SUPPLEMENTAL METHODS AND FIGURE LEGENDS

ASM RNA-Seq Experimental Methods

We obtained RNA-Seq results for transcripts of SYK corresponding to a previously published study [1]. Briefly, primary ASM cells were isolated from twelve white non-smoking donors with no chronic illness or medication use. ASM cell cultivation was described previously [2, 3]. Passages 4 to 7 ASM cells maintained in Ham's F12 medium supplemented with 10% FBS, $CaCl_2$, buffered with HEPES, penicillin/streptomycin, primocin, and additional L-glutamine were used in all experiments. The F12 medium was used for culture because it provides Ca^{2+} levels that are consistent with seeing contractility of muscles in that media. Total RNA was extracted from cells using the miRNAeasy mini kit (Qiagen Sciences, Inc., Germantown, MD). Approximately 1 μg of RNA from each sample was used to generate RNA-Seq cDNA libraries for sequencing using the TruSeq RNA Sample Prep Kit v2 (Illumina, Inc., San Diego, CA). Sequencing of 75 bp paired-end reads was performed with an Illumina HiSeq 2000 instrument at Partners Personalized Medicine (Boston, MA). Taffeta scripts (https://github.com/blancahimes/taffeta) were used to analyze RNA-Seq data, which included trimming of adapters using trimmomatic (v.0.32) [4] and using FastQC [5] (v.0.11.2) to obtain overall QC metrics. Trimmed reads for each sample were used to estimate transcript counts with Kallisto software and the hg38 human genome as reference [6]. The RNA-Seq data is available at the Gene Expression Omnibus Web site (http://www.ncbi.nlm.nih.gov/geo/) under accession GSE58434.

PCR and immunoblot analysis

Non-asthmatic HASM (passage 3-4) was stimulated overnight with TNFα (10 ng/ml), IFNγ (1000 U), or TNFα/IFNγ (Roche Life Sciences, Indianapolis, IN). Total RNA was purified from ASM cells and Ramos cells using the RNAeasy kit (Qiagen, Gaithersburg, MD) and 550 ng of RNA was reverse transcribed using iScript reverse transcriptase (Biorad, Hercules, CA). Quantitative transcript analysis was carried out using Taqman assays (Invitrogen, Grand Island, NY) for Syk (Hs00895384_m1), and GAPDH (Hs99999905_m1) on an ABI7900 quantitative PCR machine, and relative gene expression determined using ddCT analysis. Whole cell lysates from HASM and Ramos were generated using RIPA buffer. 70 μg of total protein was loaded into Criterion gels (Biorad). Gels were transferred to nitrocellulose via the iBlot system (Invitrogen), blocked, immunoblotted for Syk (Santa Cruz, Dallas, TX), and imaged and analyzed using the Odyssey Imager and software (LI-COR, Lincoln, NE).

Airway constriction assays

Slices were incubated in the presence or absence of Syk inhibitor (SYKi, 1 μM) for 20 min prior to airway constriction to C5a (1 μM), methacholine (10^{-8} – 10^{-4} M, inhibitor present at every dose), or histamine (10^{-8} – 10^{-4} M, inhibitor present at every dose).

References

1. Himes BE, Koziol-White C, Johnson M, Nikolos C, Jester W, Klanderman B, et al. Vitamin D Modulates Expression of the Airway Smooth Muscle Transcriptome in Fatal Asthma. PLoS One. 2015;10(7):e0134057. doi: 10.1371/journal.pone.0134057. PubMed PMID: 26207385; PubMed Central PMCID: PMC4514847.
2. Panettieri RA, Murray RK, DePalo LR, Yadvish PA, Kotlikoff MI. A human airway smooth muscle cell line that retains physiological responsiveness. Am J Physiol. 1989;256(2 Pt 1):C329-35. Epub 1989/02/01. PubMed PMID: 2645779.
3. Cooper PR, Mesaros AC, Zhang J, Christmas P, Stark CM, Douaidy K, et al. 20-HETE mediates ozone-induced, neutrophil-independent airway hyper-responsiveness in mice. PLoS One. 2010;5(4):e10235. Epub 2010/04/28. doi: 10.1371/journal.pone.0010235. PubMed PMID: 20422032.
4. Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics. 2014. Epub 2014/04/04. doi: 10.1093/bioinformatics/btu170. PubMed PMID: 24695404.
5. Andrews S. FastQC A Quality Control tool for High Throughput Sequence Data. Available from: http://www.bioinformatics.babraham.ac.uk/projects/fastqc/
6. Bray NL, Pimentel H, Melsted P, Pachter L. Near-optimal probabilistic RNA-seq quantification. Nat Biotechnol. 2016;34(5):525-7. doi: 10.1038/nbt.3519. PubMed PMID: 27043002.

Table 1S – Demographics for hPCLS donors.

Figure 1S – Syk is expressed in primary human mast cells but not in HASM. HASM were incubated in the presence or absence of TNFα (50 ng/ml), IFNγ (1000U), or the combination of TNFα and IFNγ. Both mRNA (exposure time – A) and protein (overnight - B) expression were assessed and compared to RAMOS cells, which are known to express high levels of Syk. (C) Expression of *syk* in HASM as assessed by RNAseq. Data are representative of 6 individual donors, with bars representing mean + SEM.

Figure 2S – Chemical structure and IUPAC name for SYKi.

Figure 3S – Effect of SYKi on (A) methacholine-, (B) histamine-, and (C) C5a-induced constriction of human small airways. Data in (A) and (B) are represented as % of KCl-induced airway constriction, where SYKi no effect on KCl-induced constriction.