



LAT alleviates Th2/Treg imbalance in an OVA-induced allergic asthma mouse model through LAT-PLC- γ 1 interaction



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ABSTRACT

Introduction: Low expression of linker for activation of T cells (LAT) is observed in asthma. LAT and its downstream regulator, phospholipase C-gamma 1 (PLC- γ 1) play important roles in the T cell antigen receptor signaling pathway, and their interaction is associated with CD4⁺ cell polarization. Here, we investigated whether LAT can alleviate the imbalance among CD4⁺ cell subgroups and the possible mechanism.

Methods: An ovalbumin-induced allergic asthma mouse model was established and LAT plasmid was delivered. The pathological changes in lung were evaluated by hematoxylin and eosin and periodic acid-Schiff staining. The typical cytokines released by T helper 2 (Th2) and regulatory T (Treg) cells were measured using enzyme-linked immunosorbent assay and the number of Th1, Th2, and Treg cells were determined using flow cytometry. Lung CD4⁺ T cells were isolated by magnetic isolation. The mRNA expression of LAT and PLC- γ 1 was determined by real-time PCR. Co-Immunoprecipitation was performed to confirm the interaction between LAT and PLC- γ 1. The protein expression of LAT, PLC- γ 1 and corresponding downstream signaling factors were determined by western blotting.

Results: The delivery of LAT DNA to the lung could suppress an overactive Th2 response by decreasing allergic response and Th2 cytokine secretion, and by increasing Treg cytokine secretion. The Th2/Treg imbalance in lung and decreased phosphorylated PLC- γ 1 expression in lung CD4⁺ T cells were rectified by LAT DNA delivery. Excessive activation of the Raf-MEK-ERK and PI3K-AKT-CREB pathways after asthma is attenuated by LAT.

Conclusion: The site-specific delivery of LAT DNA to the lung could suppress an overactive Th2 response and rectify the Th2/Treg imbalance in asthmatic mouse model. LAT-PLC- γ 1 interaction may contribute to LAT activity in vivo and LAT protects against asthma partly via Raf-MEK-ERK and PI3K-AKT-CREB pathways. The delivery of LAT DNA could offer a novel and safe strategy for asthma prevention.

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1. Introduction

Asthma is a chronic inflammatory disease characterized by airway inflammation and airway hyperresponsiveness (AHR). In asthma, the imbalance in T helper cell responses, especially an overactive helper type 2 (Th2) response, leads to the production of Th2 cytokines and causes a cascade of immune-activating events resulting in airway smooth muscle dysfunction. Treg cells represent a unique subpopulation of CD4⁺ T cells, which can inhibit T cell proliferation and an autoimmune response [1]. Compared with healthy controls, Tregs in the airway of patients with allergic asthma patients are reportedly decreased, thereby reducing the suppression of effector T cell proliferation and cytokine production [2].

The linker for the activation of T cells (LAT) is a type III transmembrane molecule, which plays a critical role in coordinating the trigger for T cell activation [3]. Studies in mice with knockout or modified knock-in LAT have revealed that LAT functions in the differentiation of

T cells via the Th1 and Th2 pathways and control of lymphocyte proliferation [4]. Moreover, overproduction of Th2 cytokines was observed in LAT-deficient mice, suggesting that LAT suppresses the Th2 response [5]. PLC- γ 1 is a critical regulator of downstream of LAT and its activation could result in the LAT-based activation of PKC, RasGRP, calcium influx and stimulation of NFAT DNA binding activity [6,7]. By controlling these transcriptional factors and its partners, LAT-PLC- γ 1 signal pathway control T cell proliferation and differentiation [8].

We recently showed that LAT expression was decreased in peripheral blood T cells derived from asthmatic patients than in those from healthy controls. Overexpression of LAT mRNA decreased Th2 cytokine production, which could be prevented by the inhibition of LAT [9]. In addition, we illustrated that a low level of LAT-PLC- γ 1 interaction is associated with Th2 polarized differentiation in asthma [9]. However, although LAT overexpression could regulate Th2/Treg imbalance in asthma, its underlying mechanism in vivo has not been studied.

In present study, we investigated whether the site-specific delivery of LAT DNA to the lung could suppress the overactive Th2 response and rectify the Th2/Treg imbalance in an OVA-induced asthmatic mouse model. Furthermore, we investigated whether LAT-PLC- γ 1

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interaction alleviates LAT activity *in vivo*. We expected that the delivery of LAT DNA to inflammation sites would offer a novel and safe strategy for asthma prevention.

2. Materials and methods

2.1. DNA vectors

LAT was cloned into a pIRES2-EGFP vector (Novobio Technology Co. Ltd., Shanghai, China). Plasmids were prepared using the EndoFree Plasmid Kit (Tiangen, Shanghai, China).

2.2. Preparation of polyetherimide (PEI)-DNA complexes

Plasmid DNA was complexed with *in vivo*-jetPEI (Polyplus-transfection, Illkirch, France) according to the manufacturer's guidelines. Briefly, 1.6 mg/ml plasmid DNA solution in 5% glucose was mixed with jetPEI solution with final DNA concentration of 0.5 mg/ml and N/P ratio of 7.5. This mixture was incubated for at least 30 min at room temperature to allow formation of stable complexes before being inoculated into mice.

