

Inhibition of spleen tyrosine kinase attenuates IgE-mediated airway contraction and mediator release in human precision cut lung slices

Cynthia J. Koziol-White, PhD^{1*}, Yanlin Jia, PhD^{2*}, Gretchen A. Baltus², Philip R. Cooper, PhD¹, Dennis M. Zaller, PhD², Michael A. Crackower, PhD², Erich E. Sirkowski², Steven Smock², Alan B. Northrup², Blanca E. Himes³, Stephen E. Alves, PhD^{2**}, Reynold A. Panettieri, Jr., MD^{**1}

¹Rutgers Institute for Translational Medicine and Science, Child Health Institute, Rutgers University, New Brunswick, NJ; ²Merck Research Laboratories, Boston, MA; ³Department of Biostatistics and Epidemiology, University of Pennsylvania, Philadelphia, PA

Correspondence: Reynold A. Panettieri, Jr., Rutgers University, 89 French Street, Room 4210, New Brunswick, NJ, Phone: 732-235-6404, Fax: 732-235-7178, E-Mail: rp856@ca.rutgers.edu

*Contributed equally as first author

**Contributed equally as senior author

Source of Funding: Merck Research Laboratories

Background and purpose: Asthma manifests as a heterogeneous syndrome characterized by airway obstruction, inflammation and hyper-reactivity (AHR). Spleen tyrosine kinase (Syk) mediates allergen-induced mast cell degranulation, a central component of allergen-induced inflammation and AHR. However, the role of Syk in IgE-mediated constriction of human small airways remains unknown. In this study, we addressed whether selective inhibition of Syk attenuates IgE-mediated constriction and mast cell mediator release in human small airways.

Experimental Approach: Human precision cut lung slices (hPCLS) *ex vivo* derived from non-asthmatic donors were incubated overnight with human IgE, dexamethasone, montelukast, antihistamines or a selective Syk inhibitor (SYKi). FcεRI activation by anti-IgE cross-linking was performed, and constriction and mediator release measured. Airway constriction was normalized to that induced by maximal carbachol stimulation. Syk expression (determined by qPCR and immunoblot) was also evaluated in human primary airway smooth muscle (HASM) cells to determine whether Syk directly modulates HASM function.

Key Results: While dexamethasone had little effect on FcεR-mediated contraction, montelukast or antihistamines partially attenuated the response. SYKi completely abrogated anti-IgE-mediated contraction and suppressed the release of mast cell or basophil mediators from the IgE-treated hPCLS. In contrast, SYKi had little effect on the non-allergic contraction induced by carbachol. Syk mRNA and protein were undetectable in HASM cells.

Conclusions and Implications: Our data demonstrate that a selective Syk inhibitor, but not corticosteroids, abrogated FcεR-mediated contraction in *ex vivo* human small airways. The mechanism involves FcεRI receptor activation on mast cells or basophils that degranulate causing airway constriction, rather than direct actions on HASM.

Abbreviations: hPCLS, human precision cut lung slices; HASM, human airway smooth muscle; Syk, spleen tyrosine kinase; AHR, airway hyperresponsiveness; CysLT, cysteinyl leukotrienes; SYKi, syk inhibitor

TARGETS		
Enzymes	Nuclear Hormone Receptors	GPCRs
Syk	<u>Glucocorticoid Receptor</u>	<u>CysLT1 Receptor</u>
		<u>H1 Receptor</u>

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/bph.13550

LIGANDS
Montelukast
Carbachol
Fexofenadine
Dexamethasone

INTRODUCTION

Asthma, a disorder characterized by airway inflammation and hyper-responsiveness, globally contributes to substantial morbidity and mortality (Vijverberg *et al.*, 2013). Mast cells play a pivotal role in modulation of airway inflammation and bronchoconstriction in allergic asthma (Reuter *et al.*, 2010). Current therapeutics directed towards the actions of mast cells includes anti-IgE therapy, histamine and leukotriene receptor antagonists. Whether corticosteroids directly modulate human mast cell function remains controversial (Croxtall *et al.*, 2000; Liu *et al.*, 2007; Zhou *et al.*, 2008). Although the aforementioned therapeutic approaches are effective in asthma, heterogeneity of response, cost and adverse effects limit effectiveness. Evidence suggests that mast cells may also play an important role in promoting a severe persistent asthma phenotype since omalizumab, a monoclonal antibody that binds selectively to IgE to mitigate mast cell and basophil activation, improves clinical outcomes in these patients. Collectively, targeting the mast cell and basophils through novel signaling pathways and the use of small molecule inhibitors may offer a unique therapeutic advantage in improving asthma management.

