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Phenotypic and genotypic association of epithelial *IL1RL1* to human T_H2-like asthma

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Abstract

Background—Severe asthma remains poorly characterized, although it likely consists of at least 1 phenotype with features of T_H2-like inflammation. *IL1RL1*, encoding both the IL-33 receptor, ST2L, and decoy receptor, sST2, has been genetically associated with asthma, though the mechanism for susceptibility remains unknown.

Objective—Given previous data supporting a role for *IL1RL1* in T_H2 inflammation, we hypothesized that ST2L expression might be increased in T_H2-like asthma and that expression levels would be associated with single nucleotide polymorphisms in *IL1RL1*, possibly explaining its genetic relationship with asthma. We also sought to evaluate the regulation of ST2L and sST2 *in vitro*.

Methods—Endobronchial brushings and biopsies were obtained and expression of ST2L compared by severity levels, as well as by T_H2-like biomarkers. Subjects were genotyped and the relationship of dichotomous expression of ST2L and sST2 to single nucleotide polymorphisms in *IL1RL1* were determined. Epithelial cells were grown in air-liquid interface culture, and ST2L and sST2 responses to IFN- γ and IL-13 were evaluated.

Results—ST2L expression was increased in severe asthma ($P = .02$) and associated with multiple indicators of T_H2-like inflammation, including blood eosinophils ($P = .001$), exhaled nitric oxide ($P = .003$), and epithelial CLCA1 ($P < .0001$) and eotaxin-3 ($P = .001$) mRNA expression. Multiple single nucleotide polymorphisms in *IL1RL1* were found in relation to dichotomous expression of both ST2L and sST2. sST2 expression was associated with IFN- γ expression in bronchoalveolar lavage, while inducing its expression *in vitro* in primary human epithelial cells.

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The rest of the authors declare that they have no relevant conflicts of interest.

Conclusion—Both pathologic and genetic approaches support a role for *IL1RL1* in severe asthma, as well as T_H2-like asthma, suggesting that targeting this pathway may have therapeutic benefits.

Keywords

Asthma; *IL1RL1*; ST2L; sST2; T_H2 inflammation; single nucleotide polymorphisms

Severe asthma affects about 10% of people with asthma but remains poorly understood, with few therapeutic options.^{1,2} Although recent clinical trials are confirming the relevance of type-2 cytokine-associated inflammation in a subset of human asthma, and severe asthma, it is likely that other immune pathways are also involved.

IL-33, an IL-1 family cytokine, and its receptor ST2L are increasingly implicated in type-2 cytokine-associated inflammation and asthma.^{3,4,5} *IL1RL1* encodes 2 major splice variants, 1 containing the transmembrane domain (ST2L) and a soluble form (sST2) that is excreted and acts as a decoy receptor for IL-33.^{6,7} The receptor is reported to be present on many cells types, including mouse T-lymphocytes and fibroblasts and human endothelial cells, epithelial cells, eosinophils, and mast cells.^{8–13} Importantly, polymorphisms in *IL1RL1* (and its ligand IL-33) have been identified in genome-wide association studies as associated with asthma, particularly that of childhood onset.^{14,15}

It is now apparent that asthma cases can be divided into those with and without evidence for a T_H2-like immune process, with increases in lung eosinophils, exhaled nitric oxide (F_{ENO}), and responses to corticosteroids associated with the presence of this T_H2-like process.^{16–19} However, the relationship of this T_H2-like inflammatory process to the IL-33 pathway and its genetics is still poorly understood.

We therefore hypothesized that epithelial ST2L would be increased in asthma, specifically in severe asthma, and in association with a T_H2-like phenotype and genetic polymorphisms. To examine these relationships, we evaluated the presence of ST2L in fresh epithelial brushings and biopsies from asthmatic and healthy control subjects and related this expression to markers of type-2 cytokine-associated inflammation, including eosinophils and F_{ENO} as well as epithelial calcium-activated chloride channel regulator 1 (CLCA1) and eotaxin-3 (CCL26). We also evaluated the relationship of single nucleotide polymorphisms (SNP) *IL1RL1* associated with epithelial ST2L mRNA and sST2 bronchoalveolar lavage (BAL) expression levels. Lastly, we examined the control of sST2/ST2L expression in cultured epithelial cells in relation to stimulation with type 1 (IFN- γ) and type 2 (IL-13) cytokines.

