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Interleukin-13 enhanced Ca^{2+} oscillations in airway smooth muscle cells

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24 **Abstract**

25 Physiological mechanisms associated with interleukin-13 (IL-13), a key cytokine in asthma,
26 in intracellular Ca^{2+} signaling in airway smooth muscle cells (ASMCs) remain unclear. The
27 aim of this study was to assess effects of IL-13 on Ca^{2+} oscillations in response to
28 leukotriene D4 (LTD4) in human cultured ASMCs.

29 LTD4-induced Ca^{2+} oscillations in ASMCs pretreated with IL-13 were imaged by
30 confocal microscopy. mRNA expressions of cysteinyl leukotriene 1 receptors (CysLT1R),
31 CD38, involved with the ryanodine receptors (RyR) system, and transient receptor potential
32 canonical (TRPC), involved with store-operated Ca^{2+} entry (SOCE), were determined by
33 real-time PCR. In IL-13-pretreated ASMCs, frequency of LTD4-induced Ca^{2+} oscillations
34 and number of oscillating cells were significantly increased compared with untreated
35 ASMCs. Both xestospongine C, a specific inhibitor of inositol 1,4,5-triphosphate receptors
36 (IP_3R), and ryanodine or ruthenium red, inhibitors of RyR, partially blocked LTD4-induced
37 Ca^{2+} oscillations. Ca^{2+} oscillations were almost completely inhibited by 50 μM of 2-
38 aminoethoxydiphenyl borate (2-APB), which dominantly blocks SOCE but not IP_3R at this
39 concentration. Pretreatment with IL-13 increased the mRNA expressions of CysLT1R and
40 CD38, but not of TRPC1 and TRPC3.

41 We conclude that IL-13 enhances frequency of LTD4-induced Ca^{2+} oscillations in
42 human ASMCs, which may be cooperatively modulated by IP_3R , RyR systems and
43 possibly by SOCE.

44

45 Key words: airway smooth muscle cells, asthma, Ca^{2+} oscillation, interleukin-13,
46 leukotriene D4

47 **Abbreviations:**

48 2-APB: 2-aminoethoxydiphenyl borate

49 ASMCs: airway smooth muscle cells

50 CICR: calcium induced calcium release

51 Cys-LTs: cysteinyl leukotrienes

52 CysLT1R: cysteinyl leukotriene 1 receptor

53 GPCR: G protein-coupled receptor

54 IICR: IP₃-induced Ca²⁺ release

55 IL: interleukin

56 IP₃R: inositol 1,4,5-triphosphate receptors

57 RyR: ryanodine receptors

58 SOCE: store-operated Ca²⁺ entry

59 SR: sacroplasmic reticulum

60 TRPC: transient receptor potential canonical

61

1. Introduction

Airway inflammation is a fundamental feature of asthma. Among a number of inflammatory cytokines and mediators involved in asthma, interleukin (IL)-13, a pleiotropic Th2 cytokine, plays a pivotal role [1]. In addition to its well-established effects, such as stimulation of eosinophilic inflammation, induction of goblet cell hyperplasia and airway fibrosis, previous studies have revealed that IL-13 augments agonist-induced contraction of the tracheal ring and increases intracellular Ca^{2+} concentrations ($[\text{Ca}^{2+}]_i$) in airway smooth muscle cells (ASMCs) [2]. Cysteinyl leukotrienes (Cys-LTs; LT-C4, -D4, and -E4) are metabolites of arachidonic acid and are important agonists for ASMCs by binding the CysLT1 receptor (CysLT1R), a G protein-coupled receptor (GPCR), and activating phospholipase C, which leads to the generation of inositol 1,4,5-triphosphate (IP_3) followed by Ca^{2+} release from sarcoplasmic reticulum (SR) [3, 4]. Both IL-13 and Cys-LTs are produced by mast cells that infiltrate airway smooth muscle layer [5], and a close relationship between IL-13 and Cys-LTs in asthma pathogenesis has been suggested recently [6].

