

ORIGINAL ARTICLE

The Role of TNF Family Molecules Light in Cellular Interaction Between Airway Smooth Muscle Cells and T Cells During Chronic Allergic Inflammation

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Abstract— Interaction between T cells and airway smooth muscle (ASM) cells has been identified as an important factor in the development of asthma. LIGHT (known as TNFSF14) - mediated signaling likely contributes to various inflammatory disorders and airway remodeling. The objective of this study was to investigate the roles of LIGHT-mediated pathways in the interaction between ASM cells and T cells during chronic allergic inflammation. Mice were sensitized and challenged by ovalbumin (OVA) to induce chronic airway allergic inflammation. The control group received PBS only. The histological features and LIGHT expressions in lungs were assessed *in vivo*. Furthermore, T cells and ASM cells derived from the model mice were co-cultured both in the presence and absence of anti-LIGHT Ab for 72 h. The effects of LIGHT blockade on expressions of downstream signaling molecules, proliferation, and apoptosis of ASM cells, differentiation of T cells, and inflammatory cytokines release were evaluated. We demonstrated that LIGHT blockade strikingly inhibited the mRNA and protein expressions of HVEM, c-JUN, and NFκB. Additionally, LIGHT blockade resulted in decreased proliferation and increased apoptosis of ASM cells. Moreover, depletion of LIGHT dramatically reduced the differentiation of CD4⁺ T cells into Th1, Th2, and Th17 cells, as well as inhibited inflammatory cytokines release including IL-13, TGF-β, and IFN-γ, which are associated with CD4⁺ T cell differentiation and ASM cell proliferation. LIGHT plays an important role in the interaction between T cells and ASM cells in chronic allergic asthma. Blockade of LIGHT markedly suppressed ASM hyperplasia and inflammatory responses, which might be modulated through HVEM-NFκB or c-JUN pathways. Therefore, targeting LIGHT is a promising therapeutic strategy for airway inflammation and remodeling in chronic allergic asthma.

KEY WORDS: asthma; LIGHT; interaction; T cells; airway smooth muscle cells; airway inflammation; airway remodeling.

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INTRODUCTION

Asthma is a chronic inflammatory disease of the airways characterized by reversible airflow obstruction and airway hyper-responsiveness, and is associated with an infiltration of inflammatory cells and increased expression of inflammatory mediators [1–3]. In people with asthma, T cells have been suggested to be crucial for driving chronic inflammatory process, and have been shown to be involved in eliciting structural changes in the airways.

Although the mechanisms underlying these inflammation-fueled changes of airway remodeling remain largely unknown, intercellular communication between T cells and airway smooth muscle (ASM) cells has been identified as an important factor in the development of airway pathophysiology in asthma. Therefore, it is important to understand the complexities of this cellular interaction and seek new target for anti-inflammatory and anti-remodeling therapies in asthma.

LIGHT, known as tumor necrosis factor superfamily member 14 (TNFSF14), is an important co-stimulatory molecule for T cell immunity and plays critical roles in regulation of cell survival, apoptosis, proliferation, differentiation, and cytokine production. It is known that LIGHT is produced by activated T cells and can bind to its receptors including herpesvirus entry mediator (HVEM) and lymphotoxin β receptor (LT β R). In addition, HVEM is expressed on T cells, NK cells, DCs, and endothelial cells; LT β R is expressed on ASM cells, DCs, and endothelial cells, but is not expressed on T cells. Recently, LIGHT-HVEM and/or LIGHT-LT β R signaling has been implicated in driving various autoimmune and inflammatory disorders, and has also been implicated in the pathogenesis of airway remodeling. Thus, LIGHT-mediated pathways may be involved in inducing the interaction between airway inflammation and remodeling by regulating diverse biological activities of ASM cells and T cells.

Based on its emerging role in asthma pathogenesis, we hypothesized that LIGHT-mediated pathways may play a crucial role in the interaction between ASM cells and T cells in chronic allergic airway, and in turn promote airway inflammation and remodeling. The aim of the present study was to investigate the effects of LIGHT-mediated pathways in ovalbumin (OVA)-induced allergic asthma and the underlying intracellular mechanisms.

MATERIALS AND METHODS

Animals and Experimental Protocol

A total of 20 female 6-week old BALB/c mice were provided by the Guangdong Medical Laboratory Animal Center (Guangzhou, Guangdong, China), and housed in specific pathogen-free conditions in the Experimental Animal Unit of the Shenzhen-PKU-HKUST Medical Center (Shenzhen, Guangdong, China) for 14 days prior to subsequent experiments. The animal experimental procedures followed protocols approved by the Laboratory Animal

Care and Use Committee of the Shenzhen-PKU-HKUST Medical Center.

The mice were randomly divided into two groups: control group (PBS) ($n=10$) and asthma group (OVA) ($n=10$). For *in vivo* asthma modeling, mice were initially sensitized by intraperitoneal injection of 20 μ g OVA adsorbed with aluminum hydroxide on days 0, 14, 28, and 42, as Vieira RP (2007) described previously [4]. Then, the mice were challenged for 30 min with an aerosol of 1% OVA in PBS, three times per week until day 51. Challenging with aerosolized OVA was performed in a Plexiglas chamber (30 $\times$ 15 $\times$ 20 cm) coupled to an ultrasonic nebulizer. At 24 h after the last challenge, animals were sacrificed by cervical dislocation, and lungs and spleens were excised. The right lungs of both groups were cut into tissue sections for preparation of lung histology and immunohistochemistry, whereas the lungs and spleens of asthma group were used for *in vitro* experiments.

