects of inhaled budesonide on LIGHT production and the airway inflammatory/remodelling process in a chronic mouse model of ovalbumin-induced asthma.

Materials and methods

Animals

Specific pathogen-free female BALB/c mice were used at 6-week old mice were obtained from the Guangdong Medical Laboratory Animal Center (Guangzhou, Guangdong, China). Mice were maintained under specific pathogen-free conditions in the Experimental Animal Unit of the Shenzhen-PKU-HKUST Medical Center (Shenzhen, Guangdong, China). All animal experiments were approved by the Laboratory Animal Care and Use Committee of the Shenzhen-PKU-HKUST Medical Center.

Study design

Animals were randomly divided into 3 groups of 11 animals. The control group (Saline/Saline): saline (0.9% NaCl)-sensitized, saline-challenged, and saline-treated; The asthma model group (OVA/Saline): OVA-sensitized, OVA-challenged, and saline-treated;

The budesonide treatment group (OVA/BUD): OVA-sensitized, OVA-challenged, and budesonide-treated.

OVA Sensitization and challenges

Mice were sensitised on days 0, 14, 28, and 42 by intraperitoneal injection of 20 μ g OVA (Grade V, Sigma, St. Louis, USA) adsorbed with aluminum hydroxide, as Vieira RP (2007) described previously.^[14] On days 22 after the initial sensitisation, the mice were challenged for 30 min with an aerosol of 1% OVA or with saline, three times per week until Day 51. Challenging with aerosolized OVA was performed in a whole-body aerosol exposure device (30 $\times$ 15 $\times$ 20 cm) coupled to an ultrasonic nebulizer. Control animals were sensitized and challenged with the placebo of saline only.

Budesonide treatment

Budesonide (100 μ g/kg) was administered to mice of the budesonide treatment group by nebulization before OVA challenge as described previously in,^[15] 1 h prior OVA challenge, three times a week from day 22 until day 51. Animals of the other two groups received saline by the same administration route. On day 52, 24 h after the last aerosol challenge, bronchoalveolar lavage fluid (BALF) and lungs were removed from the mice after sacrificing. Figure 1 shows the time line of the experimental protocol.

Leukocyte counts in BALF

BALF was obtained after the lungs were lavaged with 1 ml PBS for three times, and centrifuged at 2000 $\times$ g for 5 min at 4°C. The cells were separated from the supernatant fraction, and resuspended in 50 μ l PBS. The total number of cells was counted with a hemocytometer. Cytospin preparations were made using a cell cytospin centrifuge and then stained with Wright-Giemsa stain. Cell differential counts were performed by counting at least 200 cells per slide under the light microscope.

Cytokine analysis in BALF

The concentrations of cytokines (IL-6 and IFN- γ) in BALF supernatant were measured using the corresponding ELISA kit (Biolegend, CA, USA) following manufacturer's instructions.

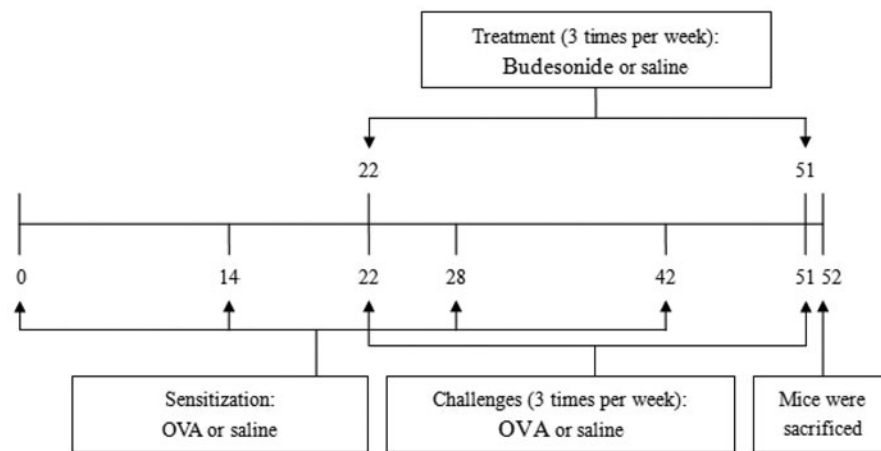


Figure 1. Time lines of the experimental protocol. The protocols of the OVA/OVA group, OVA/BUD group, and Saline group. Mice were sensitized on days 0, 14, 28, and 42 by intraperitoneal injection of 20 μ g OVA or saline. Aerosol challenges with either OVA (1% solution) or saline were performed 3 times per week starting on day 22 until day 51. Treatment with either budesonide or saline before challenge (3 times per week) was started on day 22 and was performed until day 51. Animals were sacrificed on day 52.

Light, LT β R, and HVEM protein expressions by immunohistochemistry

The tissues from the left lung were fixed in 4% paraformaldehyde and embedded in paraffin blocks. Five micrometer tissue sections were stained with hematoxylin and eosin (H&E) for pathological assessment. Images were scanned on the microscope (Leica, DM4000B) with the software (Leica IM 50 Image Manager). The protein expression of Light, LT β R, and HVEM were assessed by semi-quantitative immunohistochemistry. Paraffin sections of lung were deparaffinized, dehydrated, and the endogenous peroxidase activity was quenched with 3% H₂O₂. After boiling in sodium citrate (0.01 mol/l, pH 6.0) for antigen retrieval to unmask antigen epitopes and blocking with 3% bovine serum albumin. Sections were incubated with goat anti-mouse antibody to LIGHT (Santa Cruz, 1:100), LT β R (Santa Cruz, 1:100), or HVEM (Santa Cruz, 1:100) overnight at 4°C temperature, respectively, and then washed three times with 0.1 M PBS for 10 minutes. After washing, the sections were incubated with biotin-conjugated donkey anti-goat IgG (1:400, Vector Labs, Burlingame, CA, USA) in PB for 2 hours. Staining was performed using an ABC kit (Vector Labs), color development was achieved by incubating the sections for 5 minutes with the tyramide signal amplification system (NEN Life Science, Boston, MA, USA). The slides were examined under fluorescence microscope. For quantification of positive cells in any given experiment, at least five random slides were selected.

