



Down-regulation of secreted lymphocyte antigen-6/urokinase-type plasminogen activator receptor-related peptide-1 (SLURP-1), an endogenous allosteric $\alpha 7$ nicotinic acetylcholine receptor modulator, in murine and human asthmatic conditions

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ABSTRACT

Whereas acetylcholine (ACh) acts as a bronchoconstrictor and stimulator of mucus secretion from bronchial epithelium, it acts via $\alpha 7$ nicotinic ACh receptors (nAChRs) on macrophages in the airways to exert anti-inflammatory effects by reducing synthesis of pro-inflammatory cytokines, such as tumor necrosis factor- α (TNF- α). Moreover, the effects of ACh are modified by secreted ly-6/urokinase-type plasminogen activator receptor-related peptide-1 (SLURP-1), a positive allosteric modulator of $\alpha 7$ nAChR signaling. Our aim was to explore the roles played by SLURP-1 in the pathophysiology of asthma by assessing SLURP-1 expression in the OVA-sensitized murine asthma model and in cultured human bronchial epithelial cells. Using real-time PCR we found that expression of SLURP-1 mRNA is down-regulated in the lungs of asthmatic model mice, as compared to healthy mice. In addition, immunohistochemical studies confirmed the diminished expression of SLURP-1 in the bronchioles of asthmatic mice, and showed it was due to extensive metaplasia of mucus-secreting cells and the concomitant loss of ciliated epithelial cells. Expression of SLURP-1 mRNA and protein was also significantly down-regulated in human epithelial cells stimulated with the pro-inflammatory cytokine interleukin-13 (IL-13), which is related to asthmatic condition. Thus SLURP-1 appears to be down-regulated in both an animal model of asthma and human epithelial cells treated with an inflammatory cytokine related to asthma. Those findings suggest that diminished expression of SLURP-1 in asthma attenuates its negative regulation of airway inflammation, and that perhaps changes in SLURP-1 expression could serve as a marker of airway damage in asthma.

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1. Introduction

Airway hyperreactivity is one of the main characteristics of asthma; i.e., a lower concentration of the acetylcholine (ACh) is required for bronchial contraction in patients with asthma than in healthy controls [1]. The ACh is released from postsynaptic parasympathetic cholinergic neurons innervating the airway smooth

muscle and submucosal glands [2] and is also synthesized and released by non-neural cells, such as epithelial and inflammatory cells [2–9]. Evidence suggests this non-neural cholinergic system is involved in the regulation of a variety of cell functions, including proliferation, apoptosis and cytokine production [5,9,10], but the roles played by non-neural ACh in the pathophysiology of the lung remain unclear.

ACh exerts its effects via muscarinic and nicotinic receptors (mAChRs and nAChRs, respectively), both of which are present within the airways [4]. Among the many nAChR subtypes, $\alpha 7$ nAChRs expressed in macrophages are involved in down-regulating the production and release of pro-inflammatory cytokines,

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such as tumor necrosis factor- α (TNF- α) and high mobility group box-1 protein (HMGB-1), thereby suppressing inflammatory responses [11–13]. Moreover, $\alpha 7$ nAChRs are key regulators of airway epithelial cells and appear to play a crucial role in the differentiation of bronchial epithelial cells [14].

Secreted lymphocyte antigen-6/urokinase-type plasminogen activator receptor-related peptide-1 (SLURP-1) was first identified in a urine peptide library and purified from human blood [15]. Subsequently, Chimienti et al. [16] used *Xenopus* oocytes expressing recombinant human $\alpha 7$ nAChRs to show that SLURP-1 binds to $\alpha 7$ nAChRs and exerts an allosteric effect. In addition, we recently showed that SLURP-1 is expressed in ciliated bronchial epithelial cells, where it acts as a positive modulator of $\alpha 7$ nAChR signaling [17]. In that context, the aim of the present study was to explore the involvement of SLURP-1 in the pathophysiology of asthma. We found that SLURP-1 expression is profoundly down-regulated in a murine model of asthma due to extensive metaplasia of mucus-secreting cells in the bronchial epithelium. Moreover, we confirmed that human airway epithelial cells also express SLURP-1, and that its expression is down-regulated by the presence of interleukin (IL)-13, which is abundant in asthmatic airways and plays a key role in the pathogenesis of asthma [18,19].

