Lentiviral vector-mediated delivery of lysophosphatidylcholine acyltransferase 1 attenuates airway inflammation in ovalbumin-induced allergic asthmatic mice

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Summary

Background: Lysophosphatidylcholine (LPC) is generated through the hydrolysis of phosphatidylcholine (PC) by phospholipase A2 and is converted back to PC by lysophosphatidylcholine acyltransferase 1 (LPCAT1). Elevated levels of (LPC) are known to play a pathogenic role in the inflammatory injury of asthma. However, the role of LPCAT1 in asthma has not yet been reported.

Objective: To determine whether the exogenous expression of LPCAT1, delivered by using a recombinant lentiviral vector, could attenuate airway inflammation in asthmatic mice.

Methods: Recombinant lentivirus carrying cDNA encoding LPCAT1 (Lenti-LPCAT1), or EGFP (Lenti-EGFP) as a control, was constructed. BALB/c mice were sensitised with ovalbumin (OVA), and intratracheally pre-treated with an endobronchial administration of the recombinant lentivirus intratracheally 72 hours before the first challenge. After the last OVA challenges, the mice were assessed for airway inflammation, airway hyper-responsiveness and lipid levels.

Results: Lenti-LPCAT1-infected HEK293T cells expressed exogenous recombinant LPCAT1 protein that showed high activity of the LPC acyltransferase. OVA sensitisation and challenge significantly increased the levels of saturated species LPC 16:0 and LPC 18:0 levels in the bronchoalveolar lavage fluid (BALF) compared with wild-type mice respectively. The intratracheal Lenti-LPCAT1 instillation obviously down-regulated the OVA-induced release of LPC 16:0 and LPC 18:0. Treatment with Lenti-LPCAT1 ameliorated OVA-induced airway hyper-responsiveness and reduced airway eosinophilia infiltration in lung tissue. Furthermore, the secretion of eotaxin and Th2-associated cytokines IL-5 and IL-13 were inhibited in BALF. The level of OVA-specific IgE in serum was suppressed.

Conclusions: These results suggested that the exogenous expression of LPCAT1 may attenuate eosinophil inflammation in the airway by down-regulating the LPC 16:0 and LPC 18:0 BALF levels in asthmatic mice. (*Asian Pac J Allergy Immunol 2015;33:320-9*)

Keywords: asthma, lysophosphatidylcholine, lysophosphatidylcholine acyltransferase 1, lentiviral vector, phosphatidylcholine

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Introduction

Lysophosphatidylcholine (LPC) is a broad-spectrum bio-active lipid mediator that is thought to play a critical role in atherosclerosis.[1] Recent evidence has also implicated LPC in the pathogenesis of many tissue injuries and chronic disease, such as bronchial asthma. For instance, elevated LPC 16:0 and LPC 18:0 levels in the bronchoalveolar lavage fluids (BALF) of moderate asthmatics are associated with impaired lung function,[2] and pre-exposure of aerosolised LPC to

guinea-pigs leads to eosinophil infiltration and an increase in airway resistance.[3] Furthermore, bronchial epithelial cells are found to be a key source of endogenous LPC, which can function as an inflammatory mediator and impair the epithelial barrier.[4]

Asthma is a chronic airway inflammatory disease that is characterised by pulmonary eosinophilia and airway hyper-reactivity (AHR). In tissues, LPC is generated through phospholipase $A_2(PLA_2)$-mediated hydrolysis of phosphatidylcholine (PC). Evidence indicates that secreted PLA_2 is up-regulated in the airway of asthmatic patients, particularly in the airway epithelium, which is thought to be a likely contributor to pathological increases in LPC in the fluid lining of the lungs.[5]

Pulmonary surfactant is a complex mixture of proteins and lipids that maintains surface tension. PC comprises almost 80% of the total surfactant lipids, approximately half of which are present as dipalmitoyl-phosphatidylcholine (DPPC). Lysopho-sphatidylcholine acyltransferase 1 (LPCAT1) is a newly reported lysophospholipid acyltransferase that has been implicated in anti-inflammatory processes due to its role in converting LPC to DPPC. The LPCAT1 protein was highly expressed in alveolar type II epithelial cells of the lung.[6-8] Previous research demonstrated that mice with a hypomorphic allele of the LPCAT1 gene failed to reduce the minimum surface tension to wild-type levels, which indicates that LPCAT1 is required to achieve the levels of PC that are essential for the transition to air breathing.[9] Moreover, our previous research has shown that LPCAT1 plays a protective role in tissue injuries, such as acute lung injury, by improving the compositions of phospholipids in both BALF and lung tissue.[10]

Although LPC has been implicated as playing a pathogenic role in the inflammatory injury to the asthmatic airways, the role of LPCAT1 in the synthesis of inflammatory lipids during asthma has not yet been reported. In this study, we hypothesised that exogenous delivery of the LPCAT1 gene into the lungs may protect against airway inflammation in asthmatic mice. To prove this hypothesis, we generated a mouse model in which lung LPCAT1 protein over-expressing by using a lentiviral transgenic system. These studies demonstrate that LPCAT1 significantly improves airway inflammation by regulating LPC generation.