Spleen tyrosine kinase (Syk), a member of the ZAP70 family of non-receptor protein kinases, mediates downstream signaling of immunoreceptor tyrosine-based activation motif (ITAM)-associated receptors (Mocsai *et al.*, 2010). ITAM-associated receptors, predominantly found on hematopoietic cells, modulate innate immunity, co-activation of inflammatory cells and effector/adaptive immune responses. Evidence suggests that cross-linking at the high affinity IgE receptor (FcεR1) activates Syk and that Syk is necessary and sufficient to mediate mast cell and basophil activation and *de novo* synthesis of eicosanoids, chemokines and cytokines (Gilfillan *et al.*, 2006).

Inhibition of Syk attenuates FcεR-mediated mast cell degranulation and allergen-induced airway inflammation (Matsubara *et al.*, 2006b). The Syk inhibitor R406 inhibits allergic airway responses in mice sensitized to ovalbumin (Matsubara *et al.*, 2006a; Matsubara *et al.*, 2006b). Further, antisense oligonucleotides, and, more recently, siRNA also attenuated allergen-induced rodent responses (Stenton *et al.*, 2002; Huang *et al.*, 2013). Our data in rats and non-human primates show that inhibition of Syk abrogated FcεR-mediated tracheal extravasation, allergen-induced airway inflammatory cell trafficking and bronchoconstriction (Moy *et al.*, 2013). Given species difference in mast cell function and in allergen responses, the role of Syk in modulating human small airway function remains unclear. Using a small molecule inhibitor for Syk (Moy *et al.*, 2013), we investigated whether Syk inhibition modulated Fcε-dependent airway constriction and mediator release in hPCLS. Here, we demonstrate that Syk inhibition abrogates FcεR-mediated contraction while profoundly decreasing mediator release. These data suggest that Syk inhibition may modulate human small airway function in asthma.

METHODS

Isolation and culture of human airway smooth muscle

Human ASM cells were derived from tracheas obtained from the National Disease Research Interchange (NDRI) (Philadelphia, PA) and from the International Institute for the Advancement of Medicine (IIAM) (Edison, NJ). Human ASM cell culture was performed as described previously (Panettieri *et al.*, 1989). The cells were cultured in Ham's F-12 medium supplemented with 10% FBS, 100 U/ml penicillin, 0.1 mg/ml streptomycin, and 2.5 mg/ml amphotericin B, and this medium was replaced every 72 hours. Human ASM cells in subculture during passages 1–5 were used, because these cells retain the expression of native contractile protein, as demonstrated by immunocytochemical staining for smooth muscle actin and myosin (Panettieri *et al.*, 1989).

Generation of PCLS and airway constriction assays

Human precision cut lung slices (hPCLS) were prepared as previously described (Cooper *et al.*, 2009; Cooper *et al.*, 2011). Briefly, whole human lungs from non-asthmatic donors were dissected and inflated using 2% (wt/vol) low melting point agarose. Once the agarose set, the lobe was sectioned, and cores of 8-mm diameter were made. The cores that contained a small airway by visual inspection were sliced at a thickness of 250 μ m (Precisionary Instruments VF300 Vibratome, Greenville, NC) and collected in wells containing supplemented Ham's F-12 medium. Suitable airways ($\leq$ 1-mm diameter) on slices were selected on the basis of the following criteria: presence of a full smooth muscle wall, presence of beating cilia, and unshared muscle walls at airway branch points to eliminate possible counteracting contractile forces. Each slice contained $\sim$ 98% parenchyma tissue; hence, all airways situated on a slice had sufficient parenchymal tissue to impart basal tone. Adjacent slices containing contiguous segments of the same airway were paired and served as controls and were incubated at 37°C in a humidified air-CO₂ (95-5%) incubator. Sections were placed in fresh media every 2-3 hr during the remainder of *day 1* and all of *day 2* to remove agarose and endogenous substances released that variably confound the production of inflammatory mediators and/or alter airway tone (Cooper *et al.*, 2009; Cooper *et al.*, 2011). In some experiments, slices were pre-incubated with human IgE (4 μ g/ml) antibody for 18 hr and then incubated in the presence or absence of Syk inhibitor (SYKi, 10-1000 nM; (1S,4R)-4-hydroxy-2,2-dimethyl-4-(5-((4-methylpyrimidin-2-yl)amino)phenyl)thiazol-2-yl)cyclohexane-1-carboxylic acid; Figure 2S) for 20 min prior to assessment of contraction. A time course was performed measuring luminal area every 30 sec for 10 min or until no further constriction was observed following the administration of anti-IgE (20 μ g/ml). Slices were incubated for an additional 20 minutes, and luminal area was assessed at 30 min following Fc ϵ R-cross-linking; and culture supernatants were assessed for histamine and cysteinyl leukotriene (CysLT) levels. To assess maximum contraction and assess specificity of the SYKi, carbamoyl choline (Cch - 100 μ M) was added and the percentage of airway occlusion measured and compared to baseline.