Light, LT β R, and HVEM mRNA expressions by Real-time PCR

To investigate Light, LT β R, and HVEM mRNA expression in lung tissues from different groups, real-time PCR was performed as a quantitative analysis. Total RNA was extracted from the right lung tissue using the RIZOL reagent according to the manufacturer's instructions. Following quantification, 2 μ g of RNA was reverse-transcribed into cDNA, and real-time PCR assays were conducted using an ABI PRISM 7500 real-time PCR System (Applied Biosystems, Foster City, CA, USA) and the SYBR PrimeScript™ RT-PCR kit reagent (Takara, Dalian, China). The PCR conditions for NK-1R were 45 cycles of denaturation at 95°C for 5 s and annealing and extension at 60°C for 30 s. Target mRNA levels were normalized to those of GAPDH. Gene expression was determined by the $2^{-\Delta\Delta CT}$ method. The primers used in this study are listed as follows: LIGHT-F: 5' AACGTCTTGGAGACATAG 3'; LIGHT-R: 5' CCAATACCTATCAAGCTG 3'; LT β R-F: 5' TCCTACTCGGATACCATCTG 3'; LT β R-R: 5' AGGACAGTGGTGAAGAGCAG 3'; HVEM-F: 5' ATAGCTGGATTCTCATCTG 3'; HVEM-R: 5' AGAATTTGTTTCAGTTGGAGG 3'; GAPDH-F: 5' AGGTCGGTGTGAACGGATT 3'; GAPDH-R: 5' TGAGTGGAGTCATACTGGAACA 3'.

Statistical analysis

All data are presented as mean $\pm$ SE and analyzed by one-way analysis of variance (ANOVA) and

t test using SPSS 13.0. Pearson correlation test was applied to evaluate the correlation between the levels of LIGHT/LT β R/HVEM mRNA and cytokines. Values of $P < 0.05$ were considered statistically significant.

Results

Effects of budesonide on lung inflammation

In this study, we have used a validated mouse model of chronic asthma. In comparison to Saline/Saline mice (Figure 2A-B), OVA challenge plus saline treatment (OVA/Saline, Figure 2C-D) caused an increased airway inflammation with higher infiltration of eosinophils and lymphocytes, increased airway smooth muscle thickening and collagen deposition. The lung tissues in OVA-challenged mice treated with inhaled budesonide (OVA/BUD, Figure 2E-F) had a marked inhibition of

allergic airway inflammation than in saline treated OVA-challenged mice (OVA/Saline). Our data are consistent with previous studies.

To study the anti-inflammatory effect of budesonide, the total number of infiltrating cells, IL-6, and IFN- γ levels in BALF were determined. Furthermore, the changes of lung/body weight ratio were also measured. We found that budesonide treatment decreased the levels of inflammatory cells including eosinophils and neutrophils in BALF compared with OVA/Saline mice ($P < 0.05$; Figure 3A), and there was no significant difference in lung/body weight ratio between OVA/Saline mice and OVA/BUD mice (Figure 3B). At the same time, budesonide treatment resulted in a significant reduction of IL-6 levels ($P < 0.001$; Figure 3C), whereas an increase was detected on IFN- γ production ($P < 0.001$). No significant changes were observed in the levels of IFN- γ