METHODS

Subjects

Participants aged 18 to 65 were enrolled in the Severe Asthma Research Program (SARP) and the Electrophilic Fatty Acid Derivatives in Asthma study. Further details can be found in this article's Online Repository at www.jacionline.org. In addition, Fig E1 (in this

article's Online Repository at www.jacionline.org) outlines our study design and analysis steps.

qRT-PCR

Epithelial cell RNA was extracted in Qiazol (Qiagen, Valencia, Calif), and ST2L mRNA expression was determined by quantitative real-time PCR (qRT-PCR). The levels of each marker were determined relative to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) using the comparative CT method. Additional details can be found in this article's Online Repository at www.jacionline.org.

Immunohistochemistry

Endobronchial biopsies were fixed in 10% neutral buffered formalin, dehydrated in 70% ethanol, embedded in paraffin, and 5 μ m sections prepared. The primary antibody used was a rabbit polyclonal anti-human ST2L antibody (Millipore, Billerica, Mass) at a 1:100 dilution. Further details on immunohistochemistry and quantification are described in detail in this article's Online Repository at www.jacionline.org.

Soluble ST2 ELISA

Soluble ST2 levels in BAL fluid were measured (Elisatech, Denver, Colo) using a human ST2 Quantikine ELISA kit that measures both free and IL-33 complexed sST2 according to the manufacturer's instructions (R&D Systems, Minneapolis, Minn).

In vitro effects of IFN- γ and IL-13

Primary human bronchial epithelial cells were cultured under air-liquid interface (ALI), as previously described.²⁰ From day 0 of ALI, cells were stimulated with IFN- γ (10 ng/mL) or IL-13 (10 ng/mL) every 48 hours. On day 8, cells and supernatants were harvested and analyzed for sST2 and ST2L expression.

SNP genotyping and association with ST2L and sST2 expression

Single nucleotide polymorphism genotyping was performed with the Illumina Human1M-Duo DNA BeadChip and data quality assessed using Beadstudio (Illumina, Inc, San Diego, Calif). Hardy-Weinberg equilibrium was tested for quality control.²¹ Genotyping was performed at the Wake Forest University Center for Human Genomics.

Statistical Analysis

Statistical analysis was performed using JMP software (SAS, Cary, NC) and is described further in this article's Online Repository at www.jacionline.org.

RESULTS

Demographics

Sixty participants with mild-to-moderate and severe asthma and 22 healthy controls underwent bronchoscopic airway brushing and biopsy (Table I). Age at enrollment was highest in those with severe asthma, as was body mass index (BMI), which was significant

overall ($P = .0002$) and amongst asthmatics ($P = .01$). There were overall differences in IgE, blood eosinophils, and F_{ENO} among the groups, though intergroup comparisons did not reveal significant differences between the asthmatic groups. Leukotriene receptor antagonist (LTRA) use was different among asthmatic participants ($P = .006$). As expected, use of anti-IgE therapy was only present in those with severe asthma. Eighty percent ($n = 28$) of severe asthmatic participants were on regular oral corticosteroids (OCS).