To assess luminal area, lung slices were placed in a 12-well plate in media and held in place using a platinum weight with nylon attachments. The airway was located using a microscope (Nikon Eclipse; model no. TE2000-U; magnification, $\times$ 40) connected to a live video feed (Evolution QEi; model no. 32-0074A-130 video recorder). Airway luminal area was measured using Image-Pro Plus software (version 6.0; Media Cybernetics) and represented in units of square micrometers (Cooper *et al.*, 2009; Cooper *et al.*, 2011). After functional studies, the area of each airway at baseline and at the end of the time course or dose of agonist was calculated using Image-Pro Plus software. A maximum drug effect ($E_{\max}$) value for each airway was derived from a concentration-response curve.

Histamine and lipid analysis

Mediator release was evaluated in human lung slices according to Moy *et al.* (Moy *et al.*, 2013). The supernatant from each well was collected after the constriction recording. CysLT (Enzo Life Sciences, Farmingdale, NY) and histamine (Cisbio Assays, Bedford, MA) levels were evaluated by ELISA or HTRF, respectively.

Statistical analysis

Graph Pad Prism software was used to determine statistical significance evaluated by a paired Students *t*-test for two groups or analysis of variance (ANOVA) for multiple groups. *P* values of <0.05 were considered significant.

RESULTS

SYKi inhibits Fc ϵ R-dependent airway constriction in normal hPCLS

To address whether Syk inhibition modulates Fc ϵ R-dependent contraction, hPCLS were prepared from nine non-asthmatic donors. All subjects manifested no chronic illnesses, as shown in Table 1S (supplemental data).

We and others showed that in rodent models, Syk inhibition attenuates allergen-induced airway hyper-responsiveness (AHR) and inflammation (Moy *et al.*, 2013). Accordingly, using SYKi, we addressed whether inhibition of Syk modulates Fc ϵ R-dependent airway constriction in human

tissue. Syk inhibition attenuated FcεR-dependent contraction in hPCLS as shown in Fig 1. In a dose-dependent manner, SYKi markedly decreased FcεR-dependent contraction and nearly completely abrogated the response at 1 μM concentration. To address whether SYKi inhibition extinguished over time, experiments were performed to examine SYKi effects over 30 minutes. As shown in Fig 1B, SYKi (1 μM) sustained complete abrogation of FcεR-dependent contraction whereas the 300 nM concentration was less efficacious. At 100 nM, 300 nM and 1 μM, FcεR-induced airway constriction was also diminished as compared with that observed with 10 nM or no SYKi, as shown in Figure 1C. To characterize whether SYKi affected non-mast cell-dependent contraction, hPCLS were treated with SYKi and carbamoyl choline (Cch)-induced contraction was measured. As shown in Fig 1, panel D, SYKi had no effect on carbachol-induced contraction.

A SYKi, a CysLT inhibitor and an anti-histamine modulate FcεR-dependent airway constriction

To compare efficacy of SYKi, antihistamines and leukotriene modifiers on FcεR-dependent airway constriction, hPCLS were treated with SYKi, montelukast, fexofenadine or dexamethasone (Figure 2). The doses of the inhibitors chosen are those derived from previous studies that demonstrated maximum target inhibition with limited off-target effects (Moy *et al.*, 2013). SYKi (1 μM) markedly inhibited FcεR-dependent contraction. Fexofenadine and montelukast partially inhibited FcεR-induced contraction. The relative efficacy in the compounds inhibiting FcεR-dependent bronchoconstriction was represented as I_{max} (maximal inhibition) as shown in Fig 2, panel B. Montelukast, fexofenadine and SYKi significantly impaired FcεR-induced airway constriction whereas dexamethasone had little effect. Collectively, these data suggest that inhibition of Syk has a greater effect on FcεR-dependent contraction in human airways than treatment with montelukast, fexofenadine and dexamethasone. Since SYKi profoundly affected FcεR-dependent constriction, and since Syk and Lyn expression has been described in cultured HASM cells (Gounni *et al.*, 2005; Redhu *et al.*, 2009; Redhu *et al.*, 2011; Balhara *et al.*, 2014), we next addressed whether Syk inhibition may directly modulate HASM cell contraction. Accordingly, we examined Syk expression in HASM cells and human mast cells. Total mRNA was prepared from cultured HASM cells and Ramos cells (B lymphocyte cell line), and quantitative rtPCR was performed. Lysates were also generated from these cells following treatment with control buffer, TNFα, IFNγ, or TNFα/IFNγ for immunoblot analysis. As shown in Fig 1S, human Ramos cells robustly express Syk total mRNA (1A) and protein (1B) whereas HASM cells showed no expression at either the transcriptional or translational level. These data are consistent with our observations that SYKi has little effect on carbachol-induced contraction and that the effects of SYKi as demonstrated in hPCLS are likely mediated through mast cells or basophils found in the submucosa of the hPCLS.