2. Materials and methods

2.1. Antibodies

An anti-SLURP-1 antibody against a 13-amino acid peptide corresponding to amino acids 77–89 (CLATDPDGIGVAH) – i.e., “loop 3,” a helical protrusion of murine SLURP-1 – was raised in a New Zealand White rabbit immunized with peptide-keyhole limpet hemocyanin [20,21]. Anti-human SLURP-1 monoclonal antibody (clone 4D1) was purchased from Abnova Corp (Taipei, Taiwan). Goat anti-mouse Mucin 5AC (clone K-20) was from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Mucin 5AC (MUC5AC)/gastric mucin Ab-1 (clone 45M1) mouse monoclonal antibody was from Thermo Fisher Scientific Anatomical Pathology (Fremont, CA, USA).

2.2. Animals

Specific pathogen-free female BALB/c mice (8–10 weeks old) were purchased from SLC (Shizuoka, Japan). The animals were housed in a specific pathogen-free environment and allowed free access to food and water. All experimental protocols were approved by the Musashino University Animal Care Committee and were in accordance with the NIH Guide for the care and use of laboratory animals. Mice were initially intraperitoneally immunized twice with 10 μ g of ovalbumin (OVA) and 2 mg of aluminum hydroxide gel (on days 0 and 14), as previously reported [22,23]. After immunization, the animals were made to inhale OVA (20 mg/ml) at a flow rate of 0.3 ml/min for 10 min daily for 7 days using a micromist nebulizer (Devlbiss, Somerset, PA, USA). The mean particle diameter was 3.0 μ m. Saline-treated mice served as controls.

2.3. Histological examination

Details of the procedures for immunohistochemical examination of SLURP-1 in the lung have been reported [17]. Briefly, mice were transcardially perfused with phosphate-buffered saline (PBS), followed by 4% paraformaldehyde in 100 mM phosphate buffer (pH 7.4). The lungs were then excised, immersed in 4% paraformaldehyde for 2 h at 4 °C, washed in PBS, and soaked overnight in 30% sucrose in PBS. The specimens were then embedded in OCT compound (Miles, Elkhart, IN, USA) and quickly frozen, after which sections were cut at a thickness of 10 μ m using a Leica CM3050

cryostat and thaw-mounted on poly-L-lysine-coated glass slides (Matsunami Glass, Osaka, Japan). The lung sections were incubated first with anti-SLURP-1 (1:1000) and goat anti-Mucin 5AC (1:200) overnight, and then with Alexa Flour 488-conjugated donkey anti-IgG (Molecular Probes, Eugene, OR, USA). Finally, the sections were counterstained with 4',6'-diamidino-2-phenylindole (DAPI, Roche Diagnostics, GmbH, Mannheim, Germany) and mounted using Vectashield Mounting Medium (Vector Laboratories, Burlingame, CA, USA). Control tissues were prepared by omitting the primary antibodies from the incubation solutions, and no specific immunoreactivity was observed. Immunofluorescence images were examined using a Leica TCS-SP2 confocal microscope (Leica Microsystems, Wetzlar, Germany) with excitation wavelengths of 350, 488 and 543 nm. Images were collected and analyzed using Leica Confocal Software (Leica Microsystems).

2.4. Culture of human airway epithelial cells

Normal human bronchial epithelial cells (NHBEs) were purchased from Lonza Walkersville Inc. (Walkersville, MD, USA). The cells were cultured in bronchial epithelial growth medium supplemented with retinoic acid and epidermal growth factors (Single-Quot[®] kit, Lonza Walkersville Inc.) on collagen type-I coated dishes. For air–liquid interface cultures, cells were first cultured on collagen type-I inserts (Becton Dickinson Labware, Bedford, USA) until confluent, after which the medium was removed from the upper part of each well, and the NHBEs were cultured for 2 weeks until differentiated into ciliated cells, as previously described [24–27]. To culture in asthmatic conditions, NHBEs were cultured for 10 days after adding recombinant human IL-13 (10 ng/ml; PeproTech Inc., Rocky Hill, NJ, USA) to the lower part of each well [24].

