Involvement of thromboxane A2 in airway mucous cells in asthma-related cough

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Involvement of thromboxane A2 in airway mucous cells in asthma-related cough. J Appl Physiol 92: 763–770, 2002.—The aim of this study was to elucidate the role of thromboxane A2 (TxA2) on asthma-related cough in guinea pigs. Animals were immunosensitized and repeatedly challenged with ovalbumin as an antigen. Coughs were induced by the inhalation of 10−5 M capsaicin solution for 10 min. Thromboxane synthetase (TxS) inhibitor OKY-046 and thromboxane-receptor antagonist AA-2414 significantly inhibited cough responses in repeatedly challenged animals. Inhalation of TxA2 mimic STA-2-potentiated cough responses in normal and immunosensitized animals but not in repeatedly challenged ones. Moreover, STA-2-potentiated coughs were inhibited by administration of neurokinin-receptor antagonist FK-224. In repeatedly challenged animals, concentration of TxB2 in airway lavage fluid, expression of TxS mRNA in tracheal epithelia, and the immunostaining intensity against TxS in mucous cells of the epithelium significantly increased compared with normal and sensitized animals. These results suggest that TxA2 derived from mucous cells potentiated cough responses to capsaicin in allergic airway inflammation. We have previously reported the effect of airway inflammation on the cough response in guinea pigs (44), indicating that repeated immunochallenges induced the infiltration of inflammatory cells into the airways and increased cough sensitivity. Tachykinin-receptor antagonists inhibited the increased cough sensitivity. This finding demonstrates that the capsaicin-induced release of tachykinins plays an important role in the increased cough sensitivity of ovalbumin (OVA)-sensitized and repeatedly challenged guinea pigs.

The role of thromboxane A2 (TxA2) in the pathogenesis of asthma has been extensively studied (26, 35, 39). Thromboxane-receptor antagonist AA-2414 and selective thromboxane synthetase (TxS) inhibitor OKY-046 are commercially available as anti-asthma drugs in Japan. Moreover, it has been recently reported that a TxS inhibitor suppressed coughing induced by angiotensin-converting enzyme inhibitors and that cough threshold was increased by treatment of OKY-046 in asthmatics (38, 14). However, the precise production site and effective cells of TxA2 have not been fully determined.

TxA2 may play an important role in coughing that results from airway allergic inflammation. In the present study using a guinea pig cough model, we studied whether TxA2 was related to cough responses and investigated the relationships between TxA2 and tachykinins in cough responses. Furthermore, we elucidated the localization of TxS in guinea pig airways.

MATERIALS AND METHODS

Animals. Experiments using animals were performed in accordance with the institutional guidelines set forth by the University of Tsukuba Committee on the Use and Care of Animals. Female Hartley guinea pigs (n = 153) weighing 250–300 g (SLC Farm, Shizuoka, Japan) were used for the following experiments. Animals were sensitized as described previously (2, 37). Briefly, guinea pigs were pretreated with an intraperitoneal injection of 30 mg/kg cyclophosphamide. Two days later, they were immunized with 1 mg OVA and 100 mg of aluminum hydroxide. Three weeks after primary immunization, a booster intraperitoneal injection 10 μg OVA
together with 100 mg aluminum hydroxide was performed. Animals were used 6 wk after primary immunization.

To evaluate the effect of allergic inflammation on cough responses, we repeatedly exposed sensitized animals to aerosols of OVA (4 mg/ml saline) for 4 min daily for 7 days. Diphenhydramine (50 mg/kg) was injected intraperitoneally 30 min before each OVA challenge to protect animals from anaphylaxis.

**Measurement of cough responses.** The principle and detailed methods of measurement of cough responses were described previously (44). Unanaesthetized guinea pigs were placed in an air-tight box and exposed to aerosol production of \(10^{-5}\) M capsaicin solution for 10 min. An ultrasonic nebulizer (NEU 03, Omron, Tokyo, Japan) produced aerosols containing particles with an aerodynamic mass-median diameter of 5.0 \(\mu\)m (manufacturer’s specification) at 0.25 ml/min. Coughing was considered to have occurred if a rapid violent movement of the chest was distinguished by at least two observers. Pressure changes of the box were recorded with a differential pressure transducer (model TP-602T, Nihonkohden, Tokyo). Coughing sounds were recorded by a microphone placed in front of a face mask, and cough waves were analyzed by a computer connected with the microphone throughout the amplifier. Coughing was detected and clearly distinguished from sneezing or other body movement with these methods. In all experiments, once a guinea pig was exposed to capsaicin, it was never reused in subsequent experiments.