SYKi effects on FcεR-dependent histamine and CysLT secretion

To characterize whether SYKi modulates FcεR-dependent release of histamine and CysLT, slices were incubated with varying concentrations of SYKi. As shown in Fig 3A/B, pretreatment with SYKi profoundly decreased histamine levels in the supernatant after FcεR cross-linking. We also examined CysLT release following FcεR crosslinking. In Figure 3C, we show that SYKi pretreatment dose-dependently decreases CysLT release from hPCLS, where montelukast treatment does not significantly affect FcεR-dependent release of CysLT in hPCLS. These data suggest that SYKi that completely abrogates FcεR-dependent airway constriction also at similar doses markedly diminishes CysLT and histamine levels that are likely due to mast cell degranulation.

DISCUSSION

Allergic asthma, a chronic respiratory disorder marked by inflammation, recurrent reversible airflow obstruction, and airway hyper-responsiveness, remains a significant cause of morbidity and mortality worldwide. Despite the use of corticosteroids, long-acting bronchodilators, and leukotriene modifiers, there exists an unmet need to abrogate mast cell function in the allergic subset of this disease. Syk, a kinase that mediates much of the signaling downstream of the high affinity FcεRI, plays a key role in activation and degranulation of mast cells and basophils. Some small molecule kinase inhibitors have been developed to inhibit this kinase, showing efficacy in human trials of allergic rhinitis. Few studies have addressed the value of these therapeutics in allergic asthma. The

current study demonstrates specificity of SYKi in attenuation of FcεR crosslinking-induced bronchoconstriction and release of inflammatory mediators from hPCLS. We also show data to suggest the target of SYKi is most likely mast cells and basophils rather than HASM, as indicated by expression patterns in the primary human cells we tested.

Current therapeutics targeting mast cells or products of mast cell activation/degranulation are effective in some, but not all patients, or have been contraindicated as treatment for asthma. In the past, second generation antihistamines showed symptom scores and β_2 agonist use improved versus placebo in asthma. However, the therapeutic doses that were most effective were associated with adverse effects including fatigue and drowsiness (Grant *et al.*, 1995; Malick *et al.*, 1997; Larsen, 2001). Therefore, use of antihistamines is not considered a first line treatment for asthma. We utilized fexofenadine, a second generation antihistamine, as a comparator to partially attenuate FcεR crosslinking-induced airway constriction that provided findings consistent with our previous work (Cooper *et al.*, 2009; Cooper *et al.*, 2011). Use of montelukast, a leukotriene receptor antagonist, is effective in a subset of patients with asthma. A recent review of pediatric clinical studies (Massingham *et al.*, 2014) showed that montelukast, compared with inhaled corticosteroids (ICS), showed comparable improvement in asthma symptom scores but that improvement in pulmonary function tests favored ICS treatment over montelukast, and the risk of asthma exacerbation was greater with montelukast treatment group. Most studies concluded that first line treatment of asthma in a pediatric patient population should be ICS, but that in a subset of that population montelukast treatment may be effective, as outlined in the Expert Panel Report 3 of the Guidelines for the Diagnosis and Management of Asthma. Given the heterogeneity of the response of asthma patients to leukotriene modifiers, a therapy with broader effects may be more useful in the treatment of allergic asthma. Again we used montelukast as an industry standard in a model of FcεR crosslinking-induced contraction. We found that montelukast partially attenuated airway constriction to FcεR crosslinking that was not as effective as Syk inhibitor-induced ablation of contraction. Use of steroids in asthma therapy has been a mainstay for many years. Inhaled steroid therapy improves bronchodilator responses to β_2 agonists, to decrease inflammatory cell influx when used as maintenance therapy, and to modulate inflammatory mediator elaboration from airway cells (Chung *et al.*, 2009). Experimental evidence in human basophils suggests that glucocorticoid treatment of the cells prior to FcεR crosslinking attenuated not only IL-4 release but histamine release (Schleimer *et al.*, 1981; Schroeder *et al.*, 1997). A study using purified lung mast cells, however, dexamethasone has little effect on release of PGD₂, PGF_{2α}, PGE₂, leukotrienes, and histamine with anti-IgE treatment. Surprisingly, TxB₂ was the only mediator modulated by dexamethasone treatment prior to FcεR crosslinking (Schleimer *et al.*, 1983). We used dexamethasone to assess steroid effects on FcεR crosslinking-induced contraction and found that dexamethasone has little effect on this response. Accordingly, our data suggest that bronchoconstriction predominantly occurs with activation of mast cells and basophils that then release of an array of contractile agonists. Our data show that the Syk inhibitor inhibits not only FcεR crosslinking-induced contraction, but also release of leukotrienes and histamine. Syk inhibition provides a target to inhibit multiple allergen-triggered events, bronchoconstriction following crosslinking of FcεRI, elaboration of inflammatory mediators involved in modulation of contractile responses and perpetuation of inflammation associated with allergic asthma. Targeting Syk, a kinase proximal to the receptor, conveys therapeutic advantage over other approaches that are currently used in to alleviate symptoms of allergic asthma.