2.5. Analysis of mRNA expression

Lungs were excised from mice and immediately frozen in liquid nitrogen until used for experimentation. RNA was extracted from lung tissue or cultured cells using Trizol Reagent (Invitrogen Corporation, Carlsbad, CA, USA) and a modification of the acid guanidium–phenol–chloroform method, after which cDNA was synthesized using an iScript cDNA Synthesis kit (Bio-Rad Laboratories Inc., Hercules, CA, USA) containing RNase H⁺ reverse transcriptase and a blend of oligo-dT and random hexamer primers [20]. Levels of mRNA were quantified by real-time polymerase chain reaction (PCR) using a Light Cycler-Fast Start DNA Master SYBR Green I kit (Roche Diagnostics, Mannheim, Germany) and Light-Cycler 2.0 (Roche Applied Science, Mannheim, Germany). Quantification was accomplished using a standard curve generated with five dilutions of cDNA. Results are shown as ratios of the level of SLURP-1 mRNA normalized to the level of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) mRNA. The primers used were as follows: mouse GAPDH, 5'-TGCACCACCAACTGCTTAGC-3' (forward) and 3'-GGCATGGACTGTGGTCATGAG-5' (reverse), 87 bp; mouse SLURP-1, 5'-TTCCGAGACCTCTGCACTC-3' (forward) and 3'-ATAAGCGTGGGTATGGAAG-5' (reverse), 163 bp; human GAPDH 5'-TG CACCACCACTGCTTAGC-3' (forward) and 3'-GGCATGGACTGTGGT CATGAG-5' (reverse), 87 bp; and human SLURP-1, 5'-TTCCGAGAC CTCTGCACTC-3' (forward) and 3'-ATAAGCGTGGGTATGGAAG-5' (reverse), 163 bp.

2.6. Immunoblotting

Lung tissue or cultured cells were homogenized in Pro-prep protein extraction solution (Intron, Biotechnology Inc., Sangedae-won, Korea), after which the homogenates were centrifuged at 1000 $\times$ g for 30 min at 4 °C, and the supernatants were collected.

The proteins were separated by SDS–PAGE and transferred to a polyvinylidene difluoride membrane (Amersham Hybond-P, GE Healthcare, USA). After blocking with 5% dried, fat-free milk in PBS containing 0.05% Tween-20, the membrane was incubated first with rabbit anti-mouse-SLURP-1 antibody (1:1000) and then with horseradish-conjugated anti-rabbit antibody (GE Healthcare). We used anti-human SLURP1 (1:1000) and biotinylated polyclonal rabbit anti-mouse immunoglobulin (DAKO, Glostrup, Denmark) to detect human SLURP-1, after which the blots were developed by incubation with horseradish peroxidase-conjugated streptavidin (DAKO). As a positive control, we used human epithelial cell extracts immunoprecipitated using anti-human SLURP-1 and Catch and Release (Millipore Corporation Billerica, MA, USA). Signals were visualized using ECL reagents (GE Healthcare) and quantified using an image analyzer (LAS 3000, Fuji Film, Kanagawa, Japan).

2.7. Statistical analysis

Data were statistically analyzed using the Wilcoxon/Kruskal–Wallis test. Values of $P < 0.05$ were considered significant.

3. Results

3.1. SLURP-1 mRNA and protein expression in the murine asthma model

We initially used real-time PCR to assess the expression of SLURP-1 mRNA in lungs harvested from mice, and found that it was significantly ($P < 0.05$) suppressed in OVA-treated asthmatic mice, as compared to control (Fig. 1A). Moreover, western blot analysis confirmed that expression of SLURP-1 protein was also significantly weaker in lungs from OVA-treated mice than control mice (Fig. 1B).

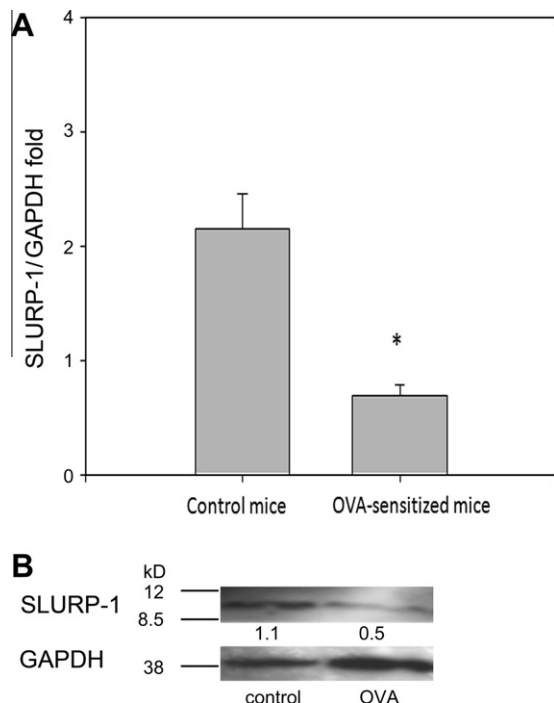


Fig. 1. SLURP-1 mRNA and protein expression in asthmatic lung in mice. (A) SLURP-1 mRNA expression was assessed using real-time PCR. Data are means $\pm$ SEM of six mice. * $P < 0.05$ vs. control. (B) Immunoblots showing the levels of SLURP-1 protein determined in lung homogenates from OVA asthmatic and control mice. Below are the relative levels of SLURP-1 normalized to those of GAPDH. Total eight samples from eight mice were used for the immunoblots. The summarized data was means $\pm$ SEM was 0.36 $\pm$ 0.047-fold (OVA/control, $P < 0.05$).