**Pharmacological effect of TXA2 on cough responses.** To evaluate whether TXA2 is related to capsaicin-induced coughing, TXA2-receptor agonist STA-2 was used instead of unstable TXA2. STA-2 was inhaled at concentrations of 3 \(\times 10^{-6}\) M, 6 \(\times 10^{-6}\) M, and 10 \(\times 10^{-6}\) M for 10 min to induce a cough response, but it did not elicit coughing in any group of guinea pigs. Next, we measured specific airway conductance as an index of airflow limitation at the three doses listed above, as described previously (19). Inhalation of STA-2 at doses of \(10^{-6}\) and \(10^{-5}\) M induced bronchoconstriction, so we used a contractile subthreshold dose of 3 \(\times 10^{-7}\) M for the experiment. Guinea pigs were pretreated with inhalation of 3 \(\times 10^{-7}\) M STA-2 for 5 min, and 30 min later they were challenged with an aerosol dose of \(10^{-5}\) M capsaicin.

To investigate the relationship between TXA2 and tachykinin, we examined the effects of FK-224, a neurokinin-receptor antagonist, on STA-2-potentiated coughing. Subject animals inhaled STA-2 at doses of \(10^{-6}\) and \(10^{-5}\) M for 5 min 30 min after pretreatment with STA-2, and 30 min later they were exposed to capsaicin.

We also examined the effects of thromboxane inhibition. Sensitized and repeatedly challenged guinea pigs were pretreated with OKY-046, a TXS inhibitor, through a mini-catheter (Alzet, Palo Alto, CA). The pump flow moderator was implanted in the skin below the rib cage of the animals. OKY-046 was at a dose of 5 \(\mu\)g/kg body wt \(\times 10^{-6}\) administered by continuous infusion for 4 days from the implantation. This dosage has been known pharmacologically to be effective enough to block TXS (6). On day 5, the exposure of capsaicin was performed. AA-2414, a TXA2-receptor antagonist, was administered orally through a gastric tube at a dose of 10 mg/kg body wt for 4 days in sensitized and repeatedly challenged animals. On day 5, animals were exposed to capsaicin aerosols.

**Enzyme immunoassay of TXB2 in airway lavage fluid.** To evaluate TXS in the airway, we measured the level of TXB2 in the airway lavage fluid of three groups \((n = 5\) in each group). Because TXA2 produced from alveolar macrophages is found in greater concentration in bronchoalveolar lavage fluid (28), we used the tracheobronchial lavage to lower this influence as much as possible. For tracheobronchial lavage, the trachea was cannulated with a 16-gauge tube under anesthesia with pentobarbital sodium (50 mg/kg ip). One milliliter of ice-cold saline, including 10 \(-5\) M indomethacin and 5 \(\times 10^{-3}\) M EDTA, was slowly injected into the trachea, and the recovered fluid was collected. These procedures were repeated three times. The recovered fluid was centrifuged at 150 \(\times g\) for 10 min at 4°C. Aliquots of the supernatants were collected and stored at \(-20°C\) until the assay. TXB2 was purified according to a manual protocol and was assayed by using a TXB2 enzyme immunoassay kit (Cayman Chemical, Ann Arbor, MI). The sensitivity is 13.3 pg/ml and the specificity is 100% for TXB2.

**Immunohistological analysis.** Normal and repeatedly challenged guinea pigs \((n = 4\) in each group) were anesthetized as described above. Their tracheas were immediately removed and fixed with 4% paraformaldehyde in phosphate buffer (pH 7.2) for 2 h, washed with PBS (pH 7.4), and incubated in 5, 10, and 20% sucrose in PBS, respectively. Then the tracheas were embedded in optimal cutting temperature compound (Miles, Elkhart, IN) and frozen. Continuous cryosections \((8 \mu m)\) were stained with immunohistochemical staining or periodic acid Schiff (PAS) to evaluate the immunohistochemical localization of TXS and the condition of mucous cells, respectively. For immunohistochemical staining, the sections were rehydrated with washing buffer (in M: 0.5 NaCl, 0.02 Tris, 0.01 CaCl2, pH 7.4) for 10 min and then incubated with 2.3% periodic acid for 5 min, 0.02% potassium borohydride for 2 min, and 0.3% H2O2 in methanol for 30 min to block endogenous peroxidase activity. After immersion in 1% normal mouse serum in caseine for 30 min, the sections were incubated for 30 min at room temperature with a biotinylated mouse anti-human TXS monoclonal antibody (BMA Medicinals, Augst, Switzerland) at a dilution of 1:20. Nonimmune mouse serum was used as a negative control. Sections were incubated with avidin-biotin-peroxidase complex (ABC kit, Vector Laboratories, Burlingame, CA). Reactions were visualized by 3,3'-diaminobenzidine tetrahydrochloride (DAB) (Dojindo, Kumamoto, Japan). Sections were used for light microscopic analysis after being counterstained with hematoxylin. For electron-microscopic analysis, sections were fixed with 2% glutaraldehyde in PBS (0.1 M, pH 7.4) after reaction with DAB and postfixed in 2% osmium tetroxide in PBS (0.1 M, pH 7.4) for 4 h. Sections were then dehydrated in a graded series of ethanol (50–100%) followed by propylene oxide, and embedded in Poly/Bed 812 resin (Polysciences, Warrington, PA) for transmission electron microscopy. Ultrathin sections of 100–150 nm were cut by using an Ultrotomy Laboratoire Kastler Brossel 2088 (LKB-Producer, Bromma, Sweden) and observed with an electron microscope (H-7000, Hitachi, Tokyo). PAS staining was performed by oxidizing sections in 0.5% periodic acid for 5 min, staining with Schiff’s reagent for 7 min and hematoxylin for 30 s, and then differentiating with 1% HCl in 70% ethanol for 3 s.

**Western blot analysis.** The specificity for the TXS antibody against guinea pigs was tested by Western blot analysis with the protein of guinea pig platelets. The blood of five guinea pigs was obtained with cardiac puncture under anesthesia, as described above, and collected into 0.1 volume, 3.8% (wt/vol) trisodium citrate. Platelet-rich plasma was prepared by centrifugation for 20 min at 200 g at 20°C. Platelet-rich plasma was then centrifuged for 10 min at 800 g. The pellet was mixed with an equal volume of loading buffer (125 mM Tris-HCl, pH 6.8; 4% SDS, 20% glycerol, 0.05% bromophenol blue, 5% β-mercaptoethanol) and boiled for 3 min. SDS-PAGE was performed on equivalent protein samples with
10% acrylamide, and protein was electrophoretically transferred to polyvinylidene difluoride membrane (Bio-Rad Laboratories, Hercules, CA). Membranes were blocked in buffer containing 5% dried milk and 1% normal mouse serum for 60 min. They were incubated with 1:20 TxsS antibody for 30 min and then with the ABC kit and stained with an AP conjugate substrate kit (Bio-Rad Laboratories).

Cloning of TxsS complement DNA fragments from guinea pig lungs. To examine the expression of TxsS mRNA, we cloned TxsS cDNA fragments from the lungs of guinea pigs. Total RNA was extracted from lungs (n = 2) with an RNaseasy total RNA kit (Qiagen, Hilden, Germany). A 514-bp DNA fragment was obtained by RT-PCR by using a GeneAmp RNA PCR kit (Perkin Elmer, Foster City, CA) with the gene-specific primers 5'-CAGACATGTACGAGAGGTG-3' and 5'-GCAATCACATCTCTAATGAG-3'. PCR product was subcloned into a cloning vector with vector pGEM-T and pGEM-T Easy Vector Systems (Promega, Madison, WI) and sequenced by the dideoxynucleotide chain-termination method by using a dideoxyterminator kit (Perkin Elmer) and a DNA sequencing model 373S (Perkin Elmer Applied Biosystems Division, Foster City, CA).