Given the inhibition that we observed in the hPCLS system, we sought to identify the potential cellular target of SYKi. Others showed that primary HASM express FcεRI, and upon incubation with IgE and subsequent stimulation with anti-IgE release IL-4, IL-5, IL-13, and eotaxin and that crosslinking of FcεRI evokes in intracellular calcium flux consistent with contraction of the muscle (Gounni 2005). In bronchial smooth muscle derived post-mortem from normal patients, attenuation of Syk expression with a lentiviral vector decreased promoter activity at the IL-8, IP-10, RANTES, and eotaxin promoters in response to FcεR crosslinking (Redhu *et al.*, 2009). To assess whether HASM are a potential target for SYKi, we examined Syk expression at both the mRNA and protein levels in primary HASM. In contrast to the findings from Redhu *et al.*, we failed to show any basal levels or TNFα-stimulated expression of mRNA or protein for Syk. Ramos cells served as a positive control for both mRNA and protein expression. A potential reason for this discrepancy could

be the sources of HASM that we used compared to the previous studies. We, however, showed in primary human mast cells expression of Syk (Moy *et al.*, 2013), suggesting that the inhibition of airway constriction that is observed following treatment of the tissue with SYKi is likely due to activation of mast cells and not a direct effect on HASM. Additionally, Wang *et al.* (Wang *et al.*, 2015) demonstrated that genetic ablation of Syk attenuated airway hyper-responsiveness following ovalbumin challenge and exposure to PM_{2.5} and ozone, but had little to no effect on methacholine-induced contractility of the lungs *in vivo* and precision cut lung slices. We demonstrate that SYKi has little effect on methacholine-, histamine-, and KCl-induced bronchoconstriction in PCLS (Figure 3S A & B, KCl – data not shown). Additionally, we show that SYKi has little effect on C5a peptide-induced airway constriction (Figure 3S C). Together, these data suggest a selective effect of SYKi on FcεRI crosslinking-induced contraction.

The hPCLS system that we utilized for the functional studies examining FcεR crosslinking-induced airway constriction has made use of a passive sensitization paradigm of FcεR sensitization (Cooper *et al.*, 2009; Cooper *et al.*, 2011). Despite limitations of this model, the hPCLS are a novel model of integrated structural cell function, devoid of trafficking leukocytes that likely contribute to the airway inflammatory response. Given this limitation, the activation of contraction in response to this stimulation is dependent upon resident immune cells, namely mast cells. Despite the dose- and time-dependent inhibition of Syk in this system, the specific effects of SYKi-mediated Syk inhibition on mast cell function are difficult to assess in this model.

Our data support Syk inhibition as a novel therapeutic target to prevent IgE-FcεRI-mediated airway constriction and mediator release from some of the cells in the airways. Further studies are needed to determine whether Syk inhibition offers therapeutic advantages over current state-of-the-art therapy in asthma.