Histological examination of the airways of OVA-treated mice revealed the presence of epithelial damage, such as metaplasia of hyper-mucus-secreting cells, and infiltration of eosinophils, as has been reported previously [23,28] (Fig. 2A). Immunohistochemical analysis showed that SLURP-1 protein was expressed exclusively in epithelial cells, and was not expressed in the mucin 5AC-positive mucus-producing cells (Fig. 2B). SLURP-1 expression was dramatically reduced in the bronchiole of OVA-treated mice, most likely because ciliated epithelial cells were extensively replaced by hyper-mucus-secreting cells (Fig. 2B).

3.2. Expression of SLURP-1 mRNA and protein in the human bronchial epithelial cells

To determine whether SLURP-1 is also expressed in human bronchial epithelial cells, we assessed its expression in NHBes. The cells were cultured at an air–liquid interface without IL-13 (reported to promote differentiation into ciliated epithelial cells), or with IL-13 in the medium (reported to promote differentiation into mucus-secreting cells, which is relevant to asthmatic conditions) [24]. We examined the effect of IL-13 on expression of SLURP-1 mRNA in NHBes using real-time PCR. We found that levels of SLURP-1 mRNA were significantly ($P < 0.01$) up-regulated when NHBes were cultured at the air–liquid interface without IL-13, as compared to the levels in cells cultured with IL-13 and in control cells (Fig. 3A). In addition, immunoblotting confirmed the expression of SLURP-1 protein in NHBes (Fig. 3B). These results are consistent with our findings in the murine asthma model, and suggest that differentiation of ciliated epithelial cells into mucus producing cells is associated with a loss of SLURP-1 expression.

4. Discussion

In the present study, we found that expression of SLURP-1 mRNA and protein was down-regulated in a murine asthma model, and we confirmed that SLURP-1 is also expressed in human bronchial epithelial cells. In normal bronchioles, the epithelium is comprised mainly of ciliated epithelial cells, with a small number of mucus-secreting cells, and SLURP-1 is exclusively expressed in the ciliated epithelium [17]. The apparent reason for the down-regulation of SLURP-1 expression in the lungs of mice with OVA-induced asthma was metaplasia of mucus-secreting cells, which replaced the ciliated epithelial cells in the bronchial epithelium. Consistent with that idea, IL-13 significantly reduced expression of both SLURP-1 mRNA and protein in cultured human bronchial epithelial cells.

SLURP-1 has been shown to act as a positive allosteric modulator of $\alpha 7$ nAChR signaling that augments the biological effects of $\alpha 7$ nAChR agonists [16,29]. For example, SLURP-1 enhances carbachol-induced effects on apoptosis and expression of cell differentiation markers in human keratinocytes [29]. Ciliated bronchial epithelial cells have the ability to synthesize ACh and express $\alpha 7$ nAChRs [4,14,17]. Moreover, $\alpha 7$ nAChR knockout mice exhibit delayed regeneration of airway epithelium following injury, suggesting that signaling via $\alpha 7$ nAChRs plays a key regulatory role in epithelial homeostasis [14]. It is thus plausible that ACh and SLURP-1 released from ciliated bronchial epithelial cells act in an autocrine fashion via $\alpha 7$ nAChRs to exert protective effects and facilitate recovery of the epithelium following injury. Down-regulation of SLURP-1 expression in asthma likely disrupts epithelial homeostasis, leading to remodeling of the bronchial epithelium and greater susceptibility to infections, such as influenza and pneumonia.