Lung Histology and Immunohistochemistry

The right middle lung from each mouse was harvested from each group. Samples were fixed in 3% formaldehyde overnight and embedded in paraffin. 5- μ m lung sections were cut and then stained with hematoxylin and eosin (H&E) as Kita T (2009) described previously [5]. Histological assessment was performed by investigators blinded to the protocol design.

Immunohistochemistry was performed on the histological sections of 5- μ m lung samples. The sections were deparaffinized and 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) overnight at 4 °C, respectively, and then washed three times with 0.1 M PBS for 10 min. Subsequently, the sections were incubated with biotin-conjugated donkey anti-goat IgG (1:400, Vector Labs, Burlingame, CA, USA) in PBS for 2 h. Staining was performed using an ABC kit (Vector Labs), and color development was achieved by incubating the sections for 5 min 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.

Isolation of ASM Cells and Splenocytes

The ASM cells were isolated from left lungs and cultured using previously described techniques [6]. Briefly, lung sections were cut in a longitudinal fashion, and then cut in cross-sections of 2–3 mm. These were placed in sterile dishes and maintained in Dulbecco's modified eagle medium (DMEM) supplemented with 20% fetal bovine serum, 4 mM glutamine, penicillin, and streptomycin. Explants were kept at 37°C, 5%CO₂, 95% air for 4 days, and then serum reduced to 10%. ASM cell cultures exhibited a typical "hill-and-valley" growth pattern, and immunofluorescence techniques for α -actin revealed that > 95% of the cells displayed the characteristics of ASM cells in culture. Cells at passage 3–6 were used for experiments.

Spleens were pressed through a wire mesh screen to obtain single-cell suspensions. The splenocytes were separated from the spleens by density centrifugation (2500 rpm, 10 min) through Lympholyte M solution (Cedarlane Laboratories, Hornby, Ontario, Canada). These cells were further washed three times in cold Hank's balanced salt solution (HBSS) and resuspended in RPMI1640 medium. T cells were isolated from the cell suspension using magnetic separation (Miltenyi Biotec, Germany) by negative depletion of non-T cells. The purity of T cells was >95% as confirmed by anti-CD3 staining using flow cytometry. Harvested T cells were cultured at 5×10^6 cells/well in RPMI 1640 supplemented with 20% fetal bovine serum and interleukin-2 (100 units/ml) for 3–4 days.

Co-culture of ASM Cells with Splenocytes

The co-culture of the splenocytes with ASM cells was carried out as previously described [7]. Briefly, splenocytes were seeded at 5×10^6 cells/well and incubated with ASM cells (5×10^4 cells/well) in 6-well plates for 24 h in RPMI 1640 medium with 10% FBS, 100 units/ml interleukin-2, 50 μ M 2-Me, 100 U/ml penicillin, 100 μ g/ml streptomycin, and 2 mM L-glutamine, at 37 °C in a humidified 5% CO₂ atmosphere. Subsequently, the splenocytes and ASM Cell cultures were randomly treated with or without 10 μ g/ml anti-LIGHT mAb (R&D Systems Inc., Minneapolis, MN). After 3 days of treatment, the lymphocytes and ASM cells were separated by rinsing three times of the co-cultures, as previously described [8], and the isolated lymphocytes, ASM cells, and the co-culture medium were collected for further analysis.

Western Blotting Analysis

Western blotting procedures were performed as described previously [9]. Briefly, the separated lymphocytes and ASM cells were lysed in the RIPA buffer (50 mM Tris-HCl, pH 7.6, 150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS). The concentration of the proteins was measured with the Bicinchoninic Acid Kit (Thermo Scientific, Rockford, USA). The primary antibodies used were: anti-HVEM Ab (Cell Signaling, Inc.), anti-c-JUN Ab (Cell Signaling, Inc.), anti-NF κ B Ab (Cell Signaling, Inc.), anti- LT β R Ab (Cell Signaling, Inc.), and anti-JNK Ab (Cell Signaling, Inc.). Relative concentration of proteins was quantitated using the Image J Software (National Institutes of Health, Bethesda, USA).

Real-Time PCR

Total RNA was extracted from the separated lymphocytes and ASM cells using the TRIzol RNA Isolation Reagents (Life Technologies), and the first-strand cDNA was converted using a the SYBR PrimeScript™ RT-PCR kit reagent (Takara, Dalian, China) according to the manufacturer's protocols. Real-time RT-PCR analysis was performed on conducted using an ABI PRISM 7500 real-time PCR System (Applied Biosystems, Foster City, CA, USA). The target mRNA levels were normalized with the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) housekeeping gene.

Proliferation and Apoptosis Assays

Cell proliferation in isolated ASM Cells was investigated by determined using BrdU ELISA according to manufacturer's protocol and as described previously [6]. Briefly, the ASM cells were plated onto 96-well plates at a density of 1000 cells/well in DMEM supplemented with 0.1% FBS for 24 h. The growth medium was then switched to DMEM supplemented with 10% FBS, and the cells were incubated in this medium for additional 24 h. On the last day, BrdU labeling solution (Roche, Germany) was added to the cultures, and the cells were incubated for further 10 h. The absorbance was measured within 5 min at 450 nm with a reference wavelength at 690 nm. Each sample was cultured in triplicate.

For detection of the separated ASM cells apoptosis, the terminal deoxynucleotidyl transferase-mediated nick-end labeling (TUNEL) assay was performed using a commercial kit (BD Biosciences, San Diego, CA, USA), as previously described [10]. TUNEL-positive cells were counterstained with 4',6-diamidino-2-phenylindole

(DAPI) and observed under fluorescence microscopy by a blinded observer.