2.3. Animals

Adult female BALB/C mice (6 weeks old, 20 ± 3 g) were purchased from Shanghai Laboratory Animal Center (Shanghai, China). All animals were housed in the animal center of Xinhua Hospital, Shanghai Jiaotong University, (Shanghai, China) with free access to food and water and a constant 12 h light/dark cycle. All experimental procedures were performed following the guidelines for the care and use of animals defined by the Ethics Committee of Xinhua Hospital Affiliated to the Shanghai Jiaotong University School of Medicine (Approval NO. XHEC-F-2016-015).

2.4. Allergen immunization/challenge protocol

Sensitization and airway challenge was performed as shown in Fig. 1A. On days 0 (day of first injection) and 14, mice were abdominally injected with $10 \mu\text{l}$ 10% chicken ovalbumin (OVA) (purity $\geq 98\%$, Sigma-Aldrich) with $250 \mu\text{l}$ 1% aluminum hydroxide $\text{Al}(\text{OH})_3$. On days 14, 25, 26, and 27, mice were anesthetized with an abdominal injection of 0.25% sodium pentobarbital (50 mg/kg) in normal saline, followed by an intranasal (i.n.) dose of $100 \mu\text{g}$ OVA in $30 \mu\text{l}$ phosphate-buffered saline (PBS). For the asthma group ($n = 5$), the immunization protocol was performed as described above. For the LAT and Plasmid control groups, in addition to the immunization protocol described above, LAT or mock plasmid delivery was performed intranasally on day 24, before a continuous OVA challenge from day 25 to 27. The corresponding control groups were intranasally injected with PBS only.

2.5. Bronchioalveolar lavage (BAL) and ELISA

BAL was performed through trachea; briefly, 2 ml PBS was injected into the mouse lung and 1 ml BAL fluid was collected. Total cells in the BAL fluid were counted by trypan blue dye exclusion, and differential cell counts were estimated using Wright's staining. Cytokines (IL-2, IL-4, IL-5, IL-10, IFN- γ and TGF- $\beta 1$) in the BAL supernatant were measured and quantified by ELISA following manufacturer's instructions (ELISA kit; eBioscience, San Diego CA, USA).

2.6. Lung histology

Mice were anesthetized and sacrificed by exsanguination. The left lobe of the lung was inflated with 5 ml 4% paraformaldehyde and immersed in 4% paraformaldehyde for 24 h to complete fixation. Thereafter, the lobes were dehydrated, embedded in paraffin, and sectioned

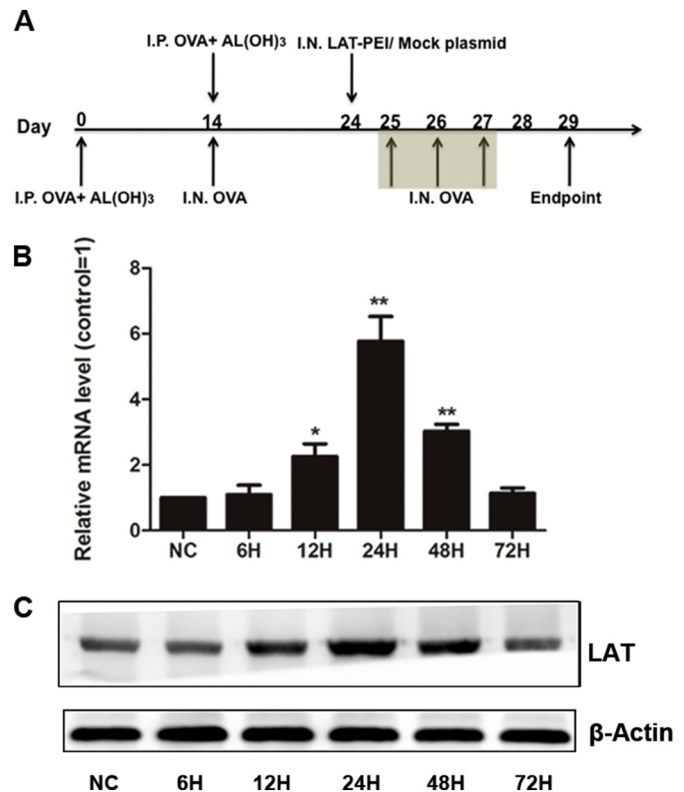


Fig. 1. Intranasal LAT plasmid delivery caused overexpression of LAT in the lung of asthmatic mice. (A) Experiment schedule for the OVA-induced model. *In vivo* transfection efficacy of LAT was determined by real-time PCR (B) and western blotting (C) at different time points. Data are presented as mean $\pm$ SEM of at least three independent experiments. * $P < 0.05$ and ** $P < 0.01$ were significant compared with the normal control group. NC: Normal control.

into 4- μm sections. After deparaffinization, sections were stained with hematoxylin and eosin (HE) and periodic acid-Schiff (PAS).