FIGURE LEGENDS

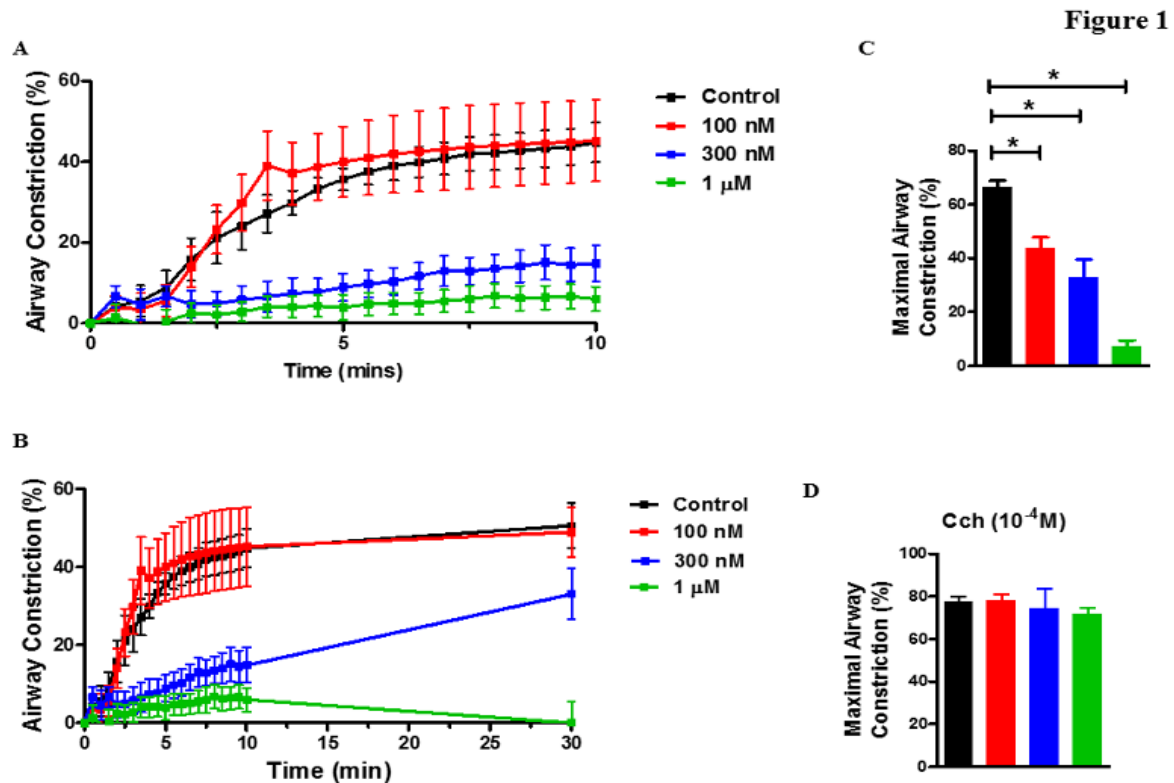


Figure 1 – SYKi attenuates Fc ϵ R crosslinking-induced airway constriction but does not attenuate cholinergic mediated contraction in hPCLS. (A) Representative time course of airway constriction to Fc ϵ R crosslinking assessed in the absence/presence of SYKi (0.1, 0.3, 1 μ M) over a course of 10 min. Constriction was measured compared to baseline luminal area and expressed as % airway constriction. (B) Representative plot of inhibition measured out to 30 min following crosslinking. (C) Maximal constriction following Fc ϵ R crosslinking was compared. (D) Following crosslinking, hPCLS were stimulated with carbamoyl choline (100 μ M) and % airway constriction measured. Data are representative of 9 individual donors, with bars representing mean + SEM with * $p \leq 0.05$.

Figure 2

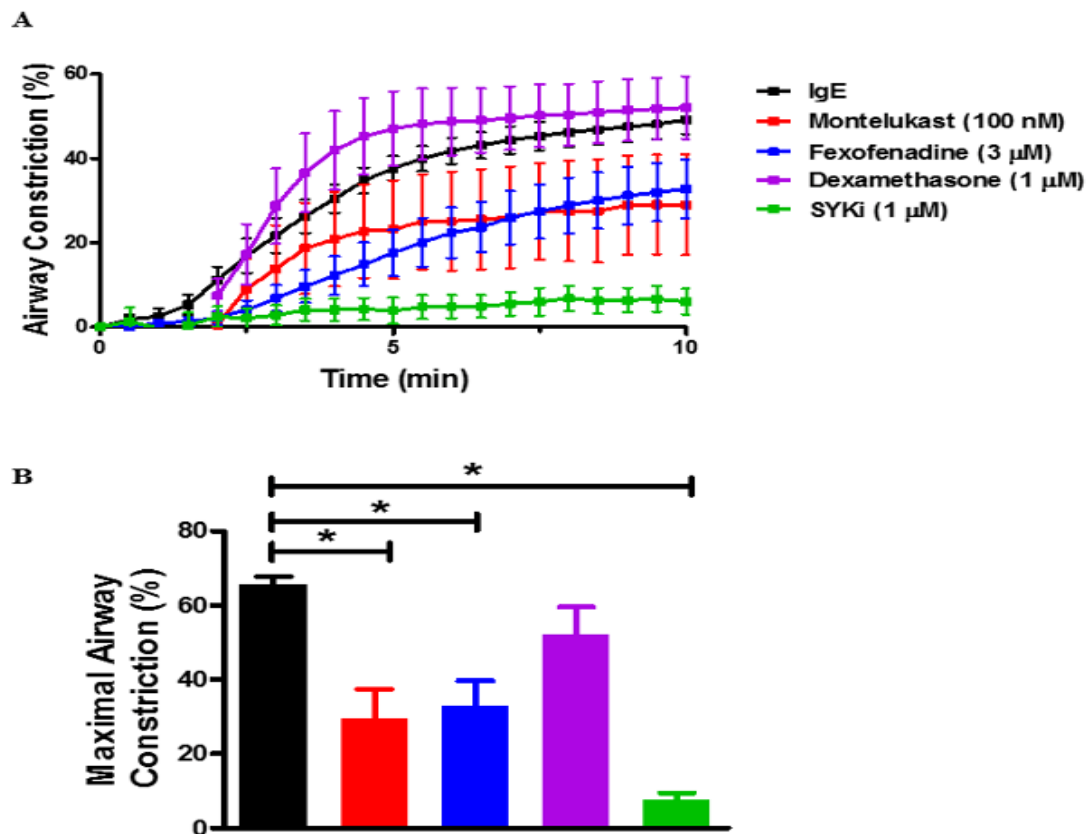


Figure 2 – SYKi markedly attenuates Fc ϵ R crosslinking-induced airway constriction in comparison with fexofenadine, montelukast, and dexamethasone. (A) The effects of montelukast (10 μ M), fexofenadine (3 μ M), and dexamethasone (1 μ M) were compared with that of SYKi (1 μ M) in four donors. (B) Maximal response following Fc ϵ R crosslinking in the presence of inhibitors was compared (I_{max}). Data are representative of 5-8 individual donors, with bars representing mean $\pm$ SEM with * p <0.05 (ANOVA p <0.0001).