Although intracellular Ca^{2+} signaling is usually assessed by $[\text{Ca}^{2+}]_i$, previous studies using mouse lung slices [7, 8] have highlighted the importance of Ca^{2+} oscillations by

showing that an increase in oscillation frequency was associated with augmentation of airway contraction. Ca^{2+} oscillation is an efficient system for the Ca^{2+} signaling pathways by reducing possible deleterious effects due to sustained increases in $[\text{Ca}^{2+}]_i$ [9]. Currently, repetitive Ca^{2+} -release systems, such as inositol 1,4,5-triphosphate receptors (IP_3R) and/or ryanodine receptors (RyR) on the SR membrane, are thought to be key systems in agonist-induced Ca^{2+} oscillations. Furthermore, an interrelationship has been recently proposed between sustained Ca^{2+} oscillations and plasma membrane Ca^{2+} influx, particularly store-operated Ca^{2+} entry (SOCE) triggered by depletion of Ca^{2+} in SR [10-12].

Thus far, however, leukotriene D4 (LTD4)-induced Ca^{2+} oscillations in ASMCs have not been demonstrated, and the effects of IL-13 on this pathway remain unknown.

In the present study, we aimed to assess the effects of IL-13 on Ca^{2+} oscillations in response to LTD4 in human cultured ASMCs, and elucidate the mechanism(s) underlying a putative augmentation of LTD4-induced Ca^{2+} oscillations with IL-13 pretreatment.

2. Methods

2.1.1 Study population and cell preparation

Human ASMCs were obtained from the lungs of 15 patients (10 males; average 68.0 years

old; lung cancer in 14 patients and granulomatous inflammation in 1) who underwent surgical resection in accordance with procedures approved by the ethics committee of Kyoto University. Airway smooth muscle bundles were dissected out and cultured as described previously [13, 14]. When cells grew to confluence, they were seeded at a density of $1 \times 10^4/\text{cm}^2$ into collagen-coated ($5 \mu\text{g}/\text{cm}^2$) glass-bottom dishes (Matsunami Glass Ind. Ltd, Osaka, Japan) for measurements of Ca^{2+} oscillations or into 6-well culture plates for measurements of mRNA. In each experiment, cells were cultured in fresh medium containing IL-13 (10 ng/ml)(Sigma Aldrich, Osaka, Japan) or diluent (PBS with 0.1% BSA) for 24 hours when they reached subconfluence. The effects of IL-13 on mRNA expression were also examined at earlier time points, i.e., after 8 hours and 12 hours of incubation with IL-13.

2.1.2 Measurements of Ca^{2+} oscillations

Pretreated ASMCs were washed and loaded with a Ca^{2+} -sensitive dye, Fluo-4 AM (5 μM) (Invitrogen, Molecular probes, CA, USA)[15] for 30 min at 37 °C. After rinsing twice, the dishes were mounted on a Zeiss LSM510 confocal microscope (Axiovert 200M, Carl Zeiss, Jena, Germany). In each experiment, 10 min perfusion with modified Krebs solution for equilibration was followed by perfusion with modified Krebs solution containing LTD4

113 (100 nM)(Cayman Chemical, Michigan, US) in the presence or absence of inhibitors: 2-
114 aminoethoxydiphenyl borate (2-APB)(Sigma Aldrich), xestospongine C (Wako, Osaka,
115 Japan), ruthenium red (Wako), or ryanodine (Calbiochem, Darmstadt, Germany). The
116 acquired images were transferred to a BV analyzer (BrainVision, Tokyo, Japan), and the
117 frequency of Ca^{2+} oscillations was analyzed. Fluorescence magnitude was expressed as the
118 ratio of fluorescence relative to the fluorescence level immediately prior to the addition of
119 an agonist. The temperature of the chamber in which glass-bottom dishes were placed to
120 measure Ca^{2+} oscillations was set at 37°C using a temperature controller (Zeiss Model CZI-
121 3, Carl Zeiss). The room temperature was set at 26°C to keep the focus plane constant. All
122 measurements were done in duplicate using at least three cell lines each obtained from a
123 different donor.

124 **2.1.3 Reverse transcription and real-time PCR analysis**

125 Total RNA was extracted from ASMCs using the RNeasy Mini Kit (Qiagen, Osaka, Japan).
126 cDNA was synthesized and real-time PCR was performed using the ABI Prism 7300
127 sequence detection system (Applied Biosystems, Tokyo, Japan) with SYBR green (Roche
128 Diagnostics, Tokyo, Japan). Specific primer sets used are shown in Table 1. The relative
129 quantity of mRNA expression level of a target molecule was normalized to the mRNA

expression levels of β -actin and β_2 microglobulin in the same sample.