2.7. Cell preparation

The lung tissue specimens were washed twice with PBS, cut into 1- mm^3 pieces, and transferred to a 15-ml centrifuge tube containing 5 ml RPMI 1640 supplemented with collagenase I (2 mg/ml; Sigma-Aldrich, St. Louis, MO, USA) and DNase I (2 mg/ml; Sigma-Aldrich). Tubes were incubated in 37°C for 2 h and shaken every 15 min. Fetal bovine serum (FBS; 1%) was added to end incubation. Tissue fragments were crushed using a 70- μm pore-size nylon filter, and cells were washed once with PBS. The lung cells were collected after centrifugation ($300 \times g$, 10 min) and resuspended in 4 ml RPMI 1640. Lymphocyte Separation Medium (4 ml; Histopaque1083, Sigma-Aldrich) was slowly added to the RPMI 1640 surface. Single lung cells were collected by density gradient centrifugation (30 min). Some cells were stained with flow cytometry antibodies for flow cytometry analysis, and others were saved for the following magnetic isolation experiments.

2.8. Magnetic isolation of lung CD4^+ cell

The single lung cells were resuspended in MACS buffer (PBS containing 1% BSA, preferentially with 2.5 mM EDTA). CD4^+ cells were directly purified from isolated single lung cells by incubation at 4°C for 15 min in $10 \mu\text{l}$ anti-mouse CD4 (L3 T4) microbeads for every 10^7 lung cells; cells were positively sorted on a MS-positive selection column according to the manufacturer's guidelines (Miltenyi Biotec, Bergisch Gladbach, Germany).

2.9. Flow cytometric analysis

Anti-Foxp3-APC (Clone FJK-16s), anti-CD25-PE (Clone PC61.5), anti-CD4-FITC (Clone GK1.5), anti-IL-4-PE (Clone 11B11), and anti-IFN- γ -APC (Clone XMG1.2) antibodies for FACS analysis were purchased from eBioscience. For intracellular IL-4 and IFN- γ staining, single lung cells were treated with cell stimulation cocktail (eBioscience) to block the secretion of these cytokines at 37 °C for 6 h. Then, all cells were washed with PBS, resuspended, and incubated at 4 °C for 45 min with the corresponding antibodies. For IL-4, IFN- γ and Foxp3 staining, cells were permeabilized and fixed using transcription factor staining buffer set (eBioscience), washed, and incubated with anti-IL-4-PE, anti-IFN- γ -APC and anti-Foxp3 mAb according to the manufacturer's protocols (eBioscience). Stained cells were analyzed on a LSRII station (BD Biosciences, San Jose, CA, USA).

2.10. Quantitative RT-PCR

The total RNA of isolated CD4⁺ T cells was extracted using Trizol Reagent (Invitrogen Life Technologies, Carlsbad, CA, USA), followed by reverse transcription to cDNA using PrimeScript RT Master Mix (Takara, Dalian, China). cDNA (1:10 dilution with water) was amplified using SYBR premix EX Taq™ (Takara) with an ABI 7500 real-time PCR system (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions. β -actin level was used as an internal control. Quantitative PCR results from triplicate RNA samples were used to calculate the mean expression for each gene. PCR primers used were as follows: LAT, 5'-CAGTGTGGCAAGCTACGAGA-3' (Forward), 5'-TCAGGCAGCACCCTAGGTA-3' (Reverse); PLC- γ 1, 5'-AGCAGTTCCTCTGAATACCA-3' (Forward), 5'-AGTATGGCTCTCAATCTCTCG-3' (Reverse); β -actin, 5'-GTGCTATGTTGCTCTAGACTTCG-3' (Forward), 5'-ATGCCACAGGATTCATACC-3' (BBI Life Science, Shanghai, China). PCR conditions were as follows: 30 s incubation at 95 °C, followed by 40 cycles of 95 °C for 5 s and 64 °C for 34 s. Gene expression was normalized to β -actin expression and analyzed by the $2^{-\Delta\Delta C_t}$ method.

2.11. Co-immunoprecipitation

Cells were collected, washed with ice-cold PBS, and resuspended in lysis buffer (Beyotime, Shanghai, China). After centrifuged for 10 min at 12,000 $\times$ g, 500 μ l cell lysate was incubated with 2 μ g of anti-LAT antibody per 0.5 mg protein coated on the protein A sepharose beads (Santa Cruz, California, USA) overnight at 4 °C. After washed four times with the lysis buffer, the immunoprecipitates were resuspended, boiled, and then the supernatant were collected and detected by western blot for anti-LAT and anti-PLCG1 antibody.

2.12. Western blot

Isolated CD4⁺ T cells were lysed in RIPA buffer, and protein concentrations were determined using the BCA assay (Beyotime, Shanghai, China). Equal amounts of proteins (30 μ g) were separated on 8%–12% SDS-PAGE gels (Beyotime), electroblotted onto polyvinylidene fluoride membranes (Millipore, Billerica, MA, USA), blocked by incubation in 5% nonfat milk (Millipore) for 1 h, and incubated overnight with primary antibodies at 4 °C. Antibodies used were as follows: anti-LAT, p-Raf (Ser338), p-MEK (Ser217/Ser221), p-ERK (Thr202/Tyr204), and p-PI3K (Tyr458), p-AKT (S473), p-AKT (T308), and p-CREB, anti- β -actin (Cell Signaling Technology, Danvers, MA, USA), anti-PLCG1, anti-PLCG1 (Phospho-Tyr783), and anti-PLCG1 (Phospho-Tyr771) (BBI Life Science, Shanghai, China). The next day, the membranes were washed and incubated for 1.5 h at room temperature with the appropriate secondary antibody linked to horseradish peroxidase (Abcam, Cambridge, UK). Western blots were visualized using an enhanced chemiluminescence fluid (Millipore).