Nicotine, which is highly toxic to humans, suppresses certain aspects of the asthmatic phenotype, both *in vivo* and *in vitro* [30–32]. In macrophages and splenocytes, $\alpha 7$ nAChR signaling is

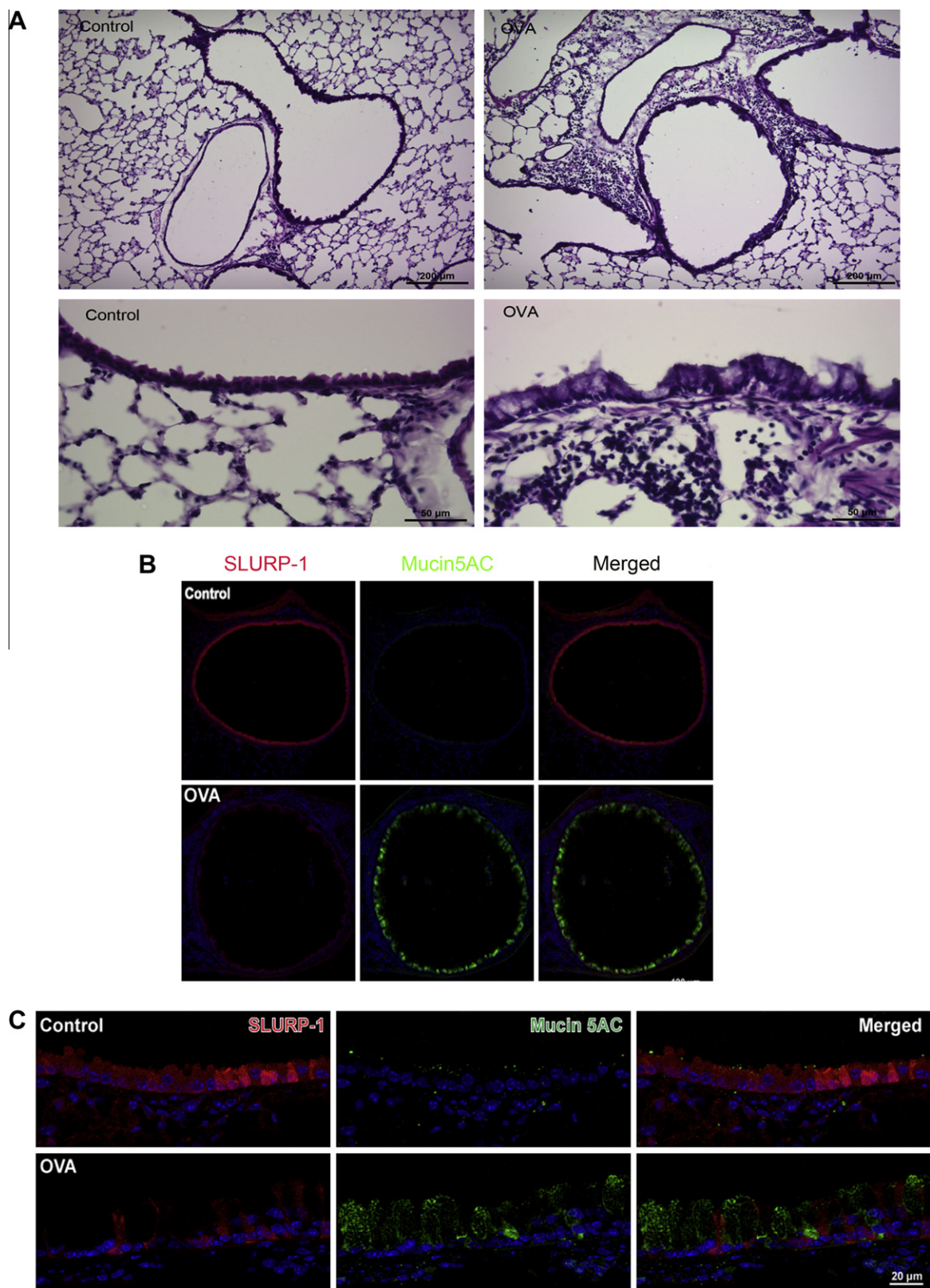


Fig. 2. (A) Hematoxylin/eosin staining of frozen lung sections: upper panels, 100×; lower panels, 400×. (B, C): Confocal micrographs of SLURP-1 (red) and MUC5AC (green) immunostaining in OVA asthmatic and control mice. DNA was counterstained with DAPI (blue). Merged images showing labeling of both SLURP-1 and MUC5AC are on the right. Low-power images (100×) are shown in panel B; high-power images (400×) are in panel C. Seven mice in each group (control and OVA) were used for the experiments.

involved in the negative regulation of pro-inflammatory cytokines [33]. Upon their release from bronchial epithelial cells, SLURP-1 and ACh diffuse over airway, where they likely function to down-

regulate expression and release of pro-inflammatory cytokines from macrophages and lymphocytes, thereby attenuating inflammatory and immune responses.