2.1.4 Western Blotting

Protein expression of CysLT1R and CD38 was analyzed by Western blotting and enhanced chemiluminescence. ASM cells were lysed in a buffer containing 50 mM Tris, 1% proteinase inhibitor cocktail (Sigma Aldrich), 1% Triton X-100. Protein concentration was determined measured by the Bio-Rad DC protein assay (Bio-Rad, Osaka, Japan). 10 μ g of protein/lane was electrophoresed through 10% SDS-PAGE and transferred to PVDF membranes (GE Healthcare, Tokyo, Japan). Membranes were blocked with 5% skimmed-milk in Tris-buffered saline and 0.05% Tween 20 and then incubated overnight at 4°C with the primary antibodies, rabbit anti-CysLT1R antibody (1:200) (Cayman Chemical) or with mouse anti-CD38 antibody (Santa Cruz Biotechnology, Inc. CA, USA) (1:200) in blocking solution overnight at 4°C. Secondary anti-rabbit or anti-mouse IgG conjugated with horseradish peroxidase for use with ECL plus (1:5,000) (GE Healthcare) was incubated for 1 hour at room temperature and visualized using ECL plus Western Blotting Detection Reagents (GE Healthcare), and quantified by densitometry using ChemiDoc XRS (Bio-Rad). Data were normalized to the values for GAPDH in the same lane.

2.2 Statistics

147 Each result for a single treatment is given as the mean $\pm$ SE. Statistical analysis used paired
148 t tests to compare untreated and treated cells. For the comparison of mRNA expression at
149 different time points, repeated measures one-way ANOVA with Fisher's protected least
150 significant difference correction was employed. p -value < 0.05 indicated statistical
151 significance.

3. Results

3.1 *LTD4 induces Ca^{2+} oscillations in ASMCs; pretreatment with IL-13 increases the number of oscillating cells and oscillation frequency*

We first found that 100 nM LTD4 induced an increase in $[Ca^{2+}]_i$ followed by Ca^{2+} oscillations in human cultured ASMCs (Fig 1a). In untreated cells, Ca^{2+} oscillations were observed in a small percentage of cells ($13.0 \pm 4.8\%$ of total cells in a visual field) with relatively low frequencies ($0.446 \pm 0.140 \text{ min}^{-1}$). However, pretreatment with IL-13 (10 ng/ml) for 24 hours significantly increased the number of oscillating ASMCs to $46.5 \pm 5.2\%$ and augmented the frequency of Ca^{2+} oscillations about 2.6-fold ($1.196 \pm 0.142 \text{ min}^{-1}$, $p=0.0037$; Fig 1b). The amplitude and frequency of Ca^{2+} oscillations were sustained during 20 min of perfusion with LTD4; the average amplitude level and frequency during the initial 10 min were maintained at $97.3 \pm 6.2\%$ and $91.9 \pm 2.6\%$ of these values, respectively, during the subsequent 10 min.

3.2 *IP₃R, RyR and SOCE dependence of LTD4-induced Ca^{2+} oscillations*

To examine the roles of IP₃R, RyR and/or SOCE for the Ca^{2+} oscillations evoked by LTD4, IP₃R, RyR or SOCE were blocked by inhibitors. Because the number of oscillating cells and the number of oscillations in each cell were too few to evaluate quantitatively without

169 IL-13 pretreatment, inhibition studies were done with ASMCs pretreated with IL-13. All
170 inhibitors were added to cell cultures during ongoing LTD4-induced Ca^{2+} oscillations.
171 Addition of xestospongin C (10 μM) [16], a specific inhibitor of IP_3R , partially inhibited
172 the amplitude and frequency of LTD4-induced Ca^{2+} oscillations to $50.6 \pm 2.3\%$ ($p < 0.001$)
173 and $43.6 \pm 12.2\%$ ($p = 0.006$) of their initial levels, respectively. Inhibition of RyR by
174 ruthenium red (200 μM) [17] also partially decreased the amplitude and frequency to $47.7 \pm$
175 6.8% ($p = 0.003$) and $47.5 \pm 17.6\%$ ($p = 0.016$) of their initial levels, respectively. Ryanodine
176 (50 μM) was also added to fully close RyR. Ryanodine decreased the amplitude and
177 frequency to $47.1 \pm 1.7\%$ ($p = 0.001$) and $65.0 \pm 3.1\%$ ($p = 0.0006$) of their initial levels,
178 respectively, but also did not abolish the LTD4-induced Ca^{2+} oscillations (Fig 2a, b).
179 Meanwhile addition of 2-APB almost completely abrogated the LTD4-induced Ca^{2+}
180 oscillations either at 50 μM wherein SOCE is dominantly blocked but not IP_3R at this
181 concentration [11] (Fig 2b) or at higher concentration (200 μM) wherein both IP_3R and
182 SOCE are blocked (Fig 2a, b).