Figure 3

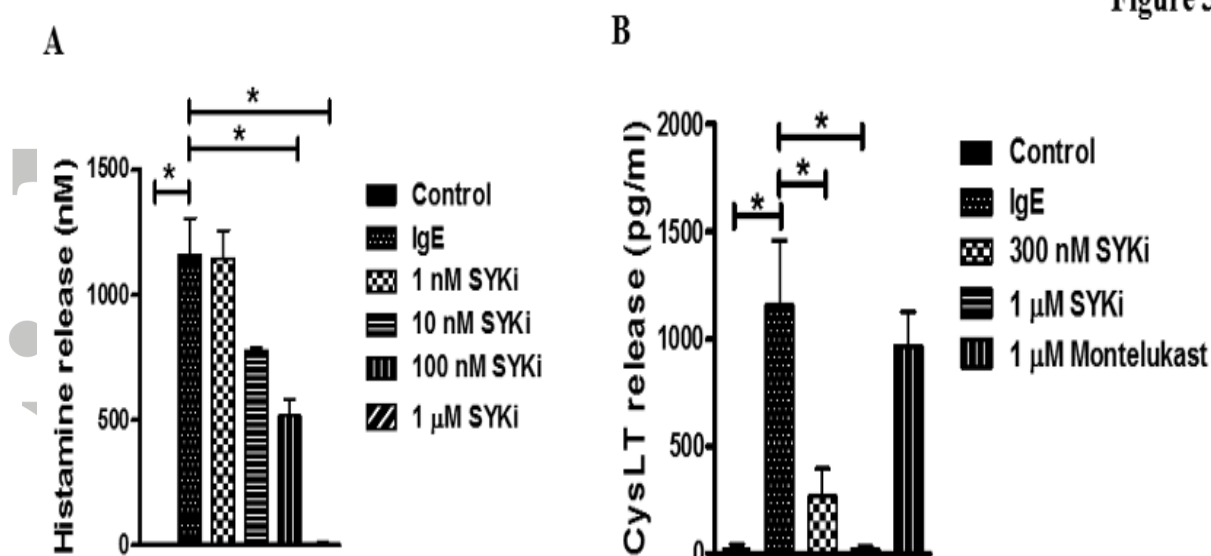


Figure 3 – Syk inhibitor attenuates histamine and leukotriene secretion in hPCLS. (A) SYKi (1 nM-1 μM) or montelukast (1 μM) were incubated with hPCLS 1 hr prior to FcεR crosslinking. Supernatants were collected at 30 minutes after crosslinking, and histamine release measured by HTRF (ANOVA $p=0.007$). (B) Leukotriene release in the presence/absence of SYKi (300 nM, 1 μM) or montelukast (1 μM) was measured by ELISA (ANOVA $p=0.0002$). Data represent lung slices from four to seven donors for each treatment. * $p<0.05$

REFERENCES

Balhara J, Redhu NS, Shan L, Gounni AS (2014). IgE regulates the expression of smMLCK in human airway smooth muscle cells. *PloS one* 9: e93946.

Chung KF, Caramori G, Adcock IM (2009). Inhaled corticosteroids as combination therapy with beta-adrenergic agonists in airways disease: present and future. *European journal of clinical pharmacology* 65: 853-871.

Cooper PR, Zhang J, Damera G, Hoshi T, Zopf DA, Panettieri RA, Jr. (2011). C-027 inhibits IgE-mediated passive sensitization bronchoconstriction and acts as a histamine and serotonin antagonist in human airways. *Allergy Asthma Proc* 32: 359-365.

Cooper PR, Lamb R, Day ND, Branigan PJ, Kajekar R, San Mateo L, *et al.* (2009). TLR3 activation stimulates cytokine secretion without altering agonist-induced human small airway contraction or relaxation. *American journal of physiology* 297: L530-537.

Croxtall JD, Choudhury Q, Flower RJ (2000). Glucocorticoids act within minutes to inhibit recruitment of signalling factors to activated EGF receptors through a receptor-dependent, transcription-independent mechanism. *British journal of pharmacology* 130: 289-298.

Gilfillan AM, Tkaczyk C (2006). Integrated signalling pathways for mast-cell activation. *Nat Rev Immunol* 6: 218-230.

Gounni AS, Wellemans V, Yang J, Bellesort F, Kassiri K, Gangloff S, *et al.* (2005). Human airway smooth muscle cells express the high affinity receptor for IgE (Fc epsilon RI): a critical role of Fc epsilon RI in human airway smooth muscle cell function. *J Immunol* 175: 2613-2621.