Northern blot analysis. Levels of TxsS mRNA in the airway epithelium were compared by Northern blot analysis among normal, sensitized, and multiply challenged groups (n = 5 in each group). Tracheas were carefully dissected free from surrounding connective tissue and incised along the ventral midline. The epithelial side of the trachea was gently scraped with a rubber scraper in the presence of PBS. Total RNA of the epithelial layer was extracted by using Trizol Reagent (Life Technologies, Grand Island, NY). Ten micrograms of total RNA obtained from each animal were separated on 4% formaldehyde-1% agarose gel and transferred to Hybond N+ nylon membrane filter (Amersham). The 514-bp TxsS cDNA fragment was labeled by 32P deoxyctydine triphosphate and further used as a probe. RNA was hybridized with the cDNA probe in ExpressHyb hybridization solution (Clontech, Palo Alto, CA) at 68°C and washed with 0.1× SSC-0.1% SDS (SSC is 0.15 M NaCl-0.15 M sodium citrate) at 50°C. The membrane was exposed to a BAS-SR imaging plate (Fuji Film, Tokyo) and analyzed with a BAS-5000 imaging analyzer (Fuji Film).

Preparation of drugs. Capsaicin (Sigma Chemical, St. Louis, MO) was dissolved in saline containing 10% ethanol and 10% Tween 80 to give stock solutions of 10⁻² M, which were stored at 4°C. FK-224 (kind gift from Fujisawa Pharmaceutical, Osaka, Japan) was dissolved in dimethyl sulfoxide to give a 0.1 M stock solution, which was stored at 4°C. These stock solutions were diluted in saline as necessary for each experiment. STA-2 (kind gift from ONO Pharmaceutical, Osaka, Japan) was dissolved in phosphate buffer (1/15 M, pH 7.2) to give a stock solution of 10⁻² M, which was stored at 4°C and diluted in the same buffer. OKY-046 (kind gift from ONO) was dissolved in ethanol to 320 mg/ml and diluted in saline to 128 mg/ml. AA-2414 (kind gift from Takeda Pharmaceutical, Osaka, Japan) was dissolved in saline containing 10% ethanol. All other drugs were obtained from Wako Pure Chemicals (Osaka, Japan) and dissolved in saline. Drugs’ doses were calculated as their free base.

Statistical analysis. Results are presented as means ± SE. Statistical analysis was performed among three groups or between two groups. Data for capsaicin-induced coughing under baseline conditions and TxB2 concentration in three groups were analyzed by one-way ANOVA and post hoc Fisher’s paired least-significant difference test. Differences between vehicle and drug pretreatment groups were evaluated with an F test and then with a Student’s t-test. Significant differences were accepted when P value was <0.05.

RESULTS
Facilitation of the effect of TxA₂ on cough responses. Effects of STA-2 on capsaicin-induced coughing are shown in Fig. 1. Values for cough under baseline conditions were significantly different in normal vs. sensitized animals (5.17 ± 1.01 vs. 10.0 ± 1.53, P < 0.05) and normal vs. repeatedly challenged ones (5.17 ± 1.01 vs. 22.5 ± 1.52, P < 0.01). Inhalation of STA-2 caused a significant increase in the number of coughs in normal and sensitized animals compared with the vehicle (22.17 ± 5.05 vs. 5.17 ± 1.01, P < 0.01 and 31.0 ± 3.0 vs. 10.0 ± 1.53, P < 0.01, respectively). Repeatedly immunochallenged guinea pigs, however, did not show a significant increase (20.17 ± 1.79 vs. 22.5 ± 1.52).

OKY-046 and AA-2414 significantly inhibited cough frequency in repeatedly challenged guinea pigs compared with the vehicle (5.17 ± 2.85 vs. 20.0 ± 1.37, P < 0.01; 4.5 ± 1.48 vs. 21.33 ± 1.89, P < 0.01) (Fig. 2). In the sensitized group, AA-2414 suppressed the number of coughs (AA-2414 vs. vehicle = 4.83 ± 1.01 vs. 10.0 ± 1.51, P < 0.05), but OKY-046 did not have a significant effect (OKY-046 vs. vehicle = 9.83 ± 1.64 vs. 10.0 ± 2.61).

To elucidate the relationship between TxA₂ and tachykinin, the effect of FK-224 after pretreatment with STA-2 was examined. FK-224 significantly inhibited an increase of cough frequency induced by pretreatment with STA-2 in normal (5.83 ± 0.83 vs. 22.17 ± 5.05, P < 0.01) and sensitized animals (2.83 ± 0.87 vs. 31.0 ± 3.0, P < 0.01) (Fig. 3).