183 ***3.3 Effects of IL-13 pretreatment on mRNA expressions of CysLT1R, IP_3R type 1, 2, 3,*** 184 ***RyR type 3, CD38, and transient receptor potential canonical (TRPC) in ASMCs***

185 We examined mRNA expressions of molecules that could be involved in IP_3R /RyR

pathways and SOCE, including CysLT1R, IP₃R type 1, 2, 3, CD38 and TRPC3 after 8, 12 and 24 hours of incubation with IL-13. CD38 is responsible for the synthesis and degradation of cyclic ADP ribose, an endogenous ligand for RyR in ASMCs [18]. mRNA expressions of CysLT1R and CD38, but not TRPC1 and TRPC3, when normalized to the mRNA expression levels of β -actin, increased significantly within 8 hours of incubation with IL-13 ($p=0.001$ for CysLT1R and $p=0.007$ for CD38) (Fig 3a). There were no significant changes in mRNA expressions of IP₃R type 1, 2, 3, or RyR type 3 (Fig 3a). Normalization to the mRNA expression levels of β_2 micro-globulin did not change the findings.

3.4 Western blotting

Western blotting analyses were performed to confirm the expressions of CysLT1R and CD38 protein. Pretreatment with IL-13 for 24 hours significantly increased the density for CysLT1R or CD38 that was normalized to GAPDH expression (Fig 3b).

4. Discussion

To the best of our knowledge, we have given the first demonstration that LTD4 induces Ca^{2+} oscillations in human cultured ASMCs. Moreover, the frequency of Ca^{2+} oscillations and the proportion of oscillating cells were significantly enhanced when ASMCs were pretreated with IL-13. This was partly explained by up-regulation of CysLT1R and CD38 expression levels after IL-13 pretreatment. Inhibition studies demonstrated that the LTD4-induced Ca^{2+} oscillations in IL-13-pretreated ASMCs were regulated cooperatively by IP_3R , RyR and SOCE.

Despite considerable evidence that LTD4 is an important mediator in airway smooth muscle contraction [4, 19] and proliferation in coupling with IL-13 in asthma [20], induction of Ca^{2+} oscillations by LTD4 and the effects of IL-13 on this pathway remain unknown. Using real-time confocal microscopy, we confirmed that LTD4 induced Ca^{2+} oscillations in human ASMCs, and pretreatment with IL-13 significantly increased the frequency of LTD4-induced Ca^{2+} oscillations and the proportion of oscillating ASMCs, which was accompanied by increases in mRNA expression as well as in protein levels of CysLT1R and CD38. The increase in CysLT1R expression is consistent with the results of a previous report that examined proliferation of ASMCs in response to LTD4 after

217 pretreatment with IL-13 [20]. This up-regulation of CysLT1R expression by IL-13
218 pretreatment in our study may have enhanced IP₃-induced Ca²⁺ release (IICR) / calcium
219 induced calcium release (CICR) *via* IP₃R as further discussed below, which resulted in an
220 increased number of oscillating cells and oscillation frequency. Consistent with a previous
221 study [21], IL-13 also increased CD38 expression that regulates cyclic ADP ribose
222 metabolism, an endogenous ligand of RyR in ASMCs. Up-regulation of CD38 expression
223 by IL-13 may have enhanced CICR *via* RyR.

224 It is well established that binding of LTD4 to CysLT1R activates phospholipase C
225 *via* the Gq protein, which leads to the generation of IP₃. After binding IP₃, IP₃Rs on SR are
226 activated and open to release Ca²⁺ from SR, which results in an initial increase in [Ca²⁺]_i.
227 This priming release of Ca²⁺ is denoted IICR and this increase of [Ca²⁺]_i promotes CICR. It
228 is currently inconclusive which of the two Ca²⁺-release systems on the SR membrane, IP₃R
229 or RyR, is responsible for agonist-induced Ca²⁺ oscillations and governs repetitive Ca²⁺
230 release. The degrees of contributions of IP₃R and RyR to CICR may vary among different
231 cell types [22], species [23] [24] and agonists used [23] [24]. In the present study, both
232 xestospongine C, a specific inhibitor of IP₃R, and ruthenium red or ryanodine, inhibitors of
233 RyR, suppressed the frequency of Ca²⁺ oscillations to a similar degree in IL-13-pretreated

234 ASMCs. These findings suggest that both IP₃R and RyR were involved in Ca²⁺ oscillations
235 in IL-13-pretreated ASMCs.