Methods

Reagents

Unless otherwise specified, all common chemicals were purchased from Sigma (St. Louis, MO). Antibodies were purchased as follows: a mouse anti-3Flag monoclonal antibody from Sigma (St. Louis, MO), a mouse anti-GAPDH monoclonal antibody from Santa Cruz Biotechnology(CA,USA), a rabbit anti-human LPCAT1 polyclonal antibody from Proteintech (Wuhan, China), and a horseradish peroxidase (HRP) -conjugated anti-rabbit IgG and anti-mouse IgG antibody from Santa Cruz Biotechnology (CA,USA). Commercial kits were purchased as follows: mouse IL-4, IL-5, IL-13 and eotaxin ELISA kits were purchased from R&D Systems Inc. (Minneapolis, MN, USA); the OVA-specific IgE ELISA kit was purchased from BioLegend (San Diego, CA); [1-^{14}C]palmitoyl-CoA (1.85-2.22GBq/mmol) was purchased from American Radiolabeled Chemicals (St. Louis, MO, USA); LPC standards from chicken eggs or soybeans, and the synthetic LPC components with a hydrolysed acyl ester bond at the *sn*-1 position (1-palmitoyl , LPC 16:0; 1-stearoyl, LPC 18:0; 1-oleoyl, LPC 18:1; 1-nonadecanoyl, LPC 19:0) and DPPC (1, 2-dipalmitoyl-sn-glycero-3-phosphocholine, PC 16:0) standards were purchased from Avanti Polar Lipids (Alabaster, AL); and OCT embedding medium was purchased from Sakura Finetek (Torrance, CA).

Animals

Specific-pathogen-free (SPF) female BALB/c mice and male Sprague-Dawley (SD) rats were purchased from the Hubei Province Center of Experimental Animals (China) and housed in an SPF animal facility. All animal protocols were reviewed and approved by the Animal Care and Use Committee of Tongji Medical College, Huazhong University of Science and Technology.

Construction of a lentiviral vector expressing mouse LPCAT1 cDNA

For the overexpression of LPCAT1, the open reading frame of mouse LPCAT1 (NCBI NM_145376) was cloned into the GV287 lentiviral vector (Ubi-MCS-3FLAG-SV40-EGFP; GeneChem Co., Ltd. Shanghai, China), and these experiments were performed by GeneChem Co., Ltd. A recombinant LPCAT1 lentiviral vector that expressed both the EGFP gene and was tagged with a 3FLAG fragment was developed and packaged into matrue lentivirus particles (Lenti-LPCAT1)

using human embryonic kidney 293T cells (HEK293T cells, 293T cells), according the manufacturer's instructions. Additionally, a lentivirus (Lenti-EGFP) that only expressed the EGFP gene (GeneChem) was used as a control. Transfections were performed using the polybrene and enhanced infection solution (GeneChem) according to the manufacturer's recommended protocol.

Exogenous recombinant LPCAT1 protein expression in HEK293T cells

The HEK293T cells were infected by Lenti-LPCAT1 or Lenti-EGFP at MOI=30, respectively. 3 days later the cells were collected and lysed. Exogenous expression of recombinant LPCAT1 protein in HEK293T cells was confirmed through Western blot conducted in cell lysate. Briefly, a fixed amount of protein from 293T cells lysed with a lysis buffer containing anti-proteinases was subjected to 4−12% Bis-Tris SDS-PAGE under reducing conditions and then transferred to a nitrocellulose membrane. The membrane was incubated with a mouse anti-3Flag monoclonal antibody (1:1,000) or a mouse anti-GAPDH antibody (1:10,000) overnight at 4°C followed by a HRP-conjugated anti-mouse IgG antibody (1:5,000) for 1 h at 37°C. Chemiluminescent detection assay was performed, and the obtained bands were developed using an autofluorography film.

Isolation of type II alveolar epithelial cells (AECII cells)

Fresh AECII cells were isolated from 8-10 weeks old male SD rats by tissue dissociation with elastase and purification on metrizamide gradients, as previously reported.[11] The intact AECII cells were used for LPCAT1 acyltransferase activity assay.

LPCAT1 acyltransferase activity assay

The LPCAT1 acyltransferase activity assay measured the transfer ability of palmitoyl-CoA to LPC to form PC. The assays were performed as previously reported.[6,10] Briefly, the microsomal cell fractions were prepared from ~ 4×10^6 Lenti-LPCAT1 or Lenti-EGFP infected 293T cells, or ~10×10^6 freshly isolated rat AECII cells independently. The liposome reaction mixture (100 mM Tris-HCl, pH 7.4, 1 mM EDTA) contained 1 mg/ml DPPC, 25μM [1-^{14}C]palmitoyl-CoA (1.85-2.22 GBq/mmol) , 0 to 100μM 1-palmitoyl-LPC, and 1μg of microsomal protein prepared in different cell experiments. The reaction mixture was incubated at 30°C for 5 min in a total volume of 0.1

ml, and then stopped by adding 0.3 ml of chloroform:methanol (1:2, v/v). Total lipids were extracted by the Bligh-Dyer method,[12] and subsequently analysed by TLC with a developing solvent chloroform:methanol:water (70:30:5, v/v). The bands at the positions corresponding to LPC and PC were excised and counted with a liquid scintillation counter.