Flow Cytometry

The isolated lymphocytes were incubated with the relevant molecule antibodies at the optimized concentrations at 4 °C for 30 min. They centrifuged, washed twice with a staining buffer, and analyzed by a FACS Calibur flow cytometer according to the manufacturer's protocol (e-Bioscience). For flow cytometry analysis of different T helper subsets (Th1, Th2, Th17, and Treg cells), anti-CD4, anti-CD25, anti-IFN- γ , anti-IL-4, anti-IL-17, and anti-Foxp3 antibodies in various fluorochrome combinations were purchased from BD Biosciences (San Diego, CA).

ELISA Assays

The co-cultured supernatants were centrifuged at 1000 rpm (4 °C) for 10 min to remove cell debris. The levels of IL-13, TGF- β , and IFN- γ were measured using commercially available enzyme-linked immunosorbent assay (ELISA) kits (BD Biosciences, San Diego, CA, USA) according to the manufacturer's instructions.

Statistical Analysis

All data are presented as mean $\pm$ SE and analyzed by one-way analysis of variance (ANOVA) and *t* test using SPSS 17.0. Values of $P < 0.05$ were considered statistically significant.

RESULTS

Impact of Chronic OVA Exposure on Airway Inflammation and Remodeling

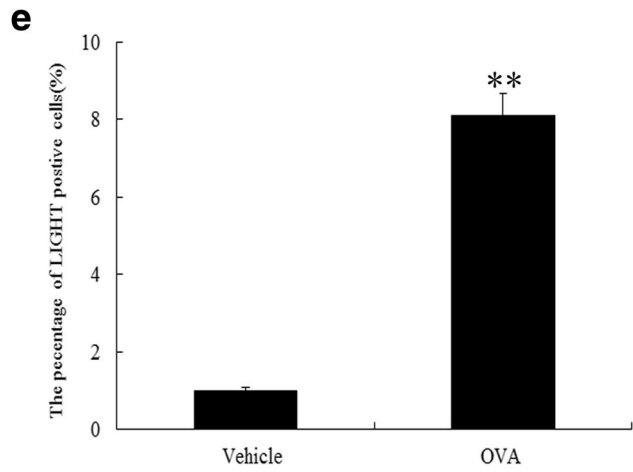
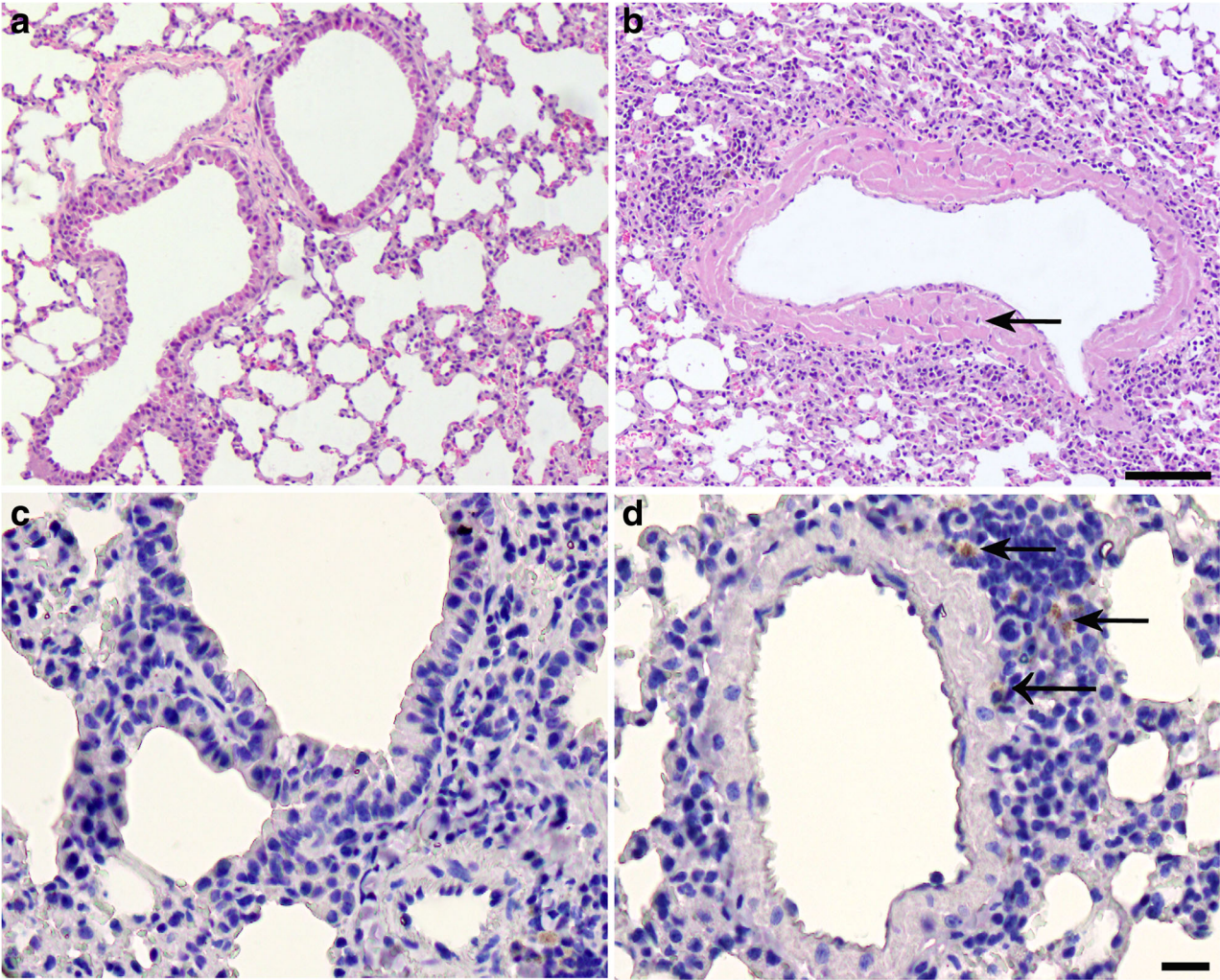
As inflammatory cells infiltration and ASM cells hyperproliferation contribute to inflammatory mechanisms and airway remodeling in chronic asthma, these interactions between ASM cells and T cells are recognized to be more important than before. We therefore assessed the success of a mouse model of chronic allergic asthma, and the inflammatory and structural abnormalities by lung histology stained with HE. As we have reported previously [6], the chronic OVA-challenged mice exhibited tachypnea and mild cyanosis symptoms during challenging with OVA, and they developed strong peribronchial infiltrate of lymphocytes and eosinophils in the peribronchial and perivascular areas. There were also areas of mild peribronchial