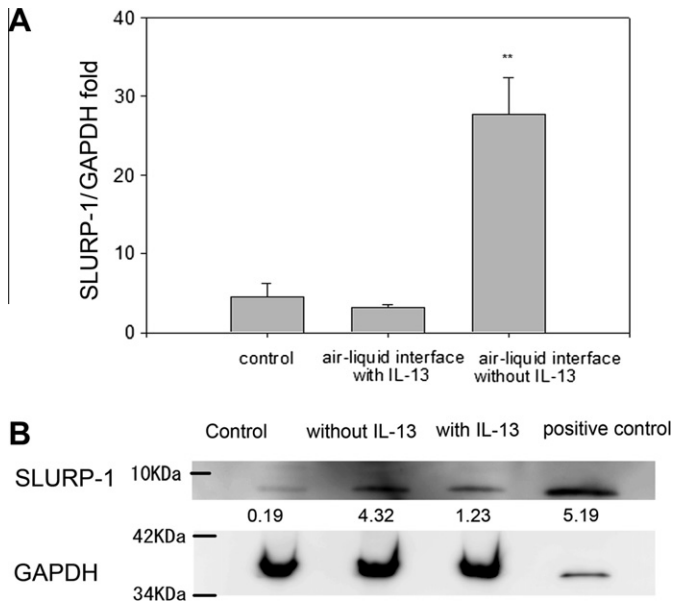


Fig. 3. Effect of IL-13 on expression of SLURP-1 mRNA and protein in NHBEs. (A) Relative levels of SLURP-1 mRNA expression in NHBEs cultured with or without IL-13. Levels of SLURP-1 mRNA were determined by real-time PCR and normalized to those of GAPDH. Bars indicate mean $\pm$ SEM of three or four samples. ** $P < 0.01$ vs. control. Control means NHBE cultured in the medium without air-liquid interface culture. (B) Immunoblot showing expression of SLURP-1 protein. As a positive control, we used human epithelial cell extracts immunoprecipitated using anti-human SLURP-1. The similar experiments were undertaken using four different samples. The summarized data was 6.9 ± 0.5 vs. 18.6 ± 1.8 -fold increase compared to control (epithelial cells with IL-13 vs. without IL-13, $P < 0.05$, $n = 4$ each group).

Taken together, our findings suggest that SLURP-1 expression is diminished in the lungs of asthma patients due to a reduction in the numbers of ciliated epithelial cells in the bronchioles. This hypothesis is currently under investigation. SLURP-1 has also been detected in the plasma, urine, sweat, saliva and tears of humans, suggesting the molecule is quite stable [21,34], which could make it possible to employ detection of SLURP-1 in plasma or urine as a marker of airway damage in asthma.

5. Conclusion

Expression of SLURP-1, a positive allosteric modulator of $\alpha 7$ nAChR signaling, is down-regulated in a murine asthma model and in humans, mainly due to extensive metaplasia of mucus-secreting cells. Restoring SLURP-1 may mitigate such remodeling of the airway epithelium and diminish production of pro-inflammatory cytokines mediated via $\alpha 7$ nAChRs.

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References

- [1] H. Meurs, R. Gosens, J. Zaagsma, Airway hyperresponsiveness in asthma: lessons from in vitro model systems and animal models, *Eur. Respir. J.* 32 (2008) 487–502.
- [2] C.R. Gwilt, L.E. Donnelly, D.F. Rogers, The non-neuronal cholinergic system in the airways: an unappreciated regulatory role in pulmonary inflammation?, *Pharmacol. Ther.* 115 (2007) 208–222.
- [3] P.J. Barnes, Distribution of receptor targets in the lung, *Proc. Am. Thorac. Soc.* 1 (2004) 345–351.