Animal study protocol

The mice aged 8-10 weeks (mean body weight 20 ± 2g) were sensitised and challenged with ovalbumin (OVA) based on a previously described procedure.[13] Briefly, the mice were sensitised on days 0 and 14 by intraperitoneal injection of 20μg OVA emulsified in 1mg of aluminium hydroxide in a total volume of 200μl. On days 24, 26 and 28 after the initial sensitisation, the mice were challenged with 200 μg OVA in 40 μl of sterile phosphate-buffered saline (PBS) by intratracheal instillation (or with saline as a control). Then, 24 hours after the last OVA challenge, the mice were sacrificed for BALF and lung tissues collection.

Purified high-titre Lenti-LPCAT1 virus [2.5×10^6 infectious units (IFU)/mouse], or the same amount of Lenti-EGFP virus as a control, was intratracheally instilled into the anaesthetised mice 72 hours before the first OVA challenge. The virus stocks were titrated by using an EGFP expression assay of HEK293T cells, and a viral titre of 2.5×10^6 IFU was used in this study.

The mice were grouped into Control group (untreated with OVA), OVA group (treated with OVA), Lenti-EGFP group (treated with empty virus Lenti-EGFP and OVA) and Lenti-LPCAT1 group (treated with Lenti-LPCAT1 virus and OVA) for these experiments. Each group contained 6 mice.

Evaluation of airway hyper-responsiveness (AHR)

At 24 hours after the last OVA challenge, the airway hyper-responsiveness (AHR), in the form of peak pulmonary resistance ($cmH_2O/ml/s$) was measured in anaesthetised mice by using the FlexiVent system (SCIREQ, Montreal, Quebec, Canada) after delivery of aerosolised methacholine. The increasing concentrations of methacholine aerosol (0, 2, 10, 25 and 50 mg/ml) were administered for 10 s, and then airway resistance was continuously monitored and recorded.

Evaluation of Bronchoalveolar lavage fluids (BALF) cells

BALF samples were performed at 24 hours after the last OVA challenge. The mice were

anaesthetised, and the trachea was cannulated while the thorax was gently massaged. The lungs were then lavaged 3 times with 0.8 ml of saline solution. The BALF samples were collected and cell counts were obtained using a haemocytometer. The remaining sample was centrifuged, and the supernatant was stored at −70°C for lipid assays and cytokines. The pellet was resuspended in PBS, and a cytospin preparation of the BALF cells was stained with Diff-Quik (Baso, Zhuhai, China). The different cell types were enumerated based on their morphology and staining profile.

Lung direct EGFP fluorescence and histopathology

At 24 hours after the last challenge, the right upper lobes of the lungs were removed from the mice after they had been sacrificed. To generate frozen sections for the direct visualisation of EGFP fluorescence from transfected mice, lung tissues were embedded in OCT embedding medium, and frozen sections were made. Confocal images were captured with Olympus fluoview image analysis. For lung histopathology, the lungs were fixed with 10% neutral phosphate-buffered formalin, sectioned at a thickness of 4μm and stained with haematoxylin-eosin (HE) to observe the airway inflammation under a light microscope at a magnification of 200×.

Endogenous and exogenous LPCAT1 protein expression in the lung of OVA-induced allergic asthmatic mice

At 24 hours after the last challenge, the lavaged left lung tissues were homogenised with protease and phosphatase inhibitors and extracted to obtain lung protein. Equal amounts of lung protein were subjected to Western blot. The specific primary antibody for mouse anti-3Flag (1:1000) was used to tag the exogenous recombinant LPCAT1 protein. The endogenous LPCAT1 protein was tagged by the commercial human polyclonal anti-LPCAT1 (1:1000). HRP-conjugated IgG was used as a secondary antibody.

Phospholipids LPC and DPPC assay in BALF

At 24 hours after the last OVA challenge, the BALF was subjected to phospholipid analysis. Total lipids were extracted from the BALF according to the Bligh and Dyer method.[12] The three most common and biologically-active LPC species (LPC 16:0, LPC 18:0, and LPC 18:1) and DPPC were quantified by high performance liquid chromatography-tandem mass spectrometry (LC-

MS-MS). The samples were spiked with LPC 19:0 (200ng/ml) as an internal standard. The system consisted of an API 3200 triple quadrupole mass spectrometer (Applied Biosystem/MDS SCIEX, Foster City, USA) that was equipped with a Shimadzu LC-20 UFLC system (USA) and a Welch Ultimate C18 column. The limits of detection were 10pg (LPC 16:0), 5pg (LPC 18:0), 10pg (LPC 18:1) and 1ng (DPPC) respectively. The LPC standard curves, over a 0.3-15μg/ml concentration range, were linear, with a coefficient of determination (R^2) >0.995. The DPPC standard curves, over a 3-150μg/ml concentration range, were linear, with a coefficient of determination (R^2) >0.995.