Epithelial ST2L mRNA and protein expression are highest in severe asthma

mRNA—Epithelial cell brushing ST2L mRNA levels differed among the 3 groups (overall $P = .02$; geometric mean = 0.05 [95% CI 0.02–0.12], 0.07 [95% CI 0.05–0.16], and 0.21 [95% CI 0.1–0.41] for healthy controls, mild-to-moderate, and severe asthmatic participants, respectively), with the highest levels in severe asthmatic participants, being significantly higher than those of the healthy controls ($P = .03$) (Fig 1). There was no significant difference between severe and mild-to-moderate asthmatic participants ($P = .11$) or healthy controls and mild-to-moderate asthmatic participants ($P = .84$). When dividing the mild/moderate asthmatic group into those on inhaled corticosteroids (ICS) ($n = 14$, relative ST2L mRNA expression, geometric mean = 0.08 [0.04–0.15]) and off ICS ($n = 11$, relative ST2L mRNA expression, geometric mean = 0.06 [0.04–0.11]), the overall difference in ST2L mRNA was no longer statistically significant ($P = .06$). There were no significant differences between these 2 groups (mild-to-moderate asthmatic participants on/off ICS; $P = .43$). In addition, ST2L mRNA levels did not differ significantly in those diagnosed with asthma at <12 years of age compared with those diagnosed at >12 years of age ($P = .43$). Soluble ST2 mRNA levels were present at extremely low levels and were not significantly different between asthmatic and control participants (total $n = 25$; $P = .6$; data not shown).

Because age at enrollment and BMI were significantly different among the groups, we examined the relationship between ST2L mRNA and these variables. There was no significant correlation between either age or BMI and ST2L mRNA levels ($r_s = 0.10$; $P = .38$ and $r_s = 0.13$; $P = .24$, respectively), supporting an absence of effect on ST2L levels.

ST2L mRNA levels were also evaluated in relationship to LTRA use, and among all subjects, ST2L mRNA was significantly higher among subjects using leukotriene receptor antagonists ($P = .04$; geometric mean 0.08 [95% CI 0.05–0.13] and 0.24 [95% CI 0.09–0.61] and for those off and on LTRAs.

Protein—ST2L immunostaining was performed and differed overall (Fig 2, A–D, $P = .0001$). Severe asthmatic participants (mean intensity 25.3 ± 1.5) had significantly more epithelial ST2L expression than mild/moderate asthmatic participants (mean intensity 14.7 ± 1.6 , $P = .0001$) and healthy controls (mean intensity 17.5 ± 1.7 , $P = .005$).

Because ST2L signaling would be expected to be regulated in part by soluble ST2 (by binding and neutralizing IL-33), BAL levels of sST2 were measured by ELISA. No significant differences were found in sST2 expression by asthma severity (Fig E2, in this article's Online Repository at www.jacionline.org) ($P = .61$), nor was there a significant correlation between sST2 levels in BAL and epithelial ST2L mRNA levels ($r_s = -0.039$; $P = .79$).

Epithelial ST2L mRNA associates with T_H2 markers (Table II)

Blood eosinophils—ST2L mRNA was increased in the eosinophil-high group ($P = .001$). In contrast, there was no significant association of ST2L mRNA to sputum or BAL eosinophils ($P = .21$ and $P = .45$, respectively).

Feno—ST2L mRNA was significantly increased in subjects with elevated F_{ENO} (>31.0 ppb; $P = .004$).

Eotaxin-3 (CCL26) and CLCA1 mRNA expression—Similar to blood eosinophils, epithelial ST2L mRNA was significantly higher in those with higher eotaxin-3 mRNA expression ($P = .001$) and CLCA1 mRNA ($P < .0001$).

Clinical relevance of epithelial ST2L mRNA

Baseline FEV₁ percent predicted did not correlate with ST2L mRNA in all participants ($r_s = -0.19$; $P = .09$). Among asthmatic participants, epithelial ST2L mRNA did not quite reach statistical significance with asthma symptoms in the last 4 weeks (increased frequency of wheezing [$P = .07$], cough [$P = .11$], shortness of breath [$P = .12$], sputum production [$P = .72$], chest tightness [$P = .43$], or nighttime symptoms [$P = .54$]). In contrast, ST2L mRNA was more strongly associated with exacerbations (Table III). ST2L mRNA was higher in participants with a history of an intensive care unit stay and/or intubation ($P = .03$), an emergency department visit or hospitalization for asthma in the last year ($P = .03$), or a need for 3 or more OCS bursts in the prior 12 months ($P = .03$) (Table III). ST2L mRNA did not differ by use of OCS ($P = .23$).