2.13. Statistics analysis

All data were analyzed by using the SPSS13.0 software (IBM, New York, NY, USA) and are expressed as mean $\pm$ SEM. Statistical significance ($P < 0.05$) was determined by one-way ANOVA or Student's *t*-test with a Mann-Whitney post-test.

3. Results

3.1. Intranasal delivery of LAT-PEI complex caused lung LAT overexpression

The LAT-PEI complex was intranasally delivered to mice to target LAT overexpression in the lung. To investigate the efficacy of lung-specific LAT DNA upregulation in modulating the asthmatic response, we first investigated the expression of the LAT-PEI complex in the mouse lung by evaluating mRNA and protein expression of LAT at different time points after the delivery of the LAT-PEI complex. As shown in Fig. 1B–C, compared to the normal control group—which received mock plasmid-PEI delivery; LAT mRNA and protein expression was elevated within 12 h and maximized at 24 h after delivery. Thereafter, LAT expression decreased until 48 h and no obvious elevation in expression was observed at 72 h. Therefore, we administered the LAT-PEI complex to mice at 24 h before the first airway challenge or immediately after the last challenge to simulate treatment before or after the onset of asthma-like symptoms.

3.2. Upregulation of LAT DNA suppresses OVA-induced allergic asthma

Compared to the asthma group, LAT-PEI delivery to the lung significantly decreased peribronchial and perivascular inflammation and decreased goblet cell metaplasia in the LAT group while no significant difference was observed in the Plasmid control group (Fig. 2A–B). Furthermore, LAT upregulation before the airway challenge significantly decreased eosinophil, neutrophil, and lymphocyte infiltration in the BAL fluid (Fig. 2C). In addition, ELISA of the BAL supernatant confirmed the typical upregulation of Th2 cytokines, IL-4 and IL-5, and downregulation of Th1 cytokines (IL-2 and IFN- γ) and Treg cytokines (IL-10 and TGF- β 1) in the asthma group; this alternation was significantly corrected following delivery of LAT DNA before the airway challenge. Furthermore, protection from cellular infiltration was also observed in LAT group suggesting that the LAT-PEI plasmid had a preventive effect (Fig. 2D).

3.3. Upregulation of LAT DNA influences the Th2/Treg ratio in OVA-induced allergic asthma

As allergic asthma is characterized by an overactive Th2 response, many studies have reported an increase in Th2 cells in asthma [10,11]. In the present study, we investigated the Th2/Treg ratio in the lung, and our findings were consistent with those of previous studies. OVA-induced allergic asthma significantly increased the number of Th2 cells while decreasing the number of Treg cells. Furthermore, LAT DNA delivery before the OVA airway challenge significantly decreased the Th2 response and elevated the number of Treg cells in LAT group compared to the asthma group while Plasmid control group showed no significant differences, indicating that LAT DNA could influence Th2 differentiation (Fig. 3).

3.4. LAT-PLC- γ 1 interaction influences the Th2/Treg ratio in OVA-induced allergic asthma

We previously found that LAT expression was decreased in peripheral monocytes derived from asthmatic patients and the lungs of an asthmatic mouse model [12]. Furthermore, our colleagues demonstrated that the low level of LAT and PLC- γ 1 expression was associated with the polarized differentiation of Th2 into naive CD4⁺ T cells in vitro [9].