Quantitation of cytokines in BALF and OVA-specific IgE in serum

The concentrations of Th2 cytokines IL-4, IL-5, IL-13 and eotaxin in the BALF were determined by enzyme-linked immunosorbent assays (ELISA). The serum was collected from mice within 24 h after the last challenge. The concentration of OVA-specific IgE was evaluated by ELISA. The ELISAs were performed as per the manufacturer's instructions.

Statistical analysis

The data were presented as the mean ± S.D. for each group. One-way analysis of variance (ANOVA) and the two-tailed t-tests were utilised for comparisons among multiple groups and pair comparisons, respectively. In all cases, a P value less than 0.05 was considered statistically significant and a P value less than 0.01 was considered highly significant.

Results

Expression and high acyltransferase activity of exogenous recombinant LPCAT1 protein delivered by Lenti-LPCAT1

Figure 1A shows an expression of exogenous recombinant LPCAT1 protein tagged by 3Flag in 293T cells by Western blot. The recombinant LPCAT1 displayed a ~60 kDa molecular weight, as previously reported.[6-8] Figure 1B illustrates the LPC acyltransferase activity of the microsomal protein derived from Lenti-LPCAT1 transfected 293T cells in the presence of 25μM [1-^{14}C]palmitoyl-CoA. The cells showed a peak acyltransferase activity at a concentration of 25μM 1-palmitoyl-LPC. This activity was approximately 3 times higher than that observed in intact rat AECII cells which are known to specifically express LPCAT1.
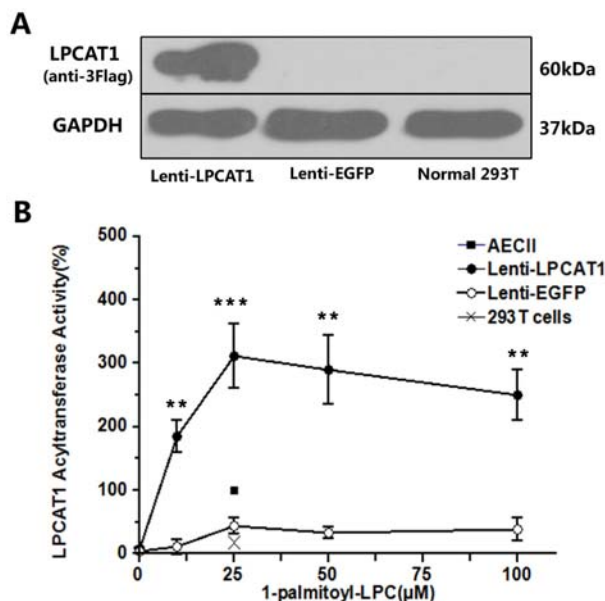
Figure 1. Lenti-LPCAT1-infected HEK293T cells express exogenous recombinant LPCAT1 protein.(A) Western blot showing 3Flag-tagged LPCAT1 protein, with a molecular weight of ~60 kDa. The 293T cells were infected with Lenti- LPCAT1 or Lenti-EGFP at an MOI of 30, and were lysed 3 days later. The 30μg cell lysate protein was loaded and assayed for Western blot using a mouse anti-3Flag monoclonal antibody (upper) or a mouse anti-GAPDH antibody (lower) as the primary antibody. (B) The recombinant LPCAT1 protein expressed in 293T cells shows high activity of the LPC acyltransferase. The acyltransferase activity asssay of LPCAT1 was measured through detecting the acyltransfer reaction of 1μg microsomal protein obtained from Lenti-LPCAT1 infected 293T cells (closed circles), Lenti-EGFP infected 293T cells (open circles), intact rat AECII cells (closed square), or intact 293T cells (cross). The measured values were normalized to those obtained in AECII cells. n= 3 independent experiments, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$vs.Lenti-EGFP .

Generation of LPCAT1-overexpressing mice

To evaluate the role of LPCAT1 in the airway, a lentiviral vector containing mouse LPCAT1 cDNA was constructed, packaged into mature lentivirus particles and instilled by a single endobronchial administration into the anaesthetised mice. Figure 2A shows the efficiency of the transfection of lentivirus vector to the lungs of mice evaluated by green fluorescence. Furthermore, we detected the endogenous and exogenous expression of LPCAT1 protein in four groups. The endogenous LPCAT1 expression was significantly decreased in the OVA

and Lenti-EGFP groups compared to the Control group (Figure 2B). Moreover, the general and exogenous expression of LPCAT1 was significantly increased in the Lenti-LPCAT1 group compared to the other 3 groups (Figures 2B & 2C). These data indicate the successful construction of an LPCAT1-overexpressing murine model.

Role of exogenous LPCAT1 in OVA-induced LPC and DPPC generation

To determine the role of LPCAT1 in OVA-induced LPC and DPPC generation, wide-type and LPCAT1-overexpressing mice were sensitised and challenged with OVA. OVA sensitisation and challenge significantly increased the saturated LPC 16:0 and LPC18:0, but not LPC18:1 in the BALF compared with the Control group ($P < 0.05$ and $P < 0.01$, respectively, Figure 3). However, LPCAT1 overexpression significantly decreased both the OVA-induced LPC 16:0 and LPC 18:0 levels in the BALF, compared to the Lenti-EGFP group ($P < 0.05$, Figure 3). There was no significant difference in the BALF DPPC levels between the 4 groups.