Level of TxB₂ in airway lavage fluid. To examine whether production of TxA₂ was augmented in sensitized- and repeatedly challenged animals, levels (pg/ml) of TxB₂ in airway lavage fluid were assayed. Immuno-reactive TxB₂ in the fluid of the repeatedly challenged group was significantly greater than that of the normal (874.40 ± 111.62 vs. 490.64 ± 88.90, P < 0.05) and sensitized groups (874.40 ± 111.62 vs. 571.04 ± 88.78, P < 0.05) (Fig. 4).

Fig. 1. Effect of STA-2 (solid bars) on capsaicin (10⁻⁵ M, 10 min)-induced coughing in normal, sensitized, and repeatedly challenged guinea pigs. Inhalation of STA-2 (3 × 10⁻² M, 5 min) was performed 30 min before capsaicin exposure. Open bars, vehicle. Values are means ± SE; n = 6 in each group. *P < 0.05 and **P < 0.01 vs. corresponding vehicle. †P < 0.05 and ††P < 0.01 vs. corresponding vehicle normal group.
Immunohistochemical localization of TxA S in the airway. The result of Western blot analysis demonstrated the specificity of anti-human TxA S antibody for guinea pigs. A single band of ~58 kDa was observed (Fig. 5).

PAS staining showed that the subtracheal gland was stained darkly (Fig. 6a), and mucous cells markedly increased in multiply challenged animals compared with normal animals (data not shown). Immunohistochemical analysis of TxA S showed that subtracheal gland and epithelial cells of the trachea were partly stained (Fig. 6, b–d). Only scattered staining was observed in normal animals. In contrast, prominent staining was seen in multiply challenged guinea pigs. No immunoreactivity was detected with nonimmune mouse serum in any group. Immunoelectron microscopic analysis revealed that immunoreactive TxA S was present in the cytosol of epithelial goblet cells, especially around secretion granules (Fig. 7).

Nucleotide sequence of a TxA S cDNA fragment from guinea pig lung. The nucleotide sequence of a 514-bp TxA S cDNA fragment of guinea pig lung was elucidated (Fig. 8). It is highly homologous to human TxA S (83.3% identity) (30), mouse TxA S (80.0% identity) (45), and rat TxA S (80.5% identity) (36). The obtained clone, therefore, was considered to encode a part of the guinea pig TxA S.

Expression of TxA S mRNA. TxA S mRNA levels of tracheal epithelial layers in normal, sensitized, and multiply challenged guinea pigs are shown in Fig. 9. Only one band with a size of ~2.1 kb was observed. TxA S mRNA expression in multiply challenged animals was markedly greater than in normal and sensitized animals. In contrast, TxA S expressions of the lung parenchyma and the whole tracheal membrane portion did not show any differences among the three groups (data not show). These data indicate that the main site of expression of TxA S mRNA is the epithelial layer in the airway.

DISCUSSION

In the present study, treatment with TxA S inhibitor OKY-046 and thromboxane-receptor antagonist AA-2414 prevented cough responses, which were shown to increase as a result of repeated immunochallenges. STA-2, a TxA 2 mimic, potentiated cough responses in normal and immunosensitized animals but not in repeatedly challenged ones. These results indicate that TxA 2 could facilitate coughing.
Coughing can be induced by the stimulation of sensory nerves in the airway (23). There are two types of cough receptors in the mucosa of the larynx and tracheobronchial tree: myelinated rapidly adapting receptors (RARs) and nonmyelinated C fiber receptors (3, 4, 5, 21, 42). Capsaicin, a powerful tussigenic agent in humans and other species, can act on those cough receptors with the release of tachykinins from the latter, further stimulating RARs and causing a cough response (41). We thought TxA2 might also induce cough responses because Karla et al. (22) reported that TxA2 could stimulate pulmonary C fiber endings predominantly and directly. However, our data show that inhalation of STA-2 enhanced capsaicin-induced cough (Fig. 1) but did not directly induce coughing. These data indicate that TxA2-facilitated cough responses are not associated with the direct stimulation of C fiber receptors.