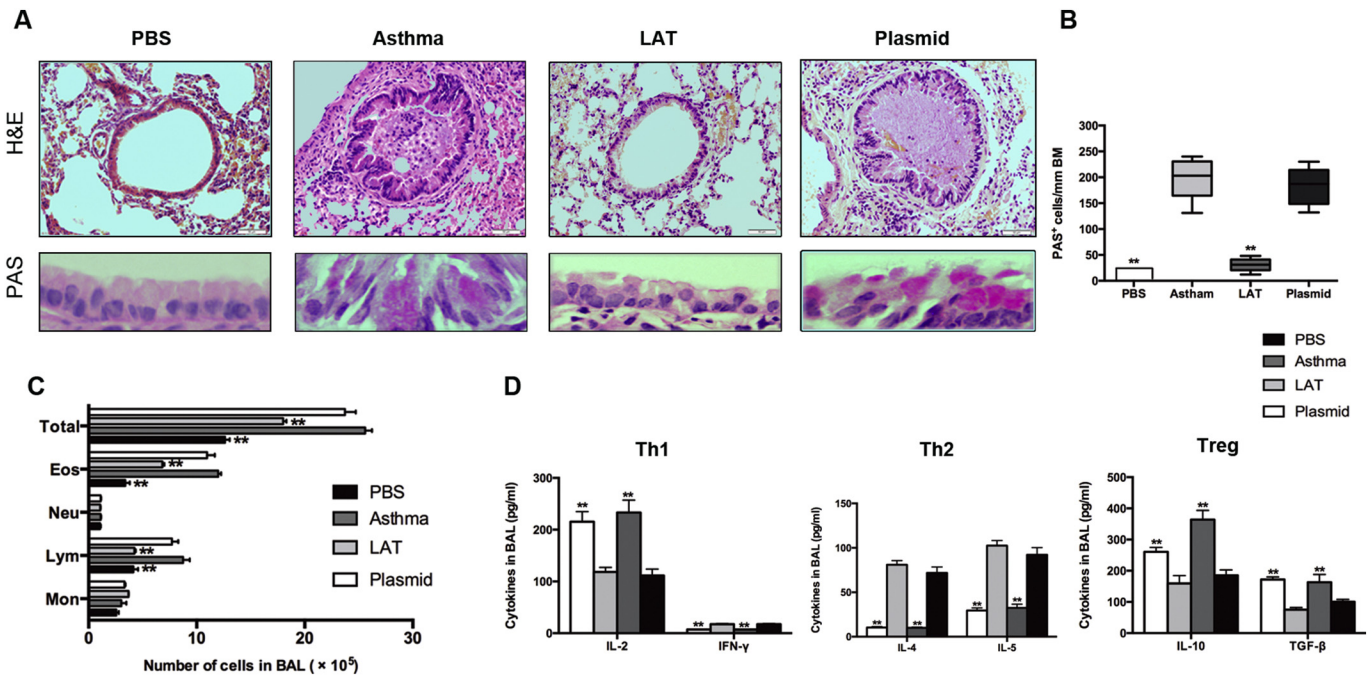


Fig. 2. LAT plasmid protects from OVA-induced allergic asthma in mice. Mice sensitized with OVA received LAT DNA either before or after the OVA challenge. Vector plasmid-sensitized mice were applied as the negative control. (A and B) Tissue inflammation and global cell metaplasia analyzed on H&E and PAS-stained lung sections. Representative micrographs of H&E staining (original magnification $\times 200$) and PAS staining (original magnification $\times 400$) are shown in A; scoring for PAS⁺ cells/mm basement membrane (BM) are shown in B. (C) Numbers of cells in BAL fluid. (D) Cytokine expression in BAL fluid. * $P < 0.05$ and ** $P < 0.01$ compared with OVA-induced allergic asthma mice. Data shown as box-and-whisker plots (minimum to maximum, with median line). $n = 5$ mice per group.

Therefore, we investigated whether LAT upregulation could reverse this differentiation in an asthmatic mouse model. To clearly elucidate the underlying mechanism, we isolated purified CD4⁺ T cells (Fig. 4A). As shown in Fig. 4B and D, intranasal delivery of LAT plasmid increased

the basal mRNA and protein expression of LAT in the LAT group compared to the asthma group. Moreover, LAT could interact with PLC-γ1 and their interaction was attenuated after asthma (Fig. 4C). Interestingly, no significant differences were found in PLC-γ1 mRNA and protein