Role of exogenous LPCAT1 in OVA-induced AHR

The effect of exogenous LPCAT1 on AHR was evaluated by measuring airway resistance in anaesthetised mice by using the FlexiVent system. No significant difference was found in baseline airway resistance among the four groups. The airway resistance generated by the administration of methacholine aerosol at doses from 0 to 50 mg/ml increased significantly in the OVA group and EGFP group. However, Lenti-LPCAT1 group showed a decrease in airway resistance (Figure 4).

Role of exogenous LPCAT1 in OVA-induced eosinophil inflammation

Figure 5A shows no obvious inflammatory cell infiltration in the BALF samples of the Control group. However, OVA sensitisation and challenge significantly increased the macrophage, eosinophil, and lymphocyte infiltration in the BALF of the OVA and Lenti-EGFP groups compared with the Control group. Significantly increased eosinophil counts in the BALF demonstrated the successful establishment of the OVA-induced asthma model. However, LPCAT1 overexpression significantly decreased the total cell number and eosinophil deferential counts compared to those of the Lenti-EGFP group ($P < 0.05$ and $P < 0.01$, respectively).

Figures 5B-E show the release of Th2 cytokines and eotaxin in the BALF. There was a significant
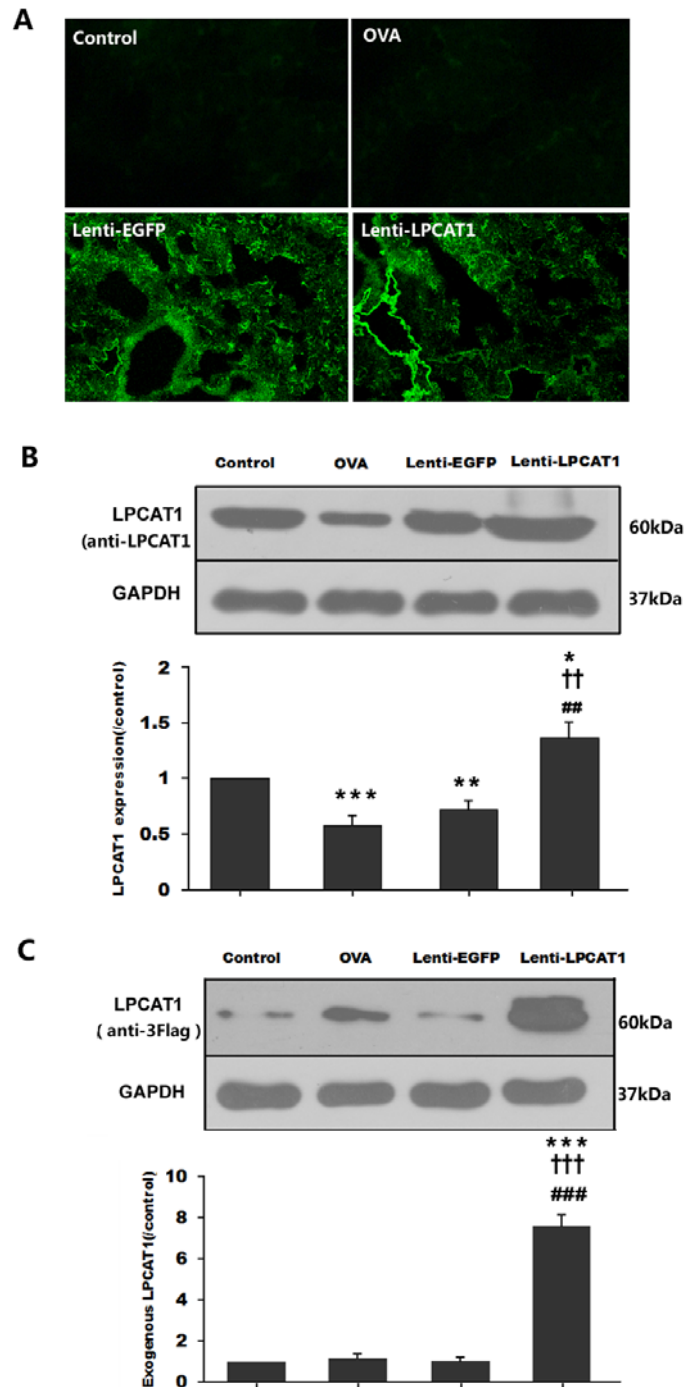
Figure 2. Generation of LPCAT1-overexpressing mice by Lenti-LPCAT1 vector intracheally administration. (A) Efficiency of the transfection by a single endobronchial administration of lentivirus to the lungs evaluated by green fluorescence.The lungs were embedded, frozen sectioned, and observed in confocal microscope, 100×. (B)Western blot showing endogenous LPCAT1 protein expression in the lungs of OVA-induced asthma mice. 24h after the last OVA challenge, total protein of lung was extracted and 50μg protein was loaded and assayed using a human polyclonal anti-LPCAT1(upper) or a mouse anti-GAPDH antibody (lower) as the primary antibody. **(C)** Western blot showing exogenous LPCAT1 in the lungs of OVA-induced asthma mice by using a mouse anti-3Flag monoclonal antibody (upper) or a mouse anti-GAPDH antibody (lower) as the primary antibody.The bar graphs (mean ± S.D.) in the bottom panels represent quantitative results of blots. n=3 independent experiments. *P <0.05, **P <0.01, ***P <0.001vs. Control; †P <0.05, ††P <0.01,††† P <0.001vs. OVA; #P <0.05,##P <0.01, ###P <0.001vs. Lenti-EGFP
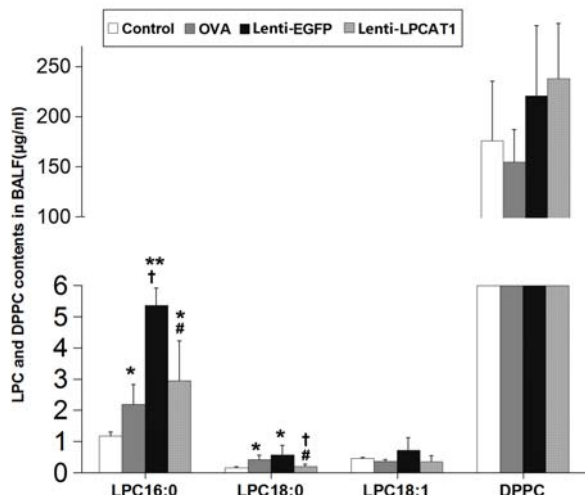
Figure 3. Effect of LPCAT1 on LPC and DPPC content in BALF of murine model of asthma. Total lipids were extracted from BALF according to the Bligh and Dyer method[12] and used to conduct LPC and DPPC measurements. LC-MS-MS chromatograph for LPC 16:0, LPC 18:0, LPC 18:1 and DPPC in BALF samples; LPC 19:0 is a internal standard. Data represent as mean ± S.D. (µg/ml). Each group contain 4-5 mice. $^*P < 0.05$,$^{**}P < 0.01$vs.Control;$^†P < 0.05$ vs.OVA ;$^#$ $P <0.05$vs.Lenti-EGFP

Figure 4. Effect of treatment with Lenti-LPCAT1 on OVA induced Airway hyperresponsiveness (AHR). AHR to inhaled a increasing dose methacholine were measured 24 hours after the last OVA challenge. **Lenti-LPCAT1 administration reduces AHR in mice.**Data are expressed as means ± S.D. Each group contain 6 mice.$^†P < 0.05$, $^{††}P < 0.01$, $^{†††}P < 0.001$ vs.OVA ; $^#P < 0.05$,$^{##}P < 0.01$, $^{###}P < 0.001$vs. Lenti-EGFP.
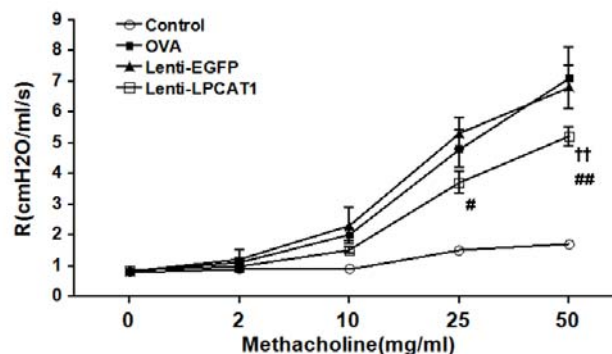
increase in the levels of IL-4, IL-5, IL-13 and eotaxin in 3 groups treated by OVA. However, the administration of Lenti-LPCAT1 showed a significant decrease in IL-5, IL-13 and eotaxin when compared to the OVA group and Lenti-EGFP group.

Figure 5F shows that OVA-specific IgE levels in the serum were elevated in the OVA group compared to the Control group. Treatment with exogenous LPCAT1 showed a significant decrease in OVA-specific IgE levels compared to the Lenti-EGFP group.

The effect of LPCAT1 on tisue inflammation was evaluated through histological examination of the lung sections. Significant perivascular and peribronchial inflammation was observed in OVA-challenged mouse lungs. In contrast, OVA-induced inflammatory injury was significantly reduced in the LPCAT1-overexpressing mice (Figure.6).

Discussion

Asthma may be classified as atopic (allergic) asthma and non-atopic asthma. Mice sensitised and challenged with OVA developed eosinophil inflammation, mucus metaplasia and AHR; this animal model has been considered to be a representative of allergic asthma. To the best of our knowledge, this study is the first to demonstrate the regulatory effect of exogenous LPCAT1 expression in an allergic asthma model *in vivo*. Lentiviral vector-mediated delivery of the exogenous LPCAT1 into the lungs showed a positive effect on the OVA-induced eosinophil inflammation in the airways in terms of the AHR, total cell number, eosinophil counts, cytokines, OVA-specific IgE and histological evaluation of inflammation.

The results indicate that significantly elevated LPC16:0 and LPC18:0 but not LPC18:1 levels are induced in the BALF of OVA asthmatic mice. These results are consistent with previous reports showing that the inherent LPC16:0 and LPC18:0 content are elevated in the BALF of asthmatic subjects with impaired lung function.[2,14]

The LPC16:0, LPC18:0, and LPC18:1 species are reported to be more pro-inflammatory active in stimulating neutrophil priming than several other LPCs such as LPC12:0, LPC14:0, and LPC17:0.[15] Furthermore, the LPC 16:0 and 18:0 species have been found to have greater pro-inflammatory activity in eosinophil adhesion, activation and recruitment than LPC 18:1.[16-18] Schistosomal-derived LPC, a mixture of bioactive LPC 16:0 and 18:0 species, induced the production of cytokines
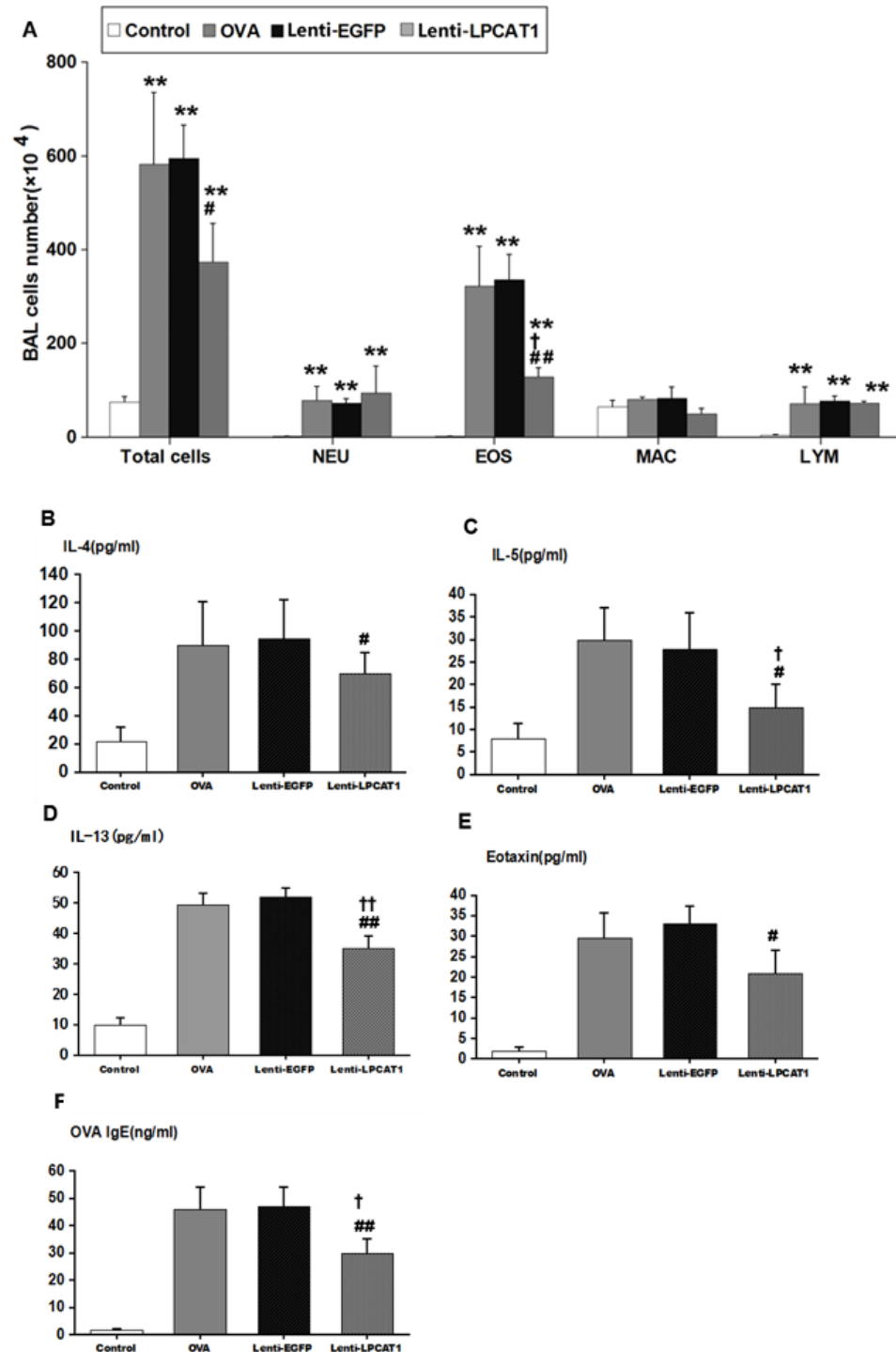
Figure 5. Effect of LPCAT1 on cell profile of BALF, cytokines and OVA specific IgE of murine model of asthma.(A)Cell counts in BALF were performed 24h after OVA challenge. NEU=Neutrophil; EOS=Eosinophil; MAC=Macrophages; LYM= Lymphocyte. **(B)-(E)**The levels of cytokine IL-4, IL-5,IL-13, etoxin in BALF were analyzed. **(F)**Serum OVA-IgE levels were analyzed. Data are expressed as means ± S.D. Each group contain 4-5 mice.$^{**}P < 0.01$ vs.Control;$^{†}P < 0.05$vs.OVA; $^{#}P < 0.05$, $^{##}P < 0.01$ vs. Lenti-EGFP.
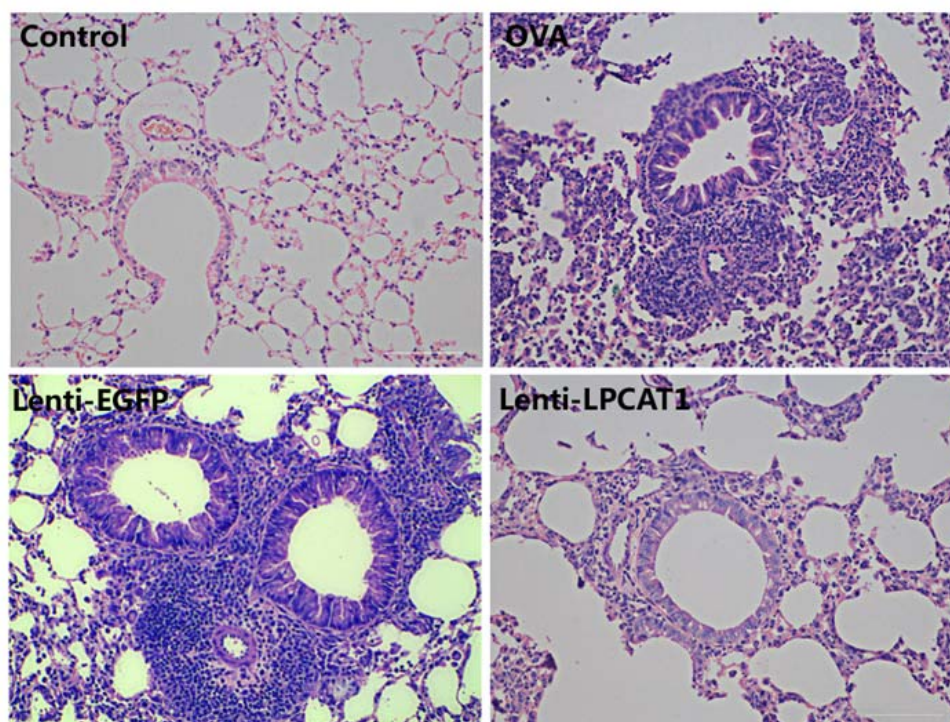
Figure 6. Effect of LPCAT1 on histopathologic study of lung tissue of murine model of asthma. Lung sections were stained with H&E for measurement of inflammatory cells around the airways (magnification × 200).

IL-5 and IL-13 and eotaxin in the peritoneal cavity by intraperitoneal administration.[18] The LPC 16:0 was shown to participate in chronic inflammation associated with asthma by an impaired epithelial barrier and increased IL-8 and MMP-1 secretion in the airways.[4] Although the LPC18:0 was found to be less efficient than LPC16:0 at the induction of T-cell activation *in vitro*, both single LPC16:0 and LPC18:0 induced mice to secrete significant amounts of IL-5 *in vivo*.[19]

In our *in vivo* OVA-induced asthma model, the levels of LPC16:0 and LPC18:0 were both elevated accompanied with increased eosinophil counts and the secretion of IL-5, IL-13 and eotaxin in BALF, which are consistent with the previous reports. Altogether, LPC16:0 and LPC18:0 are indicated to play a pathogenic role in the asthma chronic inflammation.

Our results showed exogenous LPCAT1 pre-administration significantly decreased the AHR, eosinophil counts, and levels of LPC16:0, LPC18:0 and cytokines in the BALF of OVA-induced asthma mice. Thus, it is possible that the favourable changes in the LPC compositions of Lenti-LPCAT1-treated mice were related to the amelioration of OVA-induced eosinophil inflammation. Moreover,

LPCAT1 has a preference for recognising LPC16:0 as a substrate in AECII and synthesise dipalmitoyl-phosphatidylcholine in pulmonary surfactant, which indicates a possible protective role in asthma.[6,8]

Through the acyl-transfer action of LPCAT1, LPC is converted back to PC, particularly dipalmitoyl-phosphatidylcholine (DPPC). Previous reports have speculated that the bronchial epithelium may adsorb DPPC in the airways to mask irritant receptors, inducing the bronchoconstriction that occurs during asthma. In particular, DPPC may provide a protective barrier for the airway epithelium.[20] Researchers have also reported elevated DPPC levels in the induced sputum of children with asthma, the levels of which were not influenced by inhaled steroids.[21] Thus, it is possible that inherent elevated DPPC levels in the lungs may serve a protective role, although the mechanism leading to increased DPPC levels in asthma remains to be elucidated. Our study showed that Lenti-LPCAT1 administration increased the BALF DPPC content, although not significantly, which indicates that DPPC levels may be inherent elevated during the airway inflammation phase of asthma.

In summary, lentiviral vector-mediated administration of exogenous LPCAT1 to

ovalbumin-induced asthmatic mice resulted in lower levels of LPC 16:0 and LPC 18:0 levels in the BALF, as well as reduced BALF eosinophil counts and cytokines production. Additionally, Lentivirus vector-delivered expression of LPCAT1 attenuated eosinophil airway inflammation and AHR. Thus, LPCAT1 may play a potential protective role in asthma inflammation.

Acknowledgments

Conflict of interests

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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