Fig. 1. Chronic allergic inflammation and tissue remodeling in response to repeated OVA challenge in mice. **a–b** Representative photomicrographs of lung sections stained with H&E were examined. Marked infiltration of inflammatory cells and increased smooth muscle layers were present in OVA/OVA mice (**b**) compared to the PBS/PBS mice (**a**). Images were presented at the original magnification of $\times 200$. **c–d** Expression and localization of LIGHT in the lung tissue. LIGHT protein was detected by immunohistochemistry in tissue sections from OVA/OVA mice (**c**) and PBS/PBS mice (**d**). Antigens are depicted in brown. Arrow showed LIGHT-positive cells in the lung tissues. Images were presented at the original magnification of $\times 400$. **e** Quantitative analysis showed that the percentage of LIGHT-positive cells in the OVA/OVA mice was higher than that in the PBS/PBS mice ($P < 0.05$, $n = 5$ for each group). Scale bar = 20 μ m.

muscle hypertrophy, epithelial denudation, and angiogenesis in the chronic animals (Fig. 1a). In contrast, no histological evidence of lung damage was found in the control animals. Together, these data showed that the mouse model of chronic allergic asthma was successfully established, and chronic OVA exposure was associated with notable abnormalities of ASM cells and lymphocytes. Next, we performed immunohistochemistry assay to investigate the effects of repeated OVA rechallenge on LIGHT production. As shown in Fig. 1b, c, the protein expression of LIGHT in lung tissues was higher in chronic OVA-challenged mice than in the control mice. These results demonstrate that increased LIGHT expression may be associated with airway inflammation and remodeling in chronic OVA-challenged mice.

Effects of Anti-LIGHT Ab on Signal Pathways Downstream in the Intercellular Interplay

To analyze whether LIGHT-mediated pathways have an effect on the intercellular interplay between ASM cells and T cells *in vitro*, mouse splenic T cells were co-cultivated with ASM cells and incubated with anti-LIGHT Ab for 72 h. HVEM, LT β R, c-JUN, and NF κ B productions were detected by real-time PCR and western blotting, respectively. As shown in Fig. 2a,b, c, the mRNA and protein expressions of HVEM, c-JUN, and NF κ B were inhibited by this antibody. However, the incubation with anti-LIGHT Ab had no influence on the productions of LT β R and JNK, suggesting that the bioactivity of LT β R might be not required for these intercellular processes. The results indicated that LIGHT might bind to the cells receptors and participate the interplay between ASM cells and T cells through HVEM-NF κ B/c-JUN signaling pathways.