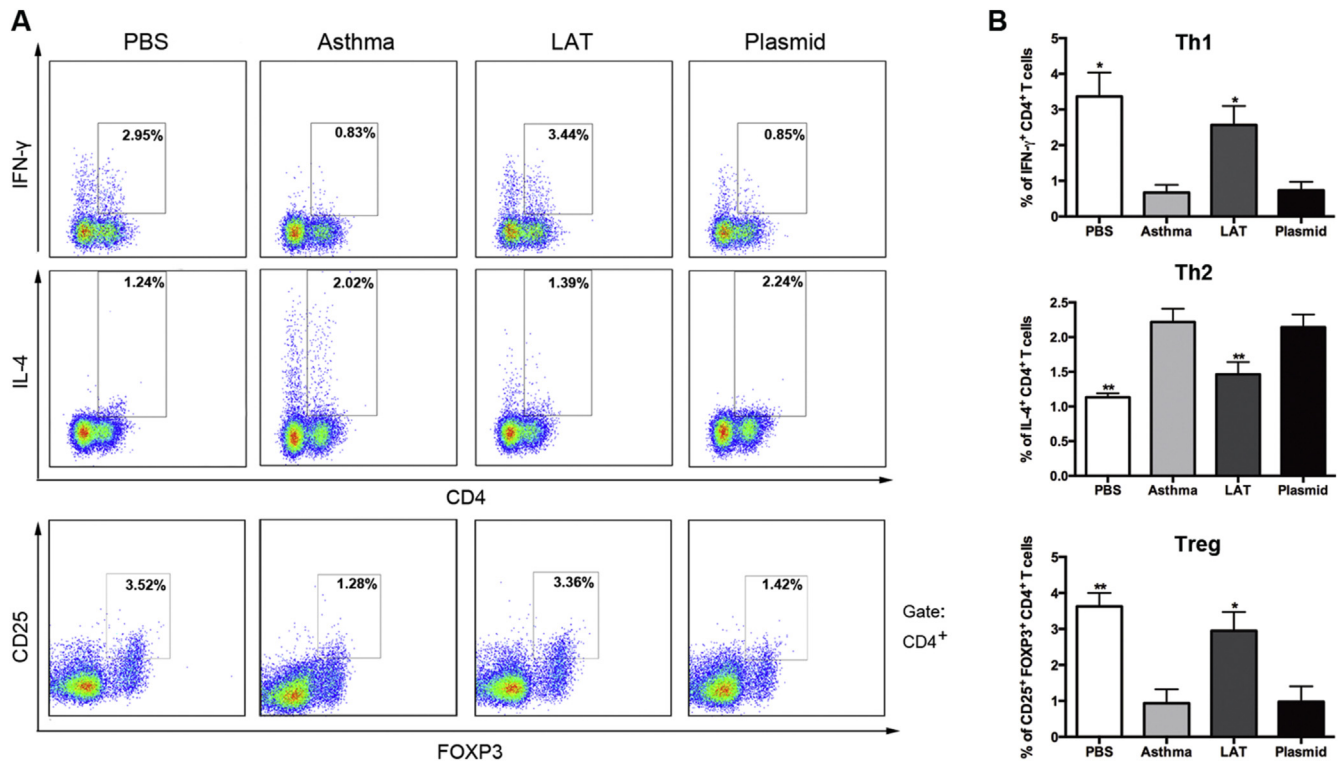


Fig. 3. LAT DNA influenced Th2/Treg and Th2/Th1 balance in OVA-induced allergic asthma mice. Intracellular expression of IFN-γ by Th1 cells, IL-4 by Th2 cells, CD25 and Foxp3 by Treg cells among CD4⁺ T cells was determined by FACS. (A) Representative results from 3 mice in each group. (B) Results are mean $\pm$ SEM from 3 independent experiments for each group. * $P < 0.05$ and ** $P < 0.01$ compared with OVA-induced allergic asthma mice.

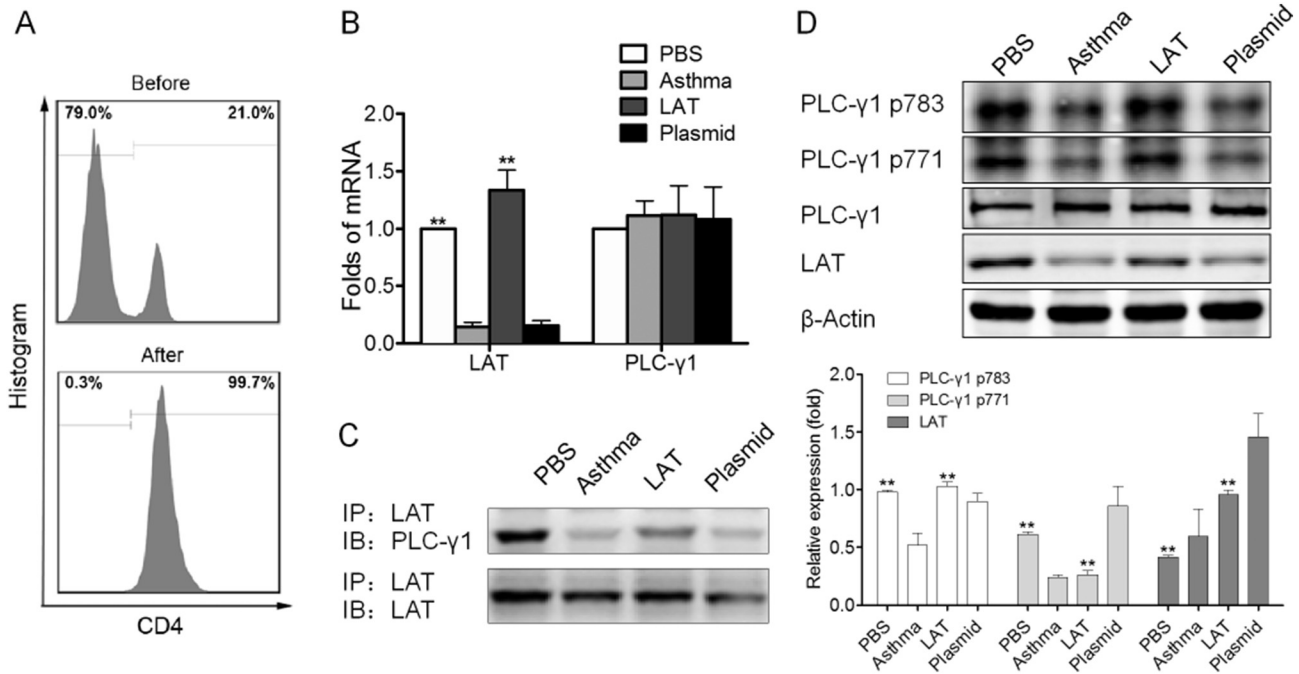


Fig. 4. LAT-PLC- γ 1 interaction is associated with Th2/Treg imbalance in OVA-induced asthma. (A) The purity of CD4⁺ T cells from lungs before and after magnetic isolation. (B) mRNA expression of LAT and PLC- γ 1 was evaluated by real-time PCR. ** $P < 0.01$ compared with OVA-induced allergic asthma mice. (C) The interaction between LAT and PLC- γ 1 was detected by co-immunoprecipitation. (D) Protein expression of LAT, PLC- γ 1, and phosphorylated-PLC- γ 1 was evaluated by western blot. Quantitative comparisons of protein bands in rat brains were analyzed by densitometry normalized to β -actin. All experiments were repeated three times with identical results. ** $P < 0.01$ versus allergic asthma group.

expression among all four groups. Because posttranscriptional modification can also influence protein function, we next determined the expression of phosphorylated PLC- γ 1. As expected, phosphorylated PLC- γ 1 (phosphorylated at amino-acid 783 and 771) expression was significantly decreased in asthma group. In addition, compared to the asthma group, phosphorylated PLC- γ 1 expression was increased in the LAT group (Fig. 4D). Taken together, these data indicate that intranasal delivery of LAT DNA could cause LAT overexpression in the mouse lung

and protect against OVA-induced allergic asthma. Additionally, this preventive effect of LAT was correlated with PLC- γ 1 phosphorylation.