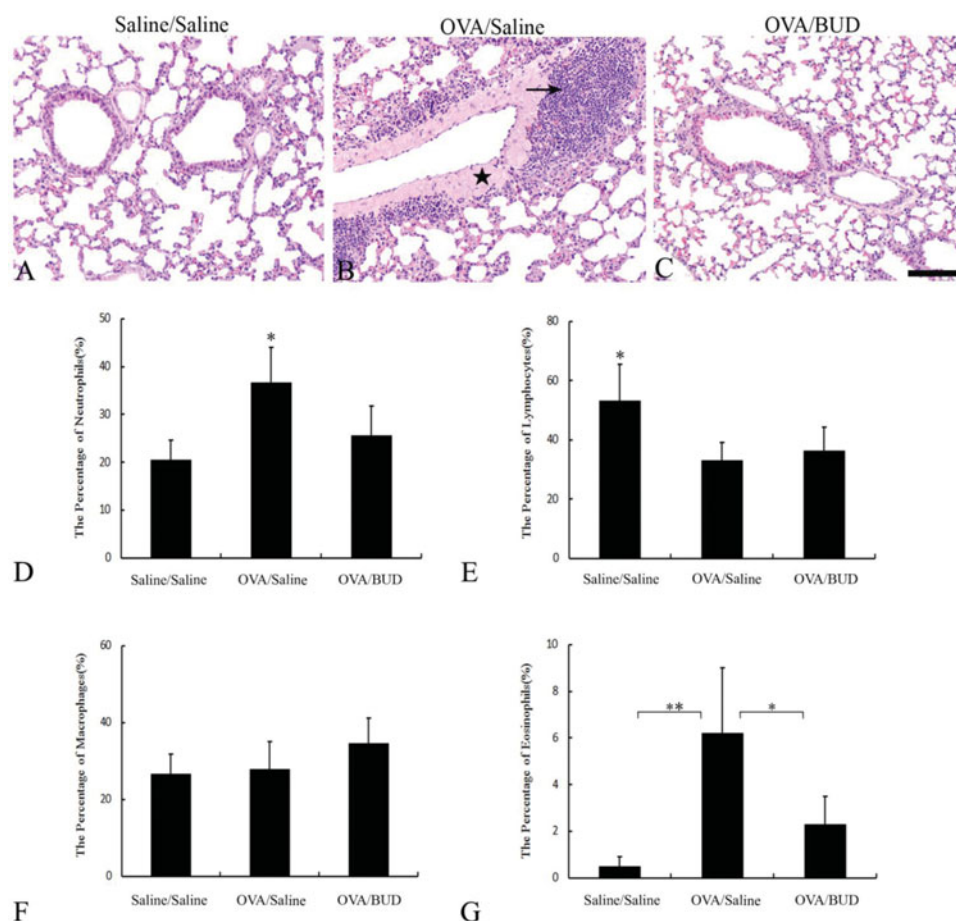


Figure 2. Effects of budesonide on airway inflammation with chronic OVA challenge. Lung tissue sections were obtained from mice 24 h after the last OVA challenge, and were stained with H&E. Increased airway inflammation (arrow) and smooth muscle layers (asterisk) in OVA/Saline mice (B) compared to the Saline/Saline mice (A) or the OVA/BUD mice (C). Cells in the BALF of three different groups were classified and counted by One-way ANOVA Test. The percentage of Neutrophils was presented in (D, * $p < 0.05$: OVA/Saline vs Saline/Saline; OVA/Saline vs OVA/BUD); Lymphocytes (E, * $p < 0.05$: OVA/Saline vs Saline/Saline; OVA/BUD vs Saline/Saline); Macrophages (F) and Eosinophils (G, ** $p < 0.001$: OVA/Saline vs Saline/Saline; * $p < 0.05$, OVA/Saline vs OVA/BUD). Scale Bar = 20 μ m.

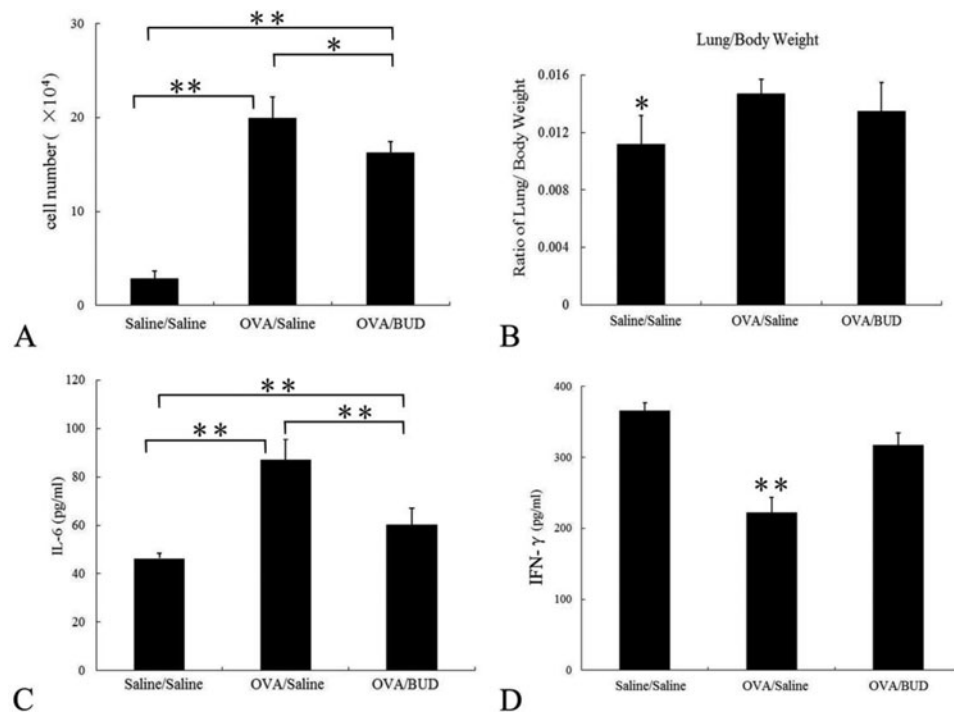


Figure 3. The levels of infiltrating cells number, ratio of lung/body weight, secretion of IL-6 and IFN- γ in the three groups, respectively. (A) The infiltrating cells number was the highest in OVA/Saline mice than that of in Saline/Saline or in OVA/BUD mice (One-way ANOVA Test, ** $p < 0.001$: OVA/Saline vs Saline/Saline, or OVA/Saline vs OVA/BUD; * $p < 0.05$: OVA/BUD vs Saline/Saline). (B) The ratio of lung/body weight was lower in Saline/Saline mice compared with other groups (One-way ANOVA Test, * $p < 0.05$: Saline/Saline vs OVA/Saline, or Saline/Saline vs OVA/BUD). The levels of IL-6 and IFN- γ in the BALF were measured by ELISA. (C). Secretion of IL-6 was higher in OVA/Saline mice than that of other groups, whereas the level of IFN- γ was the lowest in the three groups (D), One-way ANOVA Test, ** $p < 0.001$: OVA/Saline vs Saline/Saline, or OVA/Saline vs OVA/BUD. $n = 11$ for each group.

between Saline/Saline mice and OVA/BUD mice (Figure 3D).

Effects of budesonide on expressions of LIGHT, LT β R, and HVEM proteins in lung tissues

In lung tissues, several different cell types including T cells, B cells, dendritic cells, and epithelial cells, can express LIGHT and its receptors (HVEM, and/or LT β R). Whether inhaled budesonide can inhibit LIGHT/LT β R/HVEM pathway in asthmatic models remains unclear. In present study, the protein expressions of LIGHT, LT β R, and HVEM were significantly higher in OVA/Saline mice (Figure 4B, 5B, and 6B) than in Saline/Saline mice (Figure 4A, 5A, and 6A). The negative control was shown in the "Supplementary Figure 1". It indicated that repeated OVA sensitization and challenges caused lung tissues express more LIGHT, LT β R, and HVEM proteins. Furthermore, LIGHT, LT β R, and HVEM protein expressions were significantly lower in OVA/BUD mice (Figure 4C, 5C, and 6C) than in OVA/Saline mice. Thus, inhaled budesonide reduced the expressions

of LIGHT, LT β R, and HVEM proteins in the lung tissues of OVA model ($P < 0.05$; Figure 4D, 5D, and 6D).

Effects of budesonide on expressions of LIGHT LT β R, and HVEM mRNA in lung tissues

We next determined whether inhaled budesonide could affect LIGHT, LT β R, and HVEM mRNA production in lung tissues. In OVA/Saline mice, the LIGHT, LT β R, and HVEM mRNA expressions were increased when compared with Saline/Saline mice ($P < 0.05$). In OVA/BUD mice, the LIGHT, LT β R, and HVEM mRNA expressions were significantly decreased when compared with OVA/Saline mice ($P < 0.05$). Furthermore, the expression of HVEM mRNA showed no difference when compared with Saline/Saline mice ($P > 0.05$). Yet, the LIGHT and LT β R mRNA expressions in OVA/BUD mice were still significantly greater than that of Saline/Saline mice ($P < 0.05$; Figure 7). Therefore, inhaled budesonide treatment can decrease the expressions of LIGHT, LT β R, and HVEM mRNA in asthmatic lung tissues, which is

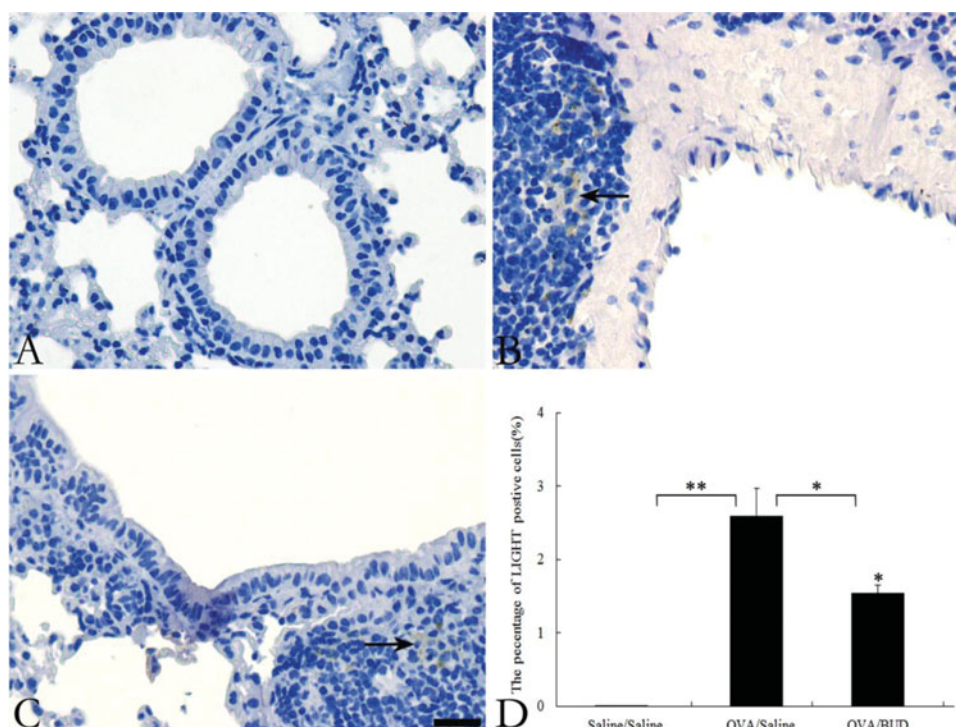


Figure 4. The expression of LIGHT protein in lung tissues in the three groups. Expression of LIGHT protein in lung tissues was determined by immunohistochemistry. Antigens are depicted in brown. Arrow showed LIGHT positive cells in the lung tissues. (A) Saline/Saline mice; (B) OVA/Saline mice; (C) OVA/BUD mice; (D) Quantitative analysis showed that the percentage of LIGHT positive cells in the OVA/Saline mice was higher than that of other groups. One-way ANOVA Test was used. ** $p < 0.001$: OVA/Saline vs Saline/Saline; * $p < 0.05$: OVA/Saline vs OVA/BUD or OVA/BUD vs Saline/Saline ($n = 5$ in each group). Scale Bar = 20 μm .

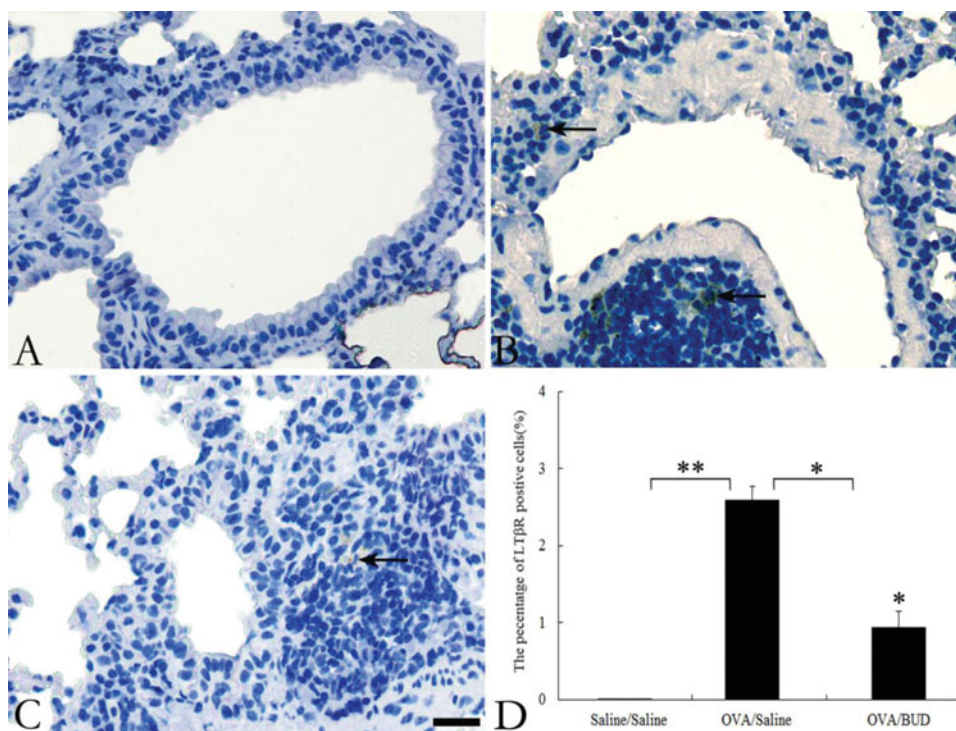


Figure 5. The expression of LTβR protein in lung tissues in the three groups. Expression of LTβR protein in lung tissues was determined by immunohistochemistry. Antigens are depicted in brown. Arrow showed LTβR positive cells in the lung tissues. (A) Saline/Saline mice; (B) OVA/Saline mice; (C) OVA/BUD mice; (D) Quantitative analysis showed that the percentage of LIGHT positive cells in the OVA/Saline mice was higher than that of other groups. One-way ANOVA Test was used. ** $p < 0.001$: OVA/Saline vs Saline/Saline; * $p < 0.05$: OVA/Saline vs OVA/BUD or OVA/BUD vs Saline/Saline ($n = 5$ in each group). Scale Bar = 20 μm .

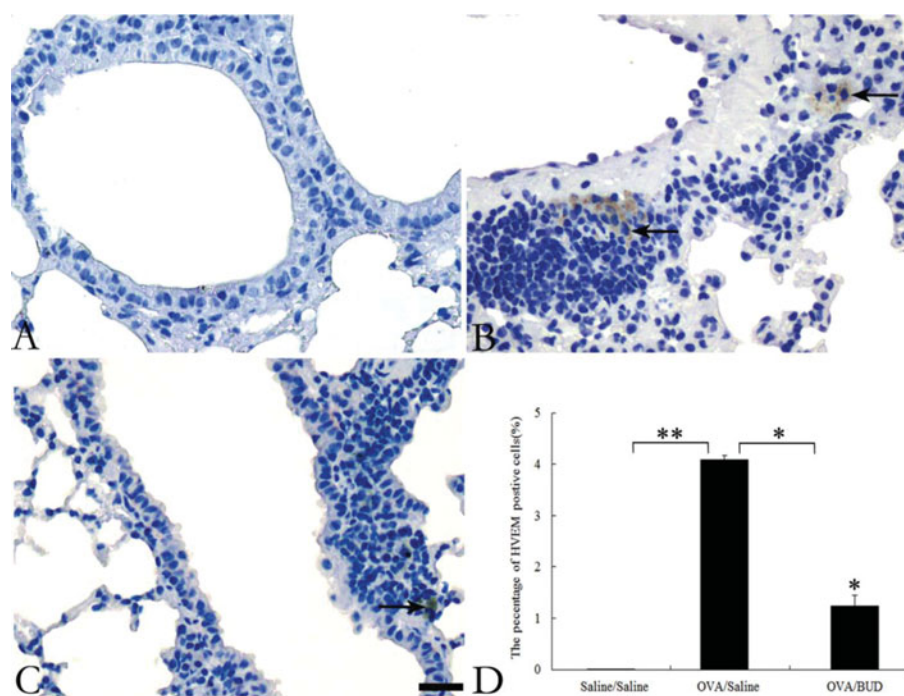


Figure 6. The expression of HVEM protein in lung tissues in the three groups. Expression of HVEM protein in lung tissues was determined by immunohistochemistry. Antigens are depicted in brown. Arrow showed HVEM positive cells in the lung tissues. (A) Saline/Saline mice; (B) OVA/Saline mice; (C) OVA/BUD mice; (D) Quantitative analysis showed that the percentage of LIGHT positive cells in the OVA/Saline mice was higher than that of other groups. One-way ANOVA Test was used. $**p < 0.001$: OVA/Saline vs Saline/Saline; $*p < 0.05$: OVA/Saline vs OVA/BUD or OVA/BUD vs Saline/Saline ($n = 5$ in each group). Scale Bar = 20 μ m.

similar to the results observed in all protein expression studies.

Correlations between levels of LIGHT/LT β R/HVEM mRNA and cytokine

To investigate the association between LIGHT/LT β R/HVEM mRNA expressions and T lymphocytes inflammatory functions in the airways treated by budesonide, we used linear regression analysis with the levels of IL-6, IFN- γ , and leukocytes count in BALF as dependents, and the expressions of LIGHT, LT β R, and HVEM mRNA in lung as covariates. We found that the levels of LIGHT and HVEM mRNA showed positive correlation with IL-6 concentration ($R^2 = 0.7955, 0.8209$). A positive correlation was also observed between the LIGHT mRNA expression and leukocytes count ($R^2 = 0.7949$) (Figure 8), whereas no significant correlation was detected between LIGHT/LT β R/HVEM mRNA and IFN- γ expressions (see Supplementary Materials).

Discussion

In the current study, we investigated changes in the expression of TNFSF member LIGHT in the lung

tissues of OVA model after inhalation of budesonide by immunohistochemistry, image analysis, and real-time PCR. We identified that LIGHT-mediated inflammation could be involved in the pathogenesis of chronic asthma, associated with an increased airway smooth muscle mass and abundance of different inflammatory cells. Furthermore, our findings indicated that inhaled budesonide could down-regulate the up-regulated LIGHT expression in asthmatic lung, which may involved in modulating airway inflammation and remodeling in chronic asthma.

T-cell inflammation is the most prominent pathophysiological feature of asthma, and it may contribute to chronic airway inflammatory response, airway remodelling, and symptomatology.^[6] LIGHT is recently recognized as a potential target for inhibiting airway remodelling and tissue fibrosis in asthma,^[16,17] and is believed to up-regulate proliferation, differentiation, and lymphokine production in T cells. Therefore, LIGHT-mediated inflammation may involved in the modulating chronic inflammatory response with airway remodelling in asthma. In the present study, we observed that an increase in allergen-driven airway inflammation characterized by an increase in inflammatory cell influx, together with an increase

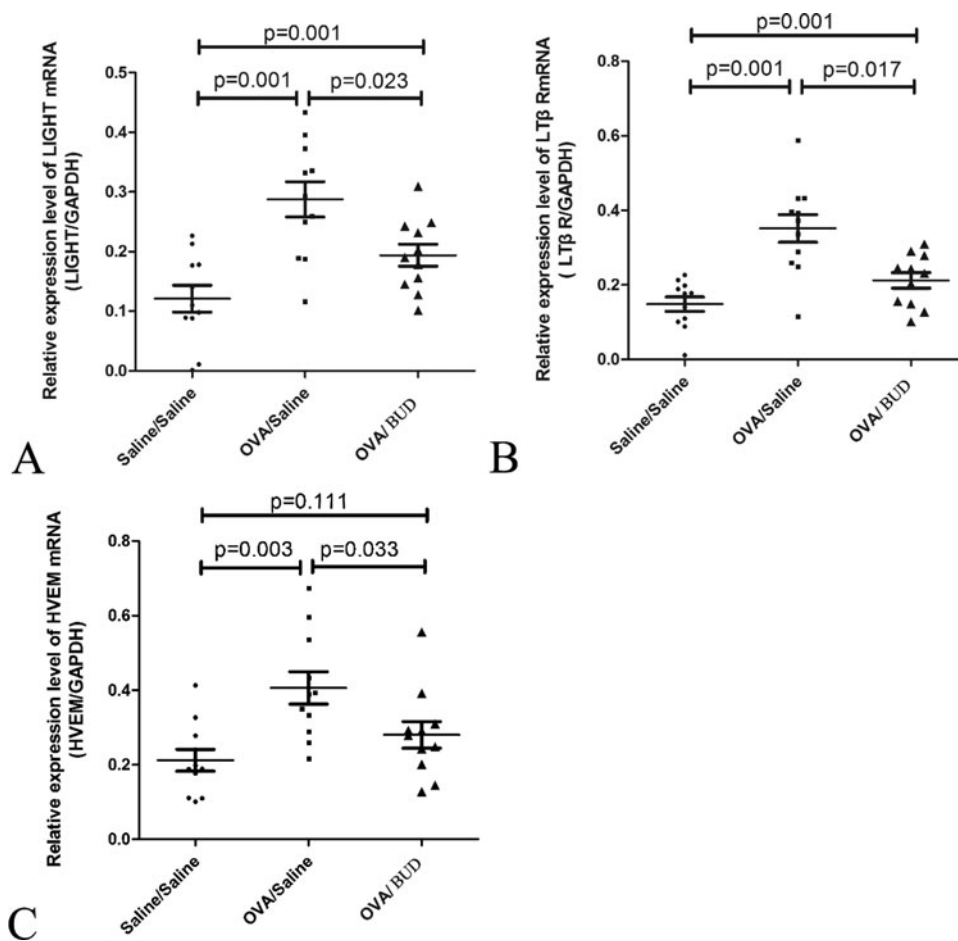


Figure 7. The expressions of LIGHT, LT β R, and HVEM mRNA in lung tissues in the three groups. Real-time polymerase chain reaction assay was carried out as described in the Materials and Methods section, and the results were obtained from the indicated group of samples. Scatterplot illustrated the relative expression of indicated mRNA as a ratio of LIGHT (A); LT β R (B) and HVEM (C) to GAPDH mRNA in each sample, $n = 11$ in each group.

in LIGHT, LT β R, and HVEM expressions. Our data showed LIGHT might contribute to exacerbations of airway inflammation in asthma via its interaction with LT β R and/or HVEM. Since the protein expression of HVEM was higher more than LT β R in asthmatic lung tissues, LIGHT/HVEM interaction is likely to be more critical than LIGHT/LT β R interaction in asthma.

Budesonide is a commonly used therapeutic drug in the treatment of asthma, and it can suppress airway inflammation and components of airway remodeling.^[15,18] However, the effect of this drug on LIGHT mediated pathways in asthma is unknown. Therefore, in our study, we compared the LIGHT, LT β R, and HVEM expressions in the lung obtained from asthmatic mice to those obtained from asthmatic mice treated with budesonide. We found that the protein and mRNA expressions of LIGHT, LT β R, and HVEM were all down-regulated in the lungs of OVA/BUD

mice, which suggests that budesonide inhibits airway inflammation, at least in part, by blocking LIGHT/LT β R/HVEM interactions. Since LIGHT is a crucial mediator of airway remodeling in asthma,^[19] budesonide may ameliorate airway remodelling via the inhibition of LIGHT-mediated pathway. Additionally, the mRNA levels of HVEM but not LT β R in budesonide treated mice were similar to the control mice. We can deduce that budesonide may restrain LIGHT-mediated inflammation, primarily through its effect on HVEM expression.

In bronchial epithelial cells IL-6 expression was found to be induced by LIGHT thus showing that LIGHT may be important for IL-6 production.^[20] In our study we observed the expression levels of IL-6 and LIGHT/HVEM mRNA showed significant positive correlation in budesonide-treated mice. So far it has been claimed that IL-6, a proinflammatory marker, can

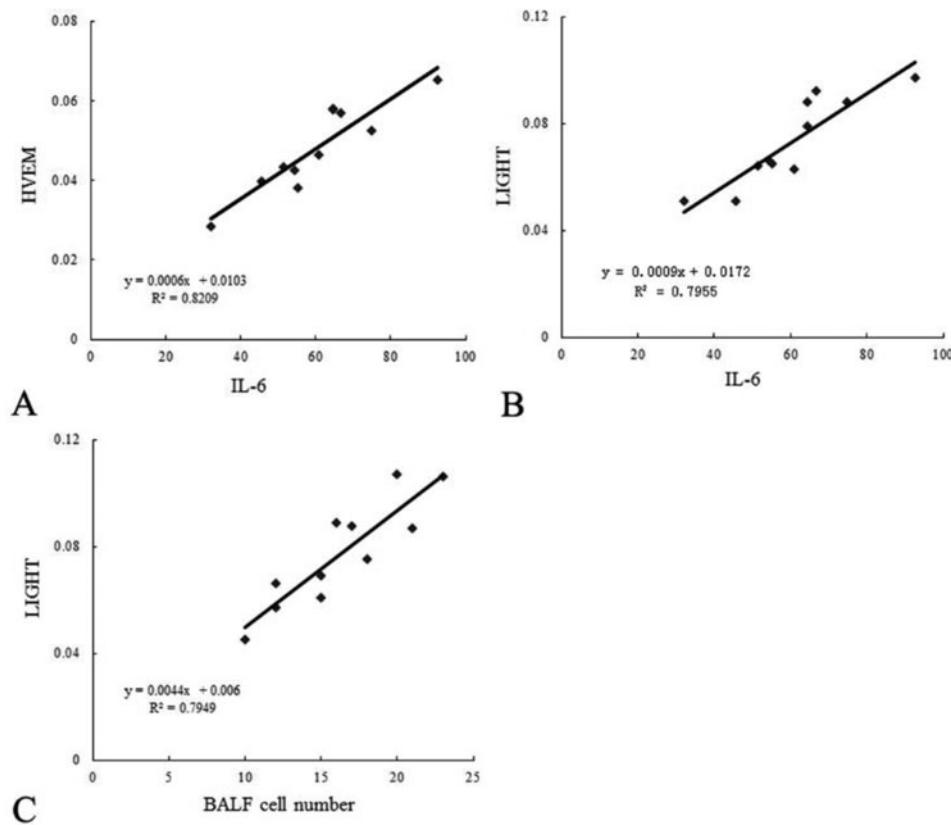


Figure 8. Correlation between LIGHT, HVEM, IL-6, and infiltration cells number in OVA/BUD mice. Scatter plot and linear-regression analysis of correlation between LIGHT, HVEM, IL-6, and infiltration cells number in OVA/BUD mice. (A) Correlation between HVEM and IL-6; (B) Correlation between LIGHT and IL-6; (C) Correlation between LIGHT and infiltration cells number.

elicit Th2 polarization and induce T cells reflux to airways.^[21,22] Additionally, LIGHT may be crucial for the release of IL-6.^[23] In light of the association studies, it can be hypothesized that budesonide may decrease the induction of IL-6 by inhibiting LIGHT/HVEM interaction and thus alleviate the airway inflammation.

Interestingly, we found no correlation between the expressions of IFN- γ and LIGHT/LT β R/HVEM mRNA. It is pointed the inadequate secretion of IFN- γ , a Th1-associated cytokine, may cause eosinophilic inflammation, and promote allergic airway inflammation.^[24] LIGHT can costimulate T cell proliferation, and induce the production of IFN- γ .^[25] Moreover, budesonide was found to up-regulate IFN- γ production in the BALF of our model. Collectively, our data suggest that LIGHT-mediated pathway isn't a major pathway through which budesonide might influence IFN- γ production. However, the precise role of budesonide in LIGHT/LT β R/HVEM interactions remains to be investigated.

In conclusion, our data suggest that budesonide treatment is effective in reducing airway inflammation by down-regulating the expressions of LIGHT, LT β R,

and HVEM, suggesting that LIGHT/LT β R/HVEM interactions may be a potentially key target for new anti-asthma therapeutic strategies. Further research about the influence of combined inhaled budesonide and blockage of LIGHT/LT β R/HVEM interactions in asthma is required.

Acknowledgments

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Conflict of interest

Conflict of interest should be mentioned or ruled out.

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Effects of Inhaled corticosteroids on the Expression of TNF Family Molecules Light in Chronic Asthma.

Supplementary Material, Zhang, et al.

Supplemental Figure S1:

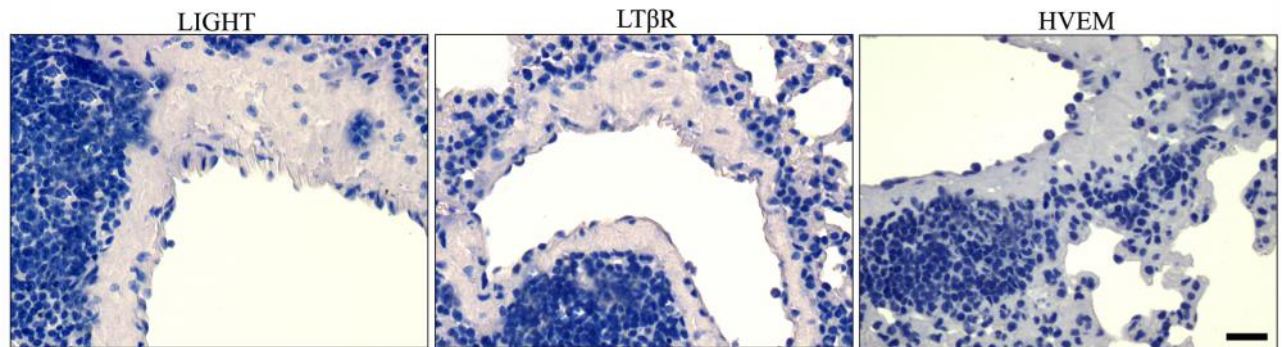


Figure S1. The expressions of LIGHT/LT β R/HVEM protein in lung tissues of negative control. Expressions of LIGHT/LT β R/HVEM protein in lung tissues were determined by immunohistochemistry. No LIGHT/LT β R/HVEM positive cells are found in the lung tissues.

Supplemental Figure S2:

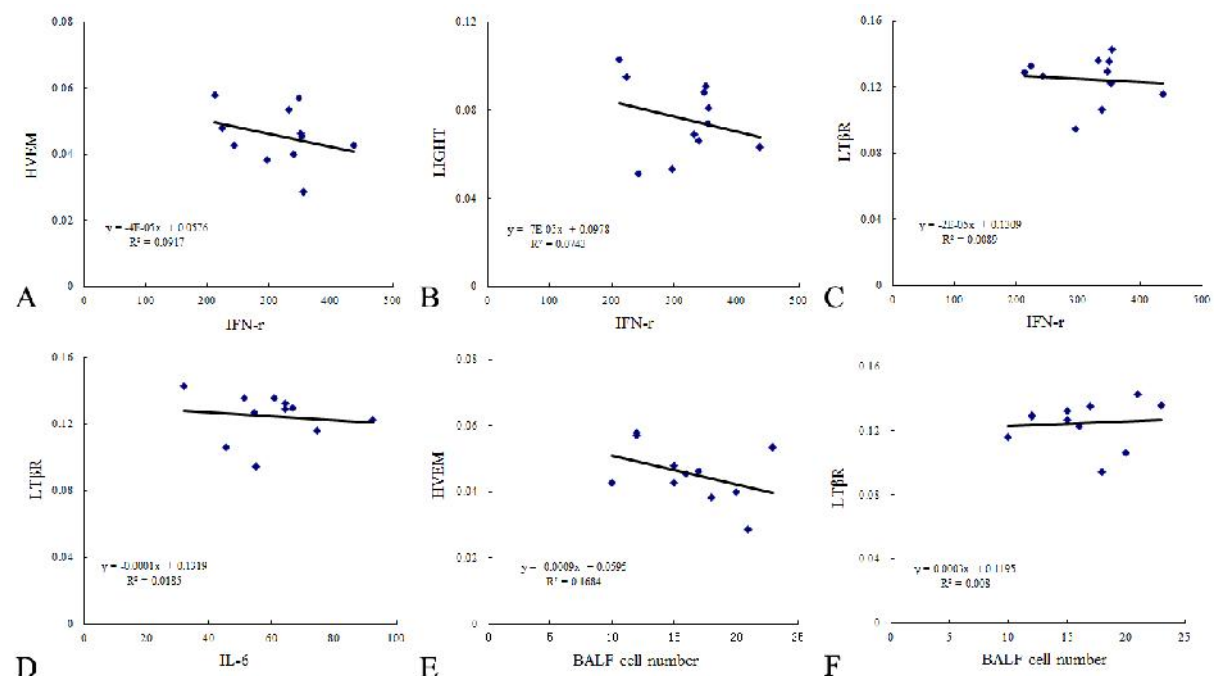


Figure S2. Correlation between LIGHT/LT β R/HVEM and IFN- γ /IL-6/infiltration cells number in OVA/BUD mice. Scatter plot and linear-regression analysis of correlation between LIGHT/LT β R/HVEM and IFN- γ /IL-6/infiltration cells number in OVA/BUD mice. (A) Correlation between HVEM and IFN- γ ; (B) Correlation

between LIGHT and IFN- γ ; (C) Correlation between LT β R and IFN- γ ; (D) Correlation between LT β R and IL-6; (E) Correlation between HVEM and infiltration cells number; (F) Correlation between LT β R and infiltration cells number.