236 For sustained Ca²⁺ oscillations, it is essential to replenish Ca²⁺ in SR and doing so
237 requires Ca²⁺ influx because some Ca²⁺ is inevitably lost to the extracellular environment.
238 In ASMCs, SOCE, but not the voltage-gated Ca²⁺ channel, plays an important role in Ca²⁺
239 influx [10]. We also found that SOCE may be involved in LTD4-induced Ca²⁺ oscillations
240 in IL-13-pretreated ASMCs because not only 200 μM of 2-APB, which blocks both IP₃R
241 and SOCE, but 50 μM of 2-APB, which dominantly blocks SOCE but not IP₃R [11],
242 inhibited the Ca²⁺ oscillations almost completely. Consistent with our findings, a recent
243 study by Gao et al. shows that pretreatment with IL-13 (10 ng/ml) for 24 hours promoted
244 SOCE in rat ASMCs [25]. However, the molecular mechanisms underlying SOCE were not
245 clarified in our study. Similar to the study of Gao et al. [25], pretreatment with IL-13 did
246 not change the mRNA expression of TRPC1. Furthermore the mRNA expression of TRPC3,
247 which was reported to be up-regulated by tumor necrosis factor-alpha [26], also did not
248 change.

249 The oscillation frequency in human cultured ASMCs in this study was slower than
250 that of ASMCs observed in mouse lung slices [7]. Another study that examined oscillations

251 in human cultured ASMCs in response to arachidonic acid also showed a slow frequency
252 (0.5-1/min) [27]. These reduced responses to LTD4 might be agonist-specific, although the
253 frequency of histamine-induced Ca^{2+} oscillations in human cultured ASMCs showed a
254 range similar to that of LTD4 (data not shown). Using cultured ASMCs might be argued
255 against because muscarinic receptor M3 is down-regulated in cultured ASMCs. Histamine
256 H1 receptor and CysLT1R are, however, reported to be retained on cultured ASMCs [28]
257 and mediate agonist-induced contraction [14]. The system using cultured ASMCs was
258 advantageous because it can evaluate direct effects of agonists or inhibitors on ASMCs
259 without the possible confounding influence of other elements, such as epithelium, vascular
260 endothelium, and inflammatory cells.

261 In conclusion, we found a substantial effect of IL-13 on LTD4-induced Ca^{2+}
262 oscillations in human cultured ASMCs *via* up-regulation of CysLT1R, which augments the
263 IP_3R system, and CD38, which may be associated with modulating the RyR system. SOCE
264 may also be involved in LTD4-induced Ca^{2+} oscillations. These systems, IP_3R , RyR, and
265 possibly SOCE cooperatively modulate agonist-induced Ca^{2+} oscillations in ASMCs. These
266 findings may partly explain altered properties of airway smooth muscle in asthma.
267

268

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355

Table 1. Sequences of primer sets for real-time PCR

CysLT1R	forward primer, 5'-GCACCTATGCTTTGTATGTCAACC-3'
	reverse primer, 5'-ATACCTACACACACAAACCTGGC-3'
CD38	forward primer, 5'-TGGCCAACTGCGAGTTCAG-3'
	reverse primer, 5'-GACGAGGATCAGGACCAGGAT-3'
IP ₃ R type1	forward primer, 5'-TCAATTTCGGGAGAGGATGTC-3'
	reverse primer, 5'-TCGACCAAGTGGATGTGGTA-3'
IP ₃ R type2	forward primer, 5'-CAACCCTCCCAAGAAGTTCA-3'
	reverse primer, 5'-GTTTGGCTTGCTTTGCTTTC-3'
IP ₃ R type3	forward primer, 5'-GCCTTCGACTCTACCACTGC-3'
	reverse primer, 5'-TTGTCTTCCCCACTCCAAAC-3'
RyR type3	forward primer, 5'- CGGATGACGTGGTAAGCTG-3'
	reverse primer, 5'- AGCCCGTCTGTGTTGAAGTTC-3'
TRPC1	forward primer, 5'- GCCCGGAATTCTCGTGA-3'
	reverse primer, 5'-AGGTGGGCTTGCGTCGGT-3'
TRPC3	forward primer, 5'-CAGGCCTAAGGGAGCAGACCATAG-3'
	reverse primer, 5'-ACTGTGATATTGGGCAGCGTGGTG-3'
β-actin	forward primer, 5'-AAGAGAGGCATCCTCACCCCT-3'
	reverse primer, 5'-TACATGGCTGGGGTGTGAA-3'
β ₂ -MG	forward primer, 5'-TGTCTTTCAGCAAGGACTGGTC-3'
	reverse primer, 5'-CA AACCTCCATGATGCTGC -3'