3.5. LAT protects against asthma partly via Raf-MEK-ERK and PI3K-AKT-CREB axis

The phosphorylation of Raf-MEK-ERK and PI3K-AKT-CREB were increased significantly, indicating that ERK and PI3K signaling pathways

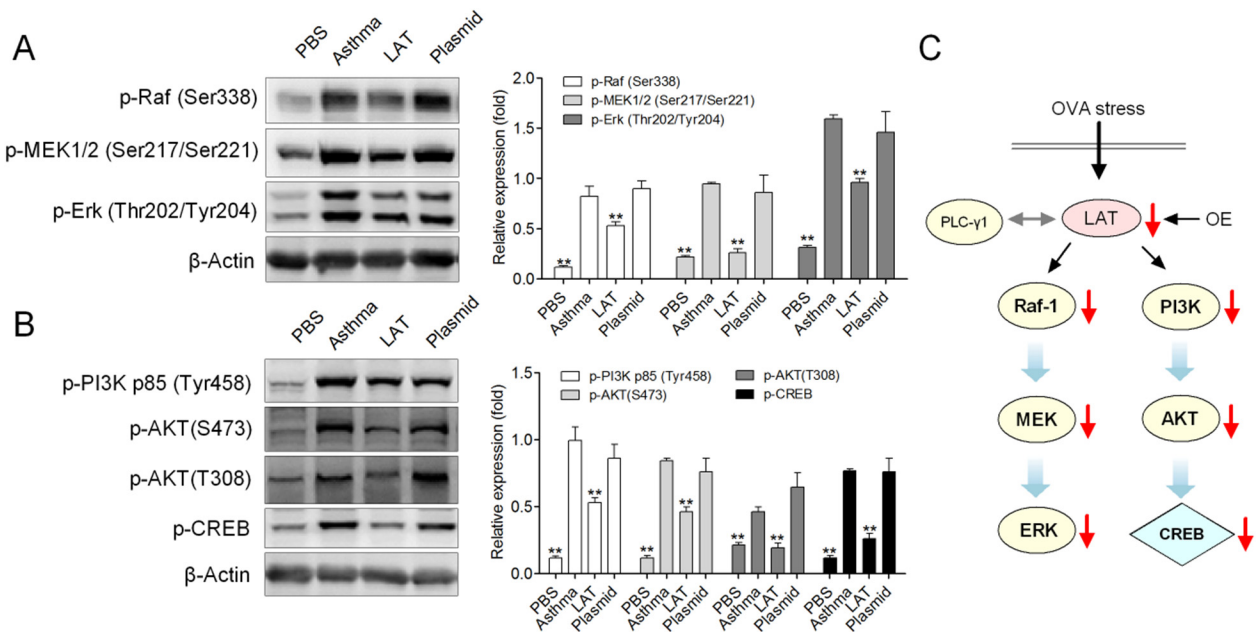


Fig. 5. LAT protects against asthma partly via Raf-MEK-ERK and PI3K-AKT-CREB pathways. (A) Raf-MEK-ERK and PI3K-AKT-CREB phosphorylation induced by asthma were measured by western blot. Quantitative comparisons of protein bands were analyzed by densitometry normalized to β -actin. Data shown are the results of three different experiments. ** $P < 0.01$ versus allergic asthma group by a one-way ANOVA analysis. (C) Schematic form of the proposed mechanisms for LAT regulated asthma-induced Raf-MEK-ERK and PI3K-AKT-CREB pathways. OE: Overexpression.

are excessively activated by asthma (Fig. 5A and B). Intranasal delivery of LAT plasmid inhibited the upregulation of phosphorylation of ERK and PI3K pathways induced by asthma. In addition, the control plasmid had no obvious effect (Fig. 5A and Fig. 5B). Altogether, these results show that excessive activation of the Raf-MEK-ERK and PI3K-AKT-CREB pathways after asthma is attenuated by LAT (Fig. 5C).

4. Discussion

The upregulation of LAT holds great protective potential against asthma because of in this condition, the restoration of the balance among helper T cell responses is required. In this study, LAT upregulation in an OVA-induced asthmatic mouse lung not only reduced the overactive Th2 response but also rectified the Th2/Treg imbalance. Furthermore, LAT-PLC- γ 1 interaction and PLC- γ 1 phosphorylation play critical roles in this regulation. In addition, LAT upregulation has a protective effect on OVA-induced asthma in mice *in vivo*.