- [4] W. Kummer, K.S. Lips, U. Pfeil, The epithelial cholinergic system of the airways, *Histochem. Cell Biol.* 130 (2008) 219–234.
- [5] K. Kawashima, T. Fujii, Extraneuronal cholinergic system in lymphocytes, *Pharmacol. Ther.* 86 (2000) 29–48.
- [6] K. Kawashima, T. Fujii, Expression of non-neuronal acetylcholine in lymphocytes and its contribution to the regulation of immune function, *Front. Biosci.* 9 (2004) 2063–2085.
- [7] T. Fujii, Y. Takada-Takatori, K. Kawashima, Basic and clinical aspects of non-neuronal acetylcholine: expression of an independent, non-neuronal cholinergic system in lymphocytes and its clinical significance in immunotherapy, *J. Pharmacol. Sci.* 106 (2008) 186–192.
- [8] S.A. Grando, K. Kawashima, C.J. Kirkpatrick, I. Wessler, Recent progress in understanding the non-neuronal cholinergic system in humans, *Life Sci.* 80 (2007) 2181–2185.
- [9] S.A. Grando, K. Kawashima, I. Wessler, Introduction: the non-neuronal cholinergic system in humans, *Life Sci.* 72 (2003) 2009–2012.
- [10] S.A. Grando, Basic and clinical aspects of non-neuronal acetylcholine: biological and clinical significance of non-canonical ligands of epithelial nicotinic acetylcholine receptors, *J. Pharmacol. Sci.* 106 (2008) 174–179.
- [11] L.V. Borovikova, S. Ivanova, M. Zhang, H. Yang, G.I. Botchkina, L.R. Watkins, H. Wang, N. Abumrad, J.W. Eaton, K.J. Tracey, Vagus nerve stimulation attenuates the systemic inflammatory response to endotoxin, *Nature* 405 (2000) 458–462.
- [12] R.W. Saeed, S. Varma, T. Peng-Nemeroff, B. Sherry, D. Balakhaneh, J. Huston, K.J. Tracey, Y. Al-Abed, C.N. Metz, Cholinergic stimulation blocks endothelial cell activation and leukocyte recruitment during inflammation, *J. Exp. Med.* 201 (2005) 1113–1123.
- [13] H. Wang, M. Yu, M. Ochani, C.A. Amella, M. Tanovic, S. Susarla, J.H. Li, H. Yang, L. Ulloa, Y. Al-Abed, C.J. Czura, K.J. Tracey, Nicotinic acetylcholine receptor $\alpha 7$ subunit is an essential regulator of inflammation, *Nature* 421 (2003) 384–388.
- [14] K. Maoche, M. Polette, T. Jolly, K. Medjber, I. Cloez-Tayarani, J.P. Changeux, H. Burlet, C. Terry, C. Coraux, J.M. Zahm, P. Birembaut, J.M. Tournier, $\alpha 7$ Nicotinic acetylcholine receptor regulates airway epithelium differentiation by controlling basal cell proliferation, *Am. J. Pathol.* 175 (2009) 1868–1882.
- [15] K. Adermann, F. Wattler, S. Wattler, G. Heine, M. Meyer, W.G. Forssmann, M. Nehls, Structural and phylogenetic characterization of human SLURP-1, the first secreted mammalian member of the Ly-6/uPAR protein superfamily, *Protein Sci.* 8 (1999) 810–819.
- [16] F. Chimienti, R.C. Hogg, L. Plantard, C. Lehmann, N. Brakch, J. Fischer, M. Huber, D. Bertrand, D. Hohl, Identification of SLURP-1 as an epidermal neuromodulator explains the clinical phenotype of Mal de Meleda, *Hum. Mol. Genet.* 12 (2003) 3017–3024.
- [17] K. Horiguchi, S. Horiguchi, N. Yamashita, K. Irie, J. Masuda, H. Takano-Ohmuro, T. Himi, M. Miyazawa, Y. Moriwaki, T. Okuda, H. Misawa, H. Ozaki, K. Kawashima, Expression of SLURP-1, an endogenous $\alpha 7$ nicotinic acetylcholine receptor allosteric ligand, in murine bronchial epithelial cells, *J. Neurosci. Res.* 87 (2009) 2740–2747.
- [18] T. Doherty, D. Broide, Cytokines and growth factors in airway remodeling in asthma, *Curr. Opin. Immunol.* 19 (2007) 676–680.
- [19] J. Mitchell, V. Dimov, R.G. Townley, IL-13 and the IL-13 receptor as therapeutic targets for asthma and allergic disease, *Curr. Opin. Investig. Drugs* 11 (2010) 527–534.
- [20] Y. Moriwaki, Y. Watanabe, T. Shinagawa, M. Kai, M. Miyazawa, T. Okuda, K. Kawashima, A. Yabashi, S. Waguri, H. Misawa, Primary sensory neuronal expression of SLURP-1, an endogenous nicotinic acetylcholine receptor ligand, *Neurosci. Res.* 64 (2009) 403–412.
- [21] R. Mastrangeli, S. Donini, C.A. Kelton, C. He, A. Bressan, F. Milazzo, V. Ciolli, F. Borrelli, F. Martelli, M. Biffoni, O. Serlupi-Crescenzi, S. Serani, E. Micangeli, N. El Tayar, R. Vaccaro, T. Renda, R. Lisciani, M. Rossi, R. Papoian, A.R.S. Component B: structural characterization, tissue expression and regulation of the gene and protein (SLURP-1) associated with Mal de Meleda, *Eur. J. Dermatol.* 13 (2003) 560–570.
- [22] Y. Matsuo, T. Ishihara, J. Ishizaki, K. Miyamoto, M. Higaki, N. Yamashita, Effect of betamethasone phosphate loaded polymeric nanoparticles on a murine asthma model, *Cell Immunol.* 260 (2009) 33–38.
- [23] N. Yamashita, H. Tashimo, Y. Matsuo, H. Ishida, K. Yoshiura, K. Sato, T. Kakiuchi, K. Ohta, Role of CCL21 and CCL19 in allergic inflammation in the ovalbumin-specific murine asthmatic model, *J. Allergy Clin. Immunol.* 117 (2006) 1040–1046.
- [24] M. Kondo, J. Tamaoki, K. Takeyama, J. Nakata, A. Nagai, Interleukin-13 induces goblet cell differentiation in primary cell culture from Guinea pig tracheal epithelium, *Am. J. Respir. Cell Mol. Biol.* 27 (2002) 536–541.
- [25] A.J. Ross, L.A. Dailey, L.E. Brighton, R.B. Devlin, Transcriptional profiling of mucociliary differentiation in human airway epithelial cells, *Am. J. Respir. Cell Mol. Biol.* 37 (2007) 169–185.
- [26] Y. You, E.J. Richer, T. Huang, S.L. Brody, Growth and differentiation of mouse tracheal epithelial cells: selection of a proliferative population, *Am. J. Physiol. Lung Cell. Mol. Physiol.* 283 (2002) L1315–L1321.
- [27] J. Laoukili, E. Perret, T. Willems, A. Minty, E. Parthoens, O. Houcine, A. Coste, M. Jorissen, F. Marano, D. Caput, F. Tournier, IL-13 alters mucociliary differentiation and ciliary beating of human respiratory epithelial cells, *J. Clin. Invest.* 108 (2001) 1817–1824.
- [28] B.D. Medoff, S.Y. Thomas, A.D. Luster, T cell trafficking in allergic asthma: the ins and outs, *Annu. Rev. Immunol.* 26 (2008) 205–232.
- [29] J. Arredondo, A.I. Chernyavsky, R.J. Webber, S.A. Grando, Biological effects of SLURP-1 on human keratinocytes, *J. Invest. Dermatol.* 125 (2005) 1236–1241.
- [30] H. Kikuchi, J. Itoh, S. Fukuda, Chronic nicotine stimulation modulates the immune response of mucosal T cells to Th1-dominant pattern via nAChR by