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CysLT1R: cysteinyl leukotriene 1 receptor, IP₃R: inositol 1,4,5-triphosphate receptors
RyR: ryanodine receptor, β₂-MG: β₂-microglobulin, TRPC: transient receptor potential canonical

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Figure legends

Fig 1. a) Top: Representative images of ASMCs pretreated with IL-13. Raw image of ASMCs loaded with Ca^{2+} -sensitive fluorescent dye, Fluo-4 AM (left), images in pseudocolor before (middle) and after (right) LTD4 stimulation. The lowest fluorescence level was assigned a value of 0 and the brightest a value of 125 in pseudocolor. **Bottom:** Representative traces of LTD4-induced Ca^{2+} oscillations in ASMCs pretreated with diluent (black) or with IL-13 (red). After 10 min perfusion with modified Krebs plain solution, 100 nM LTD4 was perfused for 30 min. Fluorescence magnitude was expressed as ratio of fluorescence relative to the fluorescence immediately prior to the addition of an agonist. **b) Top:** Proportion of oscillating ASMCs responding to LTD4 after pretreatment with either diluent (IL-13 (-)) or IL-13 (10 ng/ml).

* $p < 0.001$ **Bottom:** Average (boxes) and SE (bars) of the LTD4-induced Ca^{2+} oscillation frequency in ASMCs pretreated with either diluent (IL-13 (-)) or IL-13 ($n=6$ for each group).

† $p=0.0037$.

Fig 2. a) Representative traces of 10 μM xestospongine C, 50 μM ryanodine, 200 μM ruthenium red and 200 μM 2-APB effects on LTD4-induced Ca^{2+} oscillations of ASMCs pretreated with IL-13. After 10 min perfusion with modified Krebs plain solution, followed by 10 min with 100 nM LTD4 alone, inhibitors and 100 nM LTD4 were perfused for 20 min. **b)** Average (boxes) and SE (bars) of the amplitude and frequency of LTD4-induced Ca^{2+} oscillations with or without inhibitors ($n=6$ for xestospongine C (XC) and 2-APB (50 μM), $n=4$ for 2-APB (200 μM) and ruthenium red (RR), $n=3$ for ryanodine (Ry)). Amplitude and frequency of Ca^{2+} oscillations during the initial 10 min with an inhibitor and LTD4 (anterior half of gray bar) were normalized to those during the preceding 10 min of perfusion with LTD4 alone (horizontal white bar). * $p < 0.001$, † $p < 0.05$ vs. inhibitor (-).

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408 **Fig 3.** a) Effects of IL-13 (10 ng/ml) on mRNA expressions of cysteinyl leukotriene receptor 1
409 (CysLT1R), CD38, inositol 1,4,5-triphosphate receptors (IP₃R) type 1, 2, and 3, ryanodine
410 receptor (RyR) type 3, transient receptor potential canonical (TRPC)1, and TRPC3 assessed by
411 real time PCR (n=4) at 8, 12, and 24 hours. Relative quantity of mRNA expression of a target
412 molecule was normalized to the mRNA expression of β -actin in the same sample. mRNA level
413 of a normalized target molecule is given as the ratio to that of a sample pretreated with diluent
414 only (0 hr). By repeated measures one-way ANOVA, $p<0.01$ for CysLT1R and CD38; $p=0.07$
415 for IP3R3 and RyR3, and $p=0.09$ for TRPC1; $p>0.1$ for the others. * $p<0.05$ vs. 0 hr by post hoc
416 Fisher's protected least significant difference correction. b) Effects of IL-13 (10 ng/ml) on
417 protein levels of CysLT1R, and CD38 assessed by Western blotting (n=3 for each group).
418 Relative quantity of protein levels of a target molecule was normalized to the protein levels of
419 GAPDH in the same lane. Protein level of a normalized target molecule is given as the ratio to
420 that of a sample pretreated with diluent only. * $p<0.05$ vs. IL-13(-)

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Fig. 1a

Fig. 1b

Fig. 2a

Fig. 2b

Fig. 3a

Fig. 3b