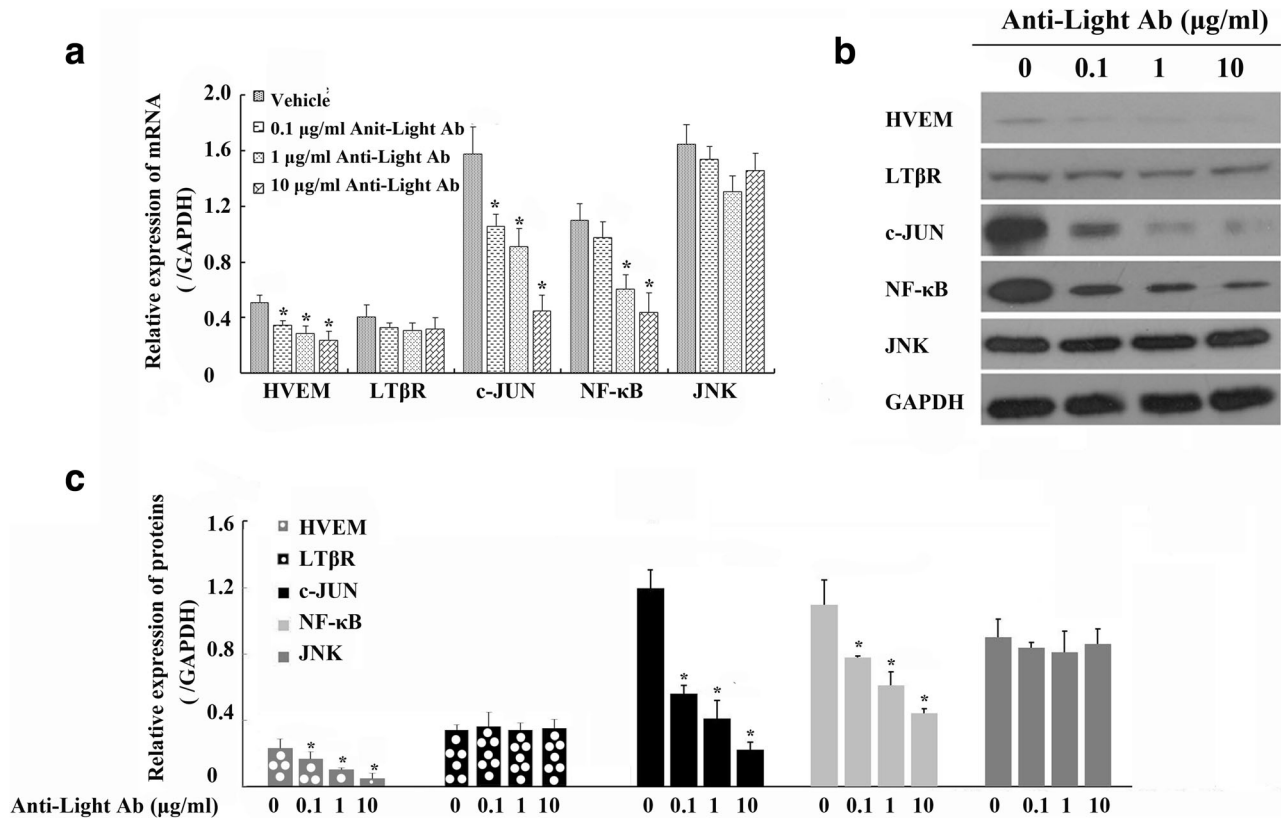


Fig. 2. The effects of anti-LIGHT Ab on HVEM, LTβR, c-JUN, NFκB, and JNK productions. **a** Real-time PCR analysis of HVEM, LTβR, c-JUN, NFκB, and JNK mRNA expressions were compared between control group and anti-LIGHT Ab group (1, 10, and 100 μg/ml). Data are presented as the mean ± SD, $n = 5$ in each group. **b** Western blot analysis of HVEM, LTβR, c-JUN, NFκB, and JNK protein levels in control or anti-LIGHT Ab group (1, 10, and 100 μg/ml). GAPDH was used as internal control. **c** Quantitative analysis of HVEM, LTβR c-JUN, NFκB, and JNK protein levels were compared between control group and anti-LIGHT Ab group (1, 10, and 100 μg/ml). * $P < 0.05$ vs. control group.

Effects of Anti-LIGHT Ab on ASM Cells Proliferation and Apoptosis in the Intercellular Interplay

LIGHT was recently found to regulate cell survival and apoptosis in several cells by binding to its receptor HVEM/LTβR [11, 12]. HVEM and LTβR can be expressed in the airway epithelium, suggesting that LIGHT-HVEM/LTβR signaling may control the smooth muscle hyperplasia in asthmatics. To investigate the effects of LIGHT on ASM cells proliferation, we performed BrdU ELISA assays *in vitro*. As expected, addition of anti-LIGHT Ab resulted in significant inhibition of ASM cells proliferation (Fig. 3a). Next, under the same pre-treatment conditions, we used TUNEL assay to examine if blocking activity of anti-LIGHT Ab could lead to ASM cell apoptosis. As shown in Fig. 3b, c, TUNEL-positive cells were significantly increased by anti-LIGHT Ab pre-treatment. Altogether, these results demonstrate that LIGHT is

involved in proliferation induction and apoptosis inhibition of ASM cells.

Anti-LIGHT Ab Selectively Inhibited CD4⁺ T Cells Differentiation in the Intercellular Interplay

Naive T cells can be differentiated to various subsets in different culture conditions. To investigate the effect of anti-LIGHT Ab on CD4⁺ T cells differentiation when co-cultured with ASM cells, the changes in different Th cell subsets were analyzed using specific flow cytometry antibodies (*i.e.*, CD4 and IFN-γ for Th1 cells, CD4 and IL-4 for Th2 cells, CD4 and IL-17 for Th17 cells, and CD4, CD25 and FOXP3 for Treg cells). Figure 4a,b, c showed the percentages of CD4⁺ IFN-γ⁺ Th1, CD4⁺ IL-4⁺ Th2, and CD4⁺ IL-17⁺ Th17 were decreased in the anti-LIGHT Ab-treated cells compared with untreated cells. In contrast, anti-LIGHT-Ab had a statistically negligible effect on