- upregulation of Th1-specific transcriptional factor, *Neurosci. Lett.* 432 (2008) 217–221.
- [31] N.C. Mishra, J. Rir-Sima-Ah, R.J. Langley, S.P. Singh, J.C. Pena-Philippides, T. Koga, S. Razani-Boroujerdi, J. Hutt, M. Campen, K.C. Kim, Y. Tesfaigzi, M.L. Sopori, Nicotine primarily suppresses lung Th2 but not goblet cell and muscle cell responses to allergens, *J. Immunol.* 180 (2008) 7655–7663.
- [32] R. Vassallo, K. Tamada, J.S. Lau, P.R. Kroening, L. Chen, Cigarette smoke extract suppresses human dendritic cell function leading to preferential induction of Th-2 priming, *J. Immunol.* 175 (2005) 2684–2691.
- [33] Y.X. Fujii, H. Fujigaya, Y. Moriwaki, H. Misawa, T. Kasahara, S.A. Grando, K. Kawashima, Enhanced serum antigen-specific IgG1 and proinflammatory cytokine production in nicotinic acetylcholine receptor alpha7 subunit gene knockout mice, *J. Neuroimmunol.* 189 (2007) 69–74.
- [34] B. Favre, L. Plantard, L. Aeschbach, N. Brakch, S. Christen-Zaech, P.A. de Viragh, A. Sergeant, M. Huber, D. Hohl, SLURP1 is a late marker of epidermal differentiation and is absent in Mal de Meleda, *J. Invest Dermatol* 127 (2007) 301–308.