We previously reported that a neurokinin 1,2-receptor antagonist FK-224 could significantly inhibit cough responses induced by capsaicin in antigen-sensitized and repeatedly challenged guinea pigs. It demonstrated that the side of action for FK-224 is in the airway and that observed cough responses are mediated by tachykinins released as a result of capsaicin exposure (44). In this study, we examined the relationship between FK-224 and TxA2 in cough responses. We observed that FK-224 significantly inhibited cough responses potentiated by STA-2 (Fig. 3). These observations confirm that tachykinins are essential in the development of a cough response to capsaicin inhalation. It seems likely that TxA2 might upregulate the sensitivity by which tachykinins could stimulate RARs and, therefore, facilitate cough responses. We found no mention of TxA2 increasing the sensitivity of tachykinins to nerve receptors. Further study is necessary to determine this hypothesis.

In this study, we investigated the actual condition of TxA2 in the airway of three groups. Concentration of TxB2 was greater in the repeatedly challenged group than in the normal and sensitized groups (Fig. 4). Our data are in agreement with results obtained in studies of asthmatic subjects by others (26, 31, 33, 40) who have shown that allergen exposure or exercise caused an increment in levels of TxA2 metabolites in plasma and urine and bronchoalveolar lavage fluid. In addition, our Northern blot analysis revealed a high expression of Txs mRNA in tracheal epithelial cells in repeatedly challenged animals compared with that in normal...
and sensitized animals (Fig. 9). Moreover, immunohistochemical study showed prominent staining in an increased number of mucus cells, such as goblet and bronchial gland cells of the epithelium, in repeatedly challenged animals (Fig. 6). Our in vivo data, which indicate that exogenous TxA2 potentiated cough responses in normal and immunosensitized guinea pigs but not in repeatedly challenged ones (Fig. 1), also demonstrates the increase of endogenous TxA2 in airway inflammation.

It has recently been reported that a TxS inhibitor suppressed coughing induced by angiotensin-converting enzyme inhibitors and that the cough threshold was increased by the treatment of OKY-046 in asthmatics (14, 38). In the present study, OKY-046 effectively decreased the number of coughs induced by capsaicin only in repeatedly challenged guinea pigs (Fig. 2). However, pretreatment with AA-2414 brought a suppressed effect on the number of coughs not only in repeatedly challenged animals but also in sensitized ones. OKY-046 is a compound that has a highly selective effect on the inhibition of TxS. In contrast, TxA2 is thought to bind a thromboxane-PG (TP) receptor (10) of which the ligands are not only TxA2 but also PGD2, PGF2α, PGE2, and PGI2 receptors (7, 9, 10). Therefore, AA-2414 could act as an inhibitor of prostanoids other than TxA2. These prostanoids are mediated by TP receptors in the human airway (8, 25). PGD2 and PGF2α have also been used as tussive agents (15). Those prostanoids could elicit coughing by stimulation of bronchial C fibers (11, 12), probably via a TP receptor.

The production site of TxA2 has been extensively examined in this study. It has been reported that TxA2 is produced from such cells as platelets, monocytes, macrophages, neutrophils, lung fibroblasts, and tracheal epithelial cells (16–18, 28, 43). However, the precise source of TxA2 in pathological conditions such as asthma has not yet been elucidated. Our data indicate that the production site of TxA2 in the airway was in the epithelial mucous cells (Fig. 7) and that repeated immunochallenges increased the number of those cells in the epithelium, thereby augmenting production of TxA2. Coughing is one of the major symptoms in patients with asthma and cough-variant asthma. Pathogenesis of asthma has recently been recognized to be a chronic airway inflammation. One of the causative factors of airflow limitation in asthma has been suggested to be airway hypersecretion produced by goblet cells and submucous glands (34). As a result of inflammation, goblet cells react to repeated exposure to antigens by increasing in number and bringing about hypersecretion into the airway, contributing to airflow limitation (1, 32, 34). Moreover, our data indicate that the augmented mucous cells were accompanied by the induction of TxS and elevation in airway thromboxane levels, which might explain why asthmatic subjects often experience chronic coughing. Nonadrenergic noncholinergic (NANC) nerves are distributed throughout the epithelium, innervating goblet cells and bronchial glands, and substance P, as a major neurotransmitter of NANC nerves, is one of the potent factors in mucous secretion (24). Further study is needed to clarify the
relationships between mucous granules, TxA2, and NANC nerves.

In conclusion, we investigated antigen inflammation enhancing TxB2 expression in the airway and how this is linked to increased capsaicin-induced cough responses. In addition, we demonstrated that mucous cells within the airway are potentially an important source of TxA2 in this model of allergen-induced cough.

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