Traditional opinion holds that the pathological consequences of uncontrolled Th1/Th2 response are related to allergic asthma [10,13]. However, Treg, which forms a subset of CD4⁺ T cells that have high suppressor activity [14], has become a hot topic of research. Previous studies have shown that Tregs are potent immunomodulators that suppress the overactive Th2 immune response in allergic disease [15,16] and the depletion of Treg populations exacerbates airway inflammation in mice [17]. Therefore, we expected that, other than a Th1/Th2 imbalance, a disrupted Th2/Treg balance might also exist in asthma. In this study, the number of Th2 cells increased while that of Treg cells significantly decreased, causing a “Th2/Treg imbalance” in the asthmatic mouse model. In addition, this imbalance was confirmed by the expression of typical cytokines secreted by these two types of cells: expression of IL-4 and IL-5, which are mainly secreted by Th2 cells, increased while that of typical Treg cytokines, IL-10 and TGF- β 1, decreased.

Because Treg cells have been proven to be able to reduce airway inflammation *in vivo* [18,19] and their typical cytokines, IL-10 and TGF- β 1, have been proven to be important in dampening inflammation caused by infectious agents, allergies, and environmental components [20–23], the regulation of the asthmatic airway response by targeting Treg cells appears promising. Chuck et al. recently demonstrated that the LAT-PLC- γ 1 interaction is important in the production of IL-10 and TGF- β 1 by Treg cells in response to T cell antigen receptor (TCR) stimulation [5]. Our team previously reported that LAT expression was significantly decreased in asthmatic patients and overexpression of LAT mRNA could decrease Th2 cytokine production [12]. These findings suggested that the LAT-PLC- γ 1 signaling pathway might regulate the disrupted Th2/Treg balance in asthma. Therefore, it is reasonable to hypothesize that the LAT-PLC- γ 1 signal may affect T cell activation and subsequently disrupt the balance among CD4⁺ T cell subgroups in asthma, which is characterized by Th2 and Treg dysregulation. Hence, transcript-based upregulation of LAT expression *in vivo* may suppress the overactive Th2 response and attenuate the Th2-driven airway inflammation.

Several studies have reported that intranasal delivery aimed at increasing number or effect of Treg cells could protect against asthma *in vivo* [11,19,24]. In this study, we found that the delivery of the LAT-PEI complex before airway challenge increased the number of Treg cells, suppressed the Th2 response, and reduced airway inflammation. This may be owing to the role played by LAT in signal transduction. Signals from antigen, co-stimulatory, and adhesion receptors induce the activation of LAT and drive downstream signaling that is critical for T cell effector function [6]. Consequently, intranasally delivered OVA could activate LAT as an allergen and subsequently set off a series of immunological reactions. These data suggest that LAT may be best applied as protective strategy in asthma.

Although we observed that the overexpression of LAT *in vivo* could rectify the Th2/Treg imbalance and attenuate airway inflammation in an asthmatic mouse, the underlying mechanism requires further

exploration. In our recent study, we found that the LAT-PLC- γ 1 signaling pathway contributes to Th2 polarization [9]. Moreover, the LAT-PLC- γ 1 interaction is essential for efficient signaling in mature CD4⁺ T cells in response to TCR crosslinking [5]. On one hand, LAT was found to have unexpected regulatory functions in T cell development and homeostasis as it cannot only induce the activation of downstream signaling pathways but can also trigger negative regulatory loop(s) that act at later time points [25]. For example, LAT-deficient Jurkat cells display defective TCR-mediated ERK activation, Ca²⁺ mobilization, and NFAT translocation [26]. In the present study, we found that excessive activation of the Raf-MEK-ERK and PI3K-AKT-CREB pathways after asthma is attenuated by LAT. On the other hand, knock-in mice with a mutation at the PLC- γ 1 binding site (Y136) of LAT develop severe lymphoproliferative syndrome and overexpress Th2 cytokines, specifically IL-4 and IL-5 [27–29]. PLC- γ 1 is required as a central regulator of intracellular signals downstream of TCR for T cell development, TCR-induced signaling, and mature T cell and Treg functions [30]. Because LAT-PLC- γ 1 has a major role in signal transduction to different signaling pathways downstream of the TCR, a key question is whether LAT-PLC- γ 1 interaction may play a role in the development of allergic asthma. In the present study, the low expression of LAT in the asthma group resulted in an overactive Th2 response consistent with previous findings. Moreover, we observed that the total PLC- γ 1 level remained unchanged in all groups while the phosphorylated PLC- γ 1 level was decreased in the asthma group. Because phosphorylation of PLC- γ 1 enhances its enzymatic activity [31,32], decreased PLC- γ 1 phosphorylation in asthmatic mice may cause the lower utilization of PLC- γ 1 and subsequently affect signal transduction. Furthermore, overexpression of LAT before the OVA challenge could reverse low PLC- γ 1 phosphorylation, while that after the OVA challenge could not, suggesting that PLC- γ 1 phosphorylation may play a key role in the regulation of the Th2/Treg imbalance and attenuation of airway inflammation by LAT in asthma.

5. Conclusion

We revealed LAT to be an important transmembrane adaptor protein that can attenuate the asthmatic response by regulating the Th2/Treg imbalance. In addition, LAT-PLC- γ 1 interaction, especially phosphorylation of PLC- γ 1, plays a critical role in this process. These results suggest the application of LAT as a protective strategy in asthma.

Conflicts of interest

Authors declare no conflict of interest.

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