Using multiple markers of T_H2-like inflammation to develop a T_H2 index

A composite “T_H2-like score” incorporating the number of T_H2-associated factors elevated in each subject was developed. Seventy-five subjects had complete data available for F_{ENO}, blood eosinophil numbers, eotaxin-3, and CLCA1 mRNA. Subjects received a score of 0 or 1 for each biomarker, depending on whether they were in the lower (= 0) or upper (= 1) half (F_{ENO}, eotaxin-3, or CLCA1 mRNA) or <300 (= 0) or ≥ 300 (= 1) eosinophils/mm³, with a maximum score of 4. As expected, asthmatic participants had significantly higher T_H2-like scores than healthy controls ($P = .01$) and there was a tendency for a higher T_H2-like score with increasing asthma severity, though it did not reach statistical significance ($P = .052$) (Table E1, in this article’s Online Repository at www.jacionline.org).

There was a significantly progressive increase in ST2L mRNA with increasing T_H2-like score (Fig 3, all subjects $P < .0001$), suggesting a possible relationship between the amount or range of T_H2-like inflammation present and the potential for ST2L activation. sST2 BAL levels were not significantly associated with any of the T_H2 markers evaluated (data not shown).

Association of SNPs in IL1RL1 with ST2L and sST2 expression

IL1RL1 has been genetically associated with asthma in multiple studies, though the nature of this relationship is undefined. Using genotype data, the relationship of SNPs in *IL1RL1* to dichotomous ST2L mRNA and sST2 BAL protein expression (based on a median split into

HI and LO expression groups) was determined. Overall genotype and dominant and recessive models were evaluated. Significant associations are outlined in Table IV (ST2L mRNA) and Table V (sST2 BAL protein). Data on all SNPs evaluated are summarized in Table E2, in this article's Online Repository at www.jacionline.org. Three SNPs were significantly associated with ST2L mRNA expression: rs12712135, located in the first intron of ST2L mRNA; rs1041973, a missense amino acid change at position 78 in both ST2L and sST2; and rs10185897, located in an intron of ST2L mRNA. In contrast (and despite a smaller "n"), 10 SNPs were significantly associated with sST2 BAL levels, with some of those SNPs located only in ST2L mRNA.

Given the relationship of ST2L to markers of T_H2 inflammation, we also evaluated SNPs in *IL1RL1* in relation to CLCA1 and eotaxin-3 epithelial mRNA levels, blood eosinophils, and F_{ENO} (all divided by a median split) and T_H2 score. No SNPs in *IL1RL1* were associated with blood eosinophils, CLCA1, or eotaxin-3 mRNA levels (data not shown). Two SNPs in *IL1RL1* associated with F_{ENO} (rs7571371) and T_H2 score (rs12999517) (see Table E3 in this article's Online Repository at www.jacionline.org).

IFN- γ is associated with and stimulates sST2 expression

Given that ST2L expression was significantly associated with T_H2-like inflammation, and SNPs in *IL1RL1* were found to be significantly associated with both ST2L and sST2 expression, we sought to determine if the presence of T_H1- or T_H2-like cytokines regulated ST2L expression in human airway epithelial cells.

In addition to increasing T_H2 score with increasing asthma severity, our laboratory has previously reported increases in BAL cell IFN- γ mRNA in severe asthma. IFN- γ mRNA was similarly significantly increased in severe asthma in the participants included in this study (53 of whose levels have been previously reported) (see Fig E3 in this article's Online Repository at www.jacionline.org; $P = .02$).²² In asthmatic subjects, and in contrast to T_H2-like biomarkers, which significantly correlated with ST2L mRNA, BAL cell IFN- γ mRNA significantly correlated with BAL sST2 protein levels ($r_s = 0.38$; $P = .03$), though neither correlated significantly with epithelial ST2L mRNA (for IFN- γ , $r_s = 0.09$; $P = .55$; for sST2, $r_s = 0.17$; $P = .32$). IL-13 BAL mRNA did not correlate significantly with either epithelial ST2L mRNA ($r_s = 0.09$; $P = .71$) or sST2 BAL levels ($r_s = -0.3$; $P = .29$).