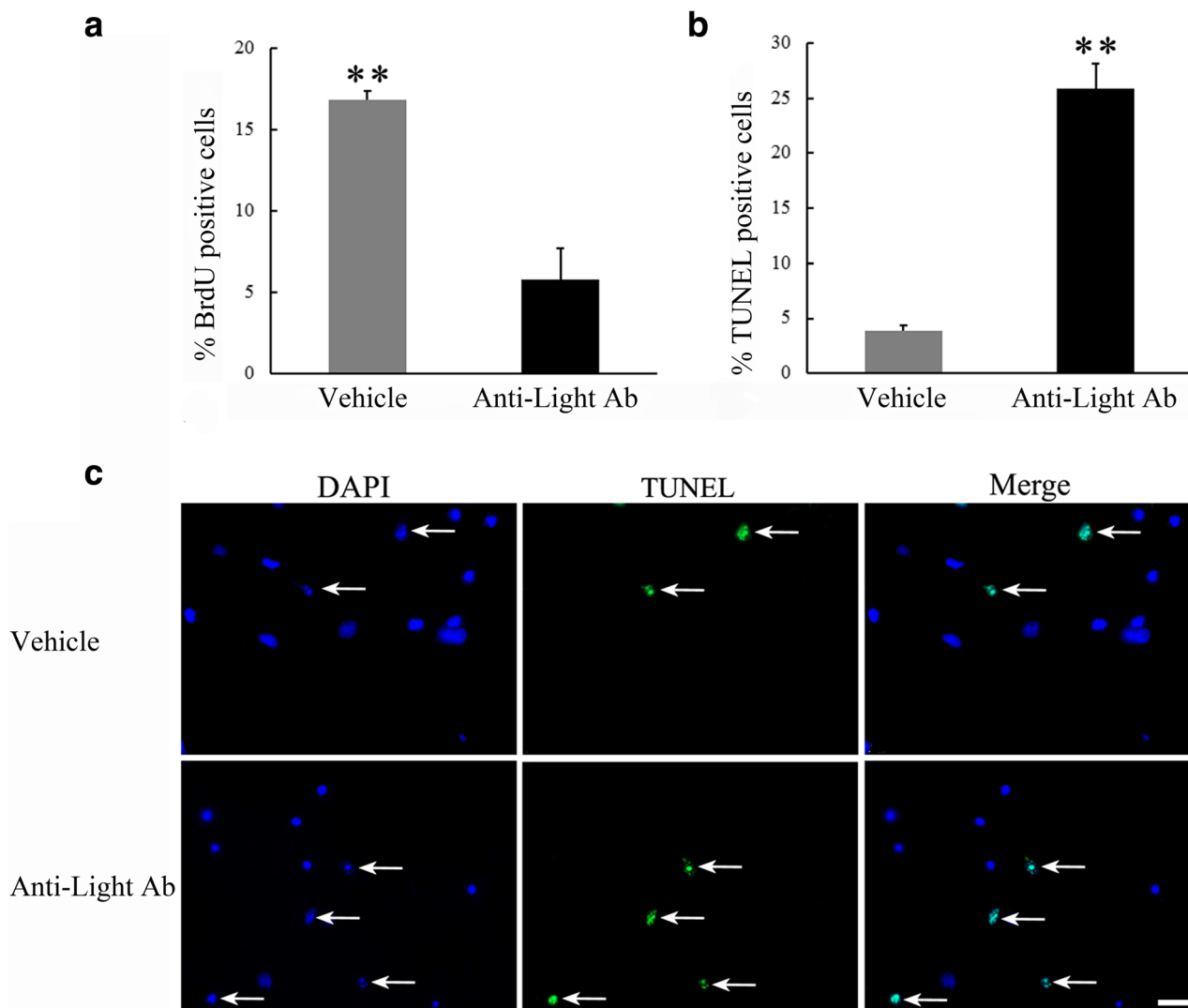


Fig. 3. Anti-LIGHT Ab reduced AMS cells proliferation and enhanced apoptosis. **a** ASM cells treated with anti-LIGHT Ab for 3 days showed a decrease in BrdU incorporation in comparison to control cells. ($P < 0.05$, $n = 5$ for each group). **b-h** TUNEL staining (green) showing quantitative data (**b**) and representative images (**c-e** in control cells, **f-h** in anti-LIGHT Ab-treated AMS cells), and nuclei are stained with DAPI.

CD4⁺CD25⁺FOXP3⁺ Treg cells development. Our result showed that anti-LIGHT Ab might have some inhibitory effects on the differentiation of CD4⁺T cells into Th cells in the intercellular interplay.

Effects of Anti-LIGHT Ab on Cytokine Levels in the Intercellular Interplay

To further explore the role of LIGHT activity in inflammatory cytokine secretion that is linked to airway inflammation and remodeling, we analyzed

the concentration of IL-13, TGF- β , and IFN- γ in co-cultured supernatants untreated or treated with anti-LIGHT Ab by ELISA. As shown in Fig. 5a, anti-LIGHT antibody showed significant reduction in IFN- γ secretion, indicating that LIGHT plays a major role in IFN- γ production predominantly from Th1 and ASM cells. Additionally, anti-LIGHT Ab down-regulated the expression of IL-13 and TGF- β (Fig. 5b, c), suggesting that LIGHT may account for Th2 cells development and ASM cells proliferation by inducing the production of IL-13 and TGF- β .

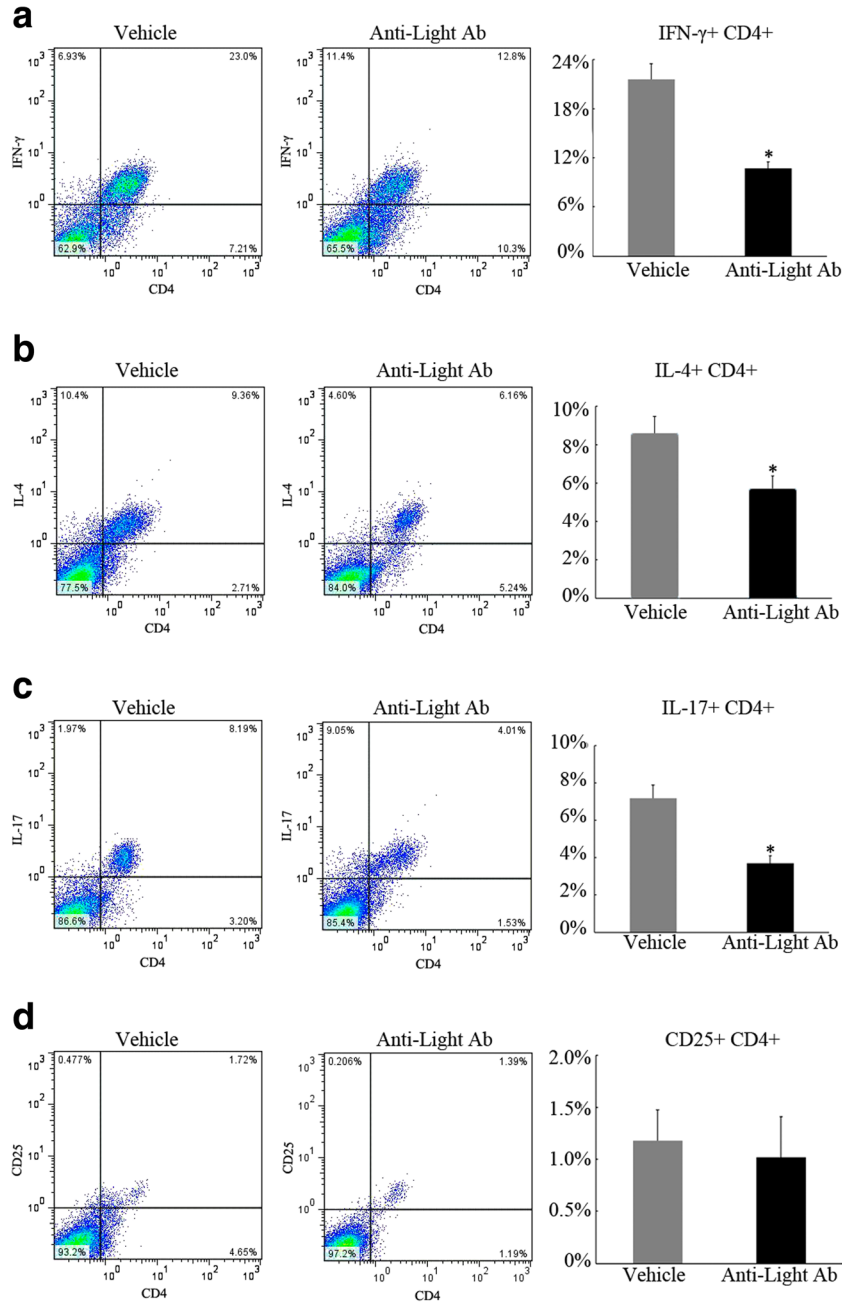


Fig. 4. Anti-LIGHT Ab selectively inhibited the differentiation of CD4⁺ T cells into Th cells. Flow cytometry analysis of Th cell subsets showed that productions of IFN- γ by Th1 cells, IL-4 by Th2, and IL-17 by Th17 were decreased in the anti-LIGHT Ab-treated T cells compared to control T cells. Representative results of control Th1 (**a**), anti-LIGHT Ab-treated Th2 (**b**), control Th2 (**d**), anti-LIGHT Ab-treated Th2 (**e**), control Th17 (**g**), anti-LIGHT Ab-treated Th17 (**h**), control Treg (**j**), and anti-LIGHT Ab-treated Treg (**k**) are shown. The geo-mean values for each Th cell subset are shown in the histogram (**c** for Th1, **f** for Th2, **i** for Th17, and **l** for Treg). Data are represented as mean $\pm$ SD, $n = 5$ for each group. * $P < 0.05$ vs. control group.

In brief, the results above indicated that intercellular signals *via* LIGHT could increase inflammatory

cytokine release, which is associated with CD4⁺ T cell differentiation and ASM cell proliferation.

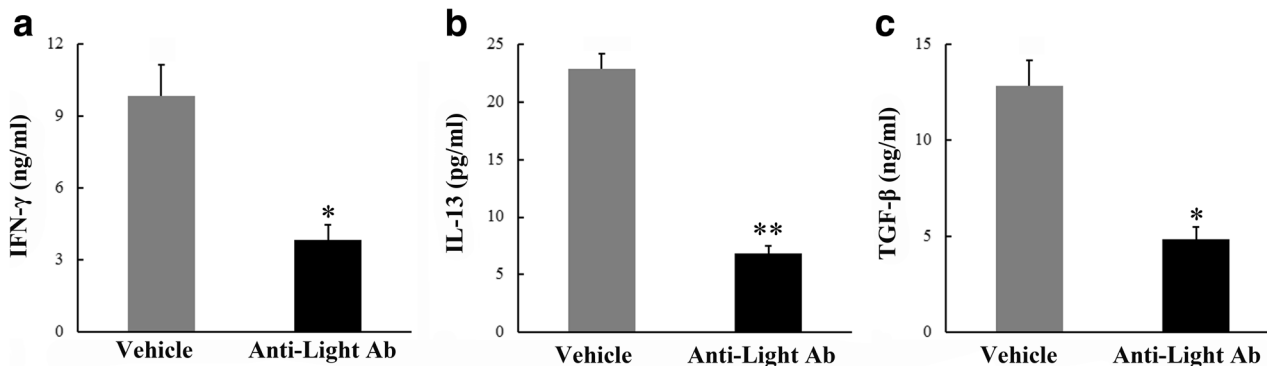


Fig. 5. Effects of anti-LIGHT Ab on cytokine levels. ELISA analyses of the expression levels of IL-13 (a), TGF- β (b), and IFN- γ (c) in the cell culture supernatants derived from anti-LIGHT Ab-treated cells or control cells. Data are represented as mean $\pm$ SD, $n = 5$ for each group. * $P < 0.05$ vs. control group.

DISCUSSION

The aim of this study was to define the contributions of LIGHT in the interplay of T cells and ASM cells derived from an OVA-induced mice model of chronic asthma. First, we identified that LIGHT protein expression was significantly higher in the lung tissue of chronic asthmatic mice, in comparison with the control mice. We also showed that anti-LIGHT Ab directly affected the expression levels of HVEM, NF κ B, and c-JUN in the co-cultivation of T cells and ASM cells. Additionally, we found that anti-LIGHT Ab treatment decreased ASM cells proliferation, increased ASM cells apoptosis, inhibited the differentiation of CD4⁺ T cells into Th cells, and reduced some inflammatory cytokines release. It was believed LIGHT binding HVEM can lead to activation of several signaling pathways, which are involved in the control of cells activation, proliferation, apoptosis, differentiation, and cytokine secretion [11, 13, 14]. These findings provide novel insight into the critical role of LIGHT-mediated signaling pathways, which may present a key target to modulate T cell-ASM cell interaction in asthma.

Doherty TA et al. reported that LIGHT plays an important role in fibrosis and smooth muscle hyperplasia in asthma [15]. In asthma-related airway remodeling, smooth muscle hyperplasia around the airways is associated with the increased proliferation and diminished apoptosis of ASM cells [16]. On the other hand, LIGHT has been shown to function as a co-stimulatory factor on lymphoid cells [17] and increase the severity of various T cell-mediated diseases [18, 19]. We found that LIGHT expression was significant enhanced in the lung tissue of chronic asthmatic mice, accompanied by the airway wall thickening and inflammation. Furthermore, our study also showed

that LIGHT blockade with a specific antibody *in vitro*, which decreased the expressions of HVEM, NF κ B, and c-JUN in the co-cultivation of T cells and ASM cells. Thus, these results suggest that LIGHT may play a major role in the interaction between T cells and ASM cells in the airways of asthma, *via* mediating positive signaling activities through HVEM activating NF κ B and c-JUN.

Airway remodeling has been considered a physiologic consequence of chronic asthma [20]. An increase in airway smooth muscle mass is one of the most striking features of airway remodeling [21]. The TNF superfamily member LIGHT, acting through its receptor, HVEM or LT β R, can provide cell proliferation and survival signals. Recent studies have highlighted the crucial roles of LIGHT in airway remodeling. It has been also postulated that LIGHT may be a target to prevent asthma-related airway remodeling. In the current study, LIGHT depletion *via* antibody resulted in a striking reduction of ASM cells proliferation, and a significant increase in ASM cells apoptosis during the co-culture *in vitro*, implying that the inhibition of LIGHT could decrease ASM mass, and subsequently attenuate airway hyper-responsiveness in asthma. The structural changes in airway are associated with asthmatic airway inflammation, and may therefore affect the innate and adaptive immune responses.

Chronic airway inflammation is another characteristic feature of asthma, and accumulation of Th cells in the airway mucosa is thought to contribute to the primary inflammatory lesion of asthma by secreting of a series of cytokines [22]. These Th cell subsets differentiate from naive CD4⁺ T cells, and they may orchestrate a chronic inflammatory response in asthma [23, 24]. In this study, we found that antibody blockade of LIGHT significantly inhibited CD4⁺ T cells differentiation into Th1, Th2, and

Th17 cells, and also reduced the levels of several inflammatory cytokines including IL-13, TGF- β , and IFN- γ . It was indicated that activated naive CD4⁺ T cells can differentiate into at least four major lineages, Th1, Th2, and Th17, as well as Treg cells, and control critical aspects of lung immunity [25]. The direction of differentiation of Th cells is crucial for the induction and persistence of asthma. Furthermore, inflammatory cytokines and growth factors released by Th cells are associated with the development of airway inflammation and remodeling. Our results showed anti-LIGHT Ab downregulated the production of specific cytokines related to CD4⁺ T cell differentiation and ASM cell proliferation in the co-culture. Thus, these findings indicated that blockade of LIGHT might have a major role in reducing cellular inflammatory response and perhaps exert protective effects on airway remodeling in asthma.

A number of transcription factors may contribute to airway inflammation and remodeling in asthma. It has been reported that NF κ B and c-JUN are involved in regulating cell proliferation, differentiation, and apoptosis in response to diverse extracellular signals in asthma [26, 27]. In addition, NF κ B and c-JUN have been shown to promote cytokine release from inflammatory cells *via* HVEM. It was also demonstrated that LIGHT serves as a co-stimulatory molecule for T cell activation, inducing CD4⁺ T cell proliferation, cytokine production, and NF κ B translocation [28, 29]. Since our data showed that, with the signaling molecules being changed, anti-LIGHT Ab could induce multiple cellular events in the co-culture system. Therefore, these findings suggested that the modulation effects of LIGHT on T cell-ASM cell interaction were correlated with HVEM-NF κ B or c-JUN signaling.

In conclusion, the present study demonstrated that LIGHT plays an important role in the interaction between T cells and ASM cells in chronic asthma. Blockade of LIGHT markedly reduced ASM cell proliferation, survival, CD4⁺ T cell differentiation, and cytokines release, which might be modulated through HVEM-NF κ B or c-JUN pathways. LIGHT may be used as a candidate target for therapeutic management of airway inflammation and remodeling in chronic allergic asthma.

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