Grant JA, Nicodemus CF, Findlay SR, Glovsky MM, Grossman J, Kaiser H, *et al.* (1995). Cetirizine in patients with seasonal rhinitis and concomitant asthma: prospective, randomized, placebo-controlled trial. *The Journal of allergy and clinical immunology* 95: 923-932.

Huang ZY, Kim MK, Kim-Han TH, Indik ZK, Schreiber AD (2013). Effect of locally administered Syk siRNA on allergen-induced arthritis and asthma. *Mol Immunol* 53: 52-59.

Larsen JS (2001). Do antihistamines have a role in asthma therapy? *Pharmacotherapy* 21: 28S-33S.

Liu C, Zhou J, Zhang LD, Wang YX, Kang ZM, Chen YZ, *et al.* (2007). Rapid inhibitory effect of corticosterone on histamine release from rat peritoneal mast cells. *Hormone and metabolic research = Hormon- und Stoffwechselforschung = Hormones et metabolisme* 39: 273-277.

Malick A, Grant JA (1997). Antihistamines in the treatment of asthma. *Allergy* 52: 55-66.

Massingham K, Fox S, Smaldone A (2014). Asthma therapy in pediatric patients: a systematic review of treatment with montelukast versus inhaled corticosteroids. *J Pediatr Health Care* 28: 51-62.

Matsubara S, Koya T, Takeda K, Joetham A, Miyahara N, Pine P, *et al.* (2006a). Syk activation in dendritic cells is essential for airway hyperresponsiveness and inflammation. *American journal of respiratory cell and molecular biology* 34: 426-433.

Matsubara S, Li G, Takeda K, Loader JE, Pine P, Masuda ES, *et al.* (2006b). Inhibition of spleen tyrosine kinase prevents mast cell activation and airway hyperresponsiveness. *Am J Respir Crit Care Med* 173: 56-63.

Mocsai A, Ruland J, Tybulewicz VL (2010). The SYK tyrosine kinase: a crucial player in diverse biological functions. *Nat Rev Immunol* 10: 387-402.

Moy LY, Jia Y, Caniga M, Lieber G, Gil M, Fernandez X, *et al.* (2013). Inhibition of spleen tyrosine kinase attenuates allergen-mediated airway constriction. *American journal of respiratory cell and molecular biology* 49: 1085-1092.

Panettieri RA, Murray RK, DePalo LR, Yadvish PA, Kotlikoff MI (1989). A human airway smooth muscle cell line that retains physiological responsiveness. *Am J Physiol* 256: C329-335.

Redhu NS, Saleh A, Lee HC, Halayko AJ, Ziegler SF, Gounni AS (2011). IgE induces transcriptional regulation of thymic stromal lymphopoietin in human airway smooth muscle cells. *The Journal of allergy and clinical immunology* 128: 892-896 e892.

Redhu NS, Saleh A, Shan L, Gerthoffer WT, Kung SK, Halayko AJ, *et al.* (2009). Proinflammatory and Th2 cytokines regulate the high affinity IgE receptor (FcεRI) and IgE-dependent activation of human airway smooth muscle cells. *PloS one* 4: e6153.

Reuter S, Stassen M, Taube C (2010). Mast cells in allergic asthma and beyond. *Yonsei Med J* 51: 797-807.

Schleimer RP, Lichtenstein LM, Gillespie E (1981). Inhibition of basophil histamine release by anti-inflammatory steroids. *Nature* 292: 454-455.

Schleimer RP, Schulman ES, MacGlashan DW, Jr., Peters SP, Hayes EC, Adams GK, 3rd, *et al.* (1983). Effects of dexamethasone on mediator release from human lung fragments and purified human lung mast cells. *The Journal of clinical investigation* 71: 1830-1835.

Schroeder JT, MacGlashan DW, Jr., MacDonald SM, Kagey-Sobotka A, Lichtenstein LM (1997). Regulation of IgE-dependent IL-4 generation by human basophils treated with glucocorticoids. *J Immunol* 158: 5448-5454.

Stenton GR, Ulanova M, Dery RE, Merani S, Kim MK, Gilchrist M, *et al.* (2002). Inhibition of allergic inflammation in the airways using aerosolized antisense to Syk kinase. *J Immunol* 169: 1028-1036.

Vijverberg SJ, Hilvering B, Raaijmakers JA, Lammers JW, Maitland-van der Zee AH, Koenderman L (2013). Clinical utility of asthma biomarkers: from bench to bedside. *Biologics* 7: 199-210.

Wang X, Khanna N, Wu J, Godri Pollitt K, Evans GJ, Chow CW, *et al.* (2015). Syk mediates airway contractility independent of leukocyte function. *Allergy* 70: 429-435.

Zhou J, Liu DF, Liu C, Kang ZM, Shen XH, Chen YZ, *et al.* (2008). Glucocorticoids inhibit degranulation of mast cells in allergic asthma via nongenomic mechanism. *Allergy* 63: 1177-1185.