T_H1- but not T_H2-like stimuli impact sST2 expression in cultured human primary epithelial cells—To evaluate if IFN- γ affected sST2 or ST2L expression *in vitro*, airway epithelial cells in ALI were stimulated with IFN- γ (10 ng/mL). IFN- γ significantly increased sST2 expression after 8 days of stimulation, both at the mRNA and protein level (Fig 4, A and B), but had no significant effect on ST2L mRNA expression ($P = .26$; see Fig E4 in this article's Online Repository at www.jacionline.org). Using Cohen's *d* to measure effect size gave values of 0.8 for sST2 mRNA and 1.7 for sST2, both indicative of a large effect of IFN- γ on sST2 expression. In contrast, IL-13 (10 ng/mL) had no significant effect on epithelial ST2L or sST2 mRNA expression (see Fig E5 in this article's Online Repository at www.jacionline.org; $P = .61$ and $P = .75$, respectively).

DISCUSSION

This study identified increased expression of epithelial ST2L in patients with severe, T_H2-like asthma and found a relationship of dichotomous expression of ST2L and sST2 to SNPs in *IL1RL1*. As the relationship is strongest in subjects with the most evidence for T_H2-related disease, targeting the IL-33/ST2 axis for therapy may lead to downregulation of T_H2-like inflammation. This approach was successful in a mouse model of asthma, whereby adenoviral-mediated delivery of sST2 was able to decrease IgE, eosinophil infiltration, and BAL levels of IL-4, IL-5, and IL-13 compared with controls.⁴ In contrast to this association with T_H2-like inflammation, sST2 expression *in vitro* and perhaps *ex vivo* was strongly regulated by a T_H1-type cytokine, IFN- γ .

Although ST2L expression and functional responses to IL-33 stimulation (namely IL-8 production) have been shown *in vitro* in normal human bronchial epithelial cells, this is the first study to examine ST2L expression *ex vivo* in fresh epithelial brushings from asthmatic subjects.⁸ In addition, although ST2L activation has been implicated in T_H2-like responses, those studies have focused on eosinophils, mast cells, and T cells and have largely ignored the epithelium.^{11–13} Multiple recent GWAS have identified SNPs in the gene encoding ST2L, *IL1RL1*, to asthma, with this study being the first to link these polymorphisms to epithelial ST2L and sST2 expression.^{14,15} This suggests the airway epithelium may play a key role in this association, especially as the epithelium is the first barrier encountered by allergens and microbes and a prime source for the ST2L ligand, IL-33.^{8,23}

Consistent with previous studies that have suggested a role for activation of ST2L in the promotion of T_H2 responses, upregulation of ST2L tracked with several known markers of T_H2 inflammation, including peripheral blood eosinophils, F_{ENO}, and epithelial eotaxin-3 and CLCA1 mRNA.^{11,12,24–26} Importantly, the greater the evidence for T_H2-like biomarkers (the highest T_H2-like score), the higher the ST2L mRNA level, suggesting that ST2L mRNA itself may be a significant indicator of the presence of T_H2-like inflammation. Notably, ST2L expression did not appear to be affected by inhaled or oral corticosteroids. ST2L mRNA was also higher in those subjects taking LTRAs, though LTRA use was highest in severe asthmatic participants, which may help to explain this association.

Although the genetic relationship between *IL1RL1* and asthma susceptibility has now been repeatedly demonstrated, the mechanism for the increase in susceptibility is unknown. In that regard, this study observed that several associations of SNPs in *IL1RL1* associated not only with ST2L or sST2 expression, but also with markers of T_H2-like inflammation, including F_{ENO} and T_H2-like score. These results support the role of ST2L pathway activation in regulating overall levels of T_H2-like inflammation. Interestingly, multiple SNPs located in ST2L (but not sST2) were associated with sST2 expression levels, suggesting that ST2L expression levels or pathway activation regulates sST2 expression, perhaps through some type of feedback mechanism. The generally similar directions of influence of these *IL1RL1* SNPs on both ST2 mRNA and sST2 would support this. This is also similar to findings by Ho, et al, who found that genetic variation in ST2L was associated with circulating levels of sST2.²⁷ Despite this overall genetic association, in the present study, sST2 mRNA or protein

levels did not associate with markers of T_H2 inflammation, perhaps suggesting there is dysregulation of sST2 in T_H2-like asthma.

In our analysis, 5 of the SNPs associated with ST2L mRNA and/or sST2 BAL levels were previously associated with asthma (rs1420101, rs1420089, rs1041973, rs1921622, and rs10185897).^{14,28} However, additional SNPs, including rs17696376 (in strong linkage disequilibrium with rs1420089 [$r^2 = 0.9006$]), rs13015714, and the LD block of rs12712135, rs10178436, and rs11685424 ($r^2 = 0.9663$) have not previously been associated with asthma. They are also not in linkage disequilibrium with previously reported SNPs, but in this study, they were associated with ST2L mRNA and/or sST2 BAL levels. Of note, linkage disequilibrium of *IL1RL1* is reported to be similar across ethnic groups, though our SNP analysis was restricted to the Caucasian population.²⁸

Very little is known to date about the regulation of ST2L expression. Although fresh epithelial brushings and tissue biopsy sections demonstrated differences in ST2L expression, baseline *in vitro* expression of ST2L was extremely low, perhaps an artifact of the culture system itself. sST2 is a decoy receptor for IL-33 and could play a key role in controlling activation of ST2L.^{6,7} Given the role of ST2L activation in T_H2-like inflammation, T_H1 cytokines might play a pivotal counter-regulatory role in control of the IL-33/ST2L axis, specifically via the IL-33 decoy receptor sST2. This hypothesis is supported by our observation that BAL IFN- γ mRNA levels and sST2 BAL protein levels are highly correlated and that IFN- γ regulated sST2 expression *in vitro*. In contrast, IL-13, a T_H2 cytokine, did not affect either ST2L or sST2 expression, suggesting that IFN- γ regulation of sST2 expression may be a primary mechanism by which ST2L activation is controlled (by blocking IL-33).

Limitations of this study include timing differences in results from complete blood count measurements, symptoms, and spirometry in relation to bronchoscopy, which could influence the results of our study. Although the sample size was quite large for a bronchoscopically focused human study, limited human samples prevented every measurement being performed on every subject. Our subject groups are not well matched, which is a product of the nature of patients with severe asthma being both older and heavier. Though there was no statistical correlation between BMI or age and ST2L mRNA, independent effects of these 2 variables cannot be determined. The lack of correlation of ST2L mRNA expression in fresh epithelial brushings and subsequently in ALI culture makes it difficult to study responses to IL-33 *in vitro*. It is possible that *in vitro* culture conditions have effects on ST2L expression. Expression of ST2L by other cell types could clearly be playing a role as well. Our immunohistochemical staining for ST2 also included a relatively small sample size. Although sST2 mRNA from fresh brushings was generally absent, our antibody against ST2 is unable to distinguish between sST2 and ST2L. In addition, our SNP analysis looked only at the relation to ST2L expression levels; it remains possible that other SNPs might affect downstream pathway activation instead of ST2L expression itself. Our small sample size for SNP analysis precluded the use of a classical expression quantitative trait loci analysis. As such, our findings of an association of SNPs in *IL1RL1* with dichotomous ST2L mRNA and sST2 BAL levels must be interpreted as exploratory, as our statistical analysis did not correct for multiple comparisons, and the

small sample size could lead to an overestimation of the genetic effect of individual SNPs. Therefore, our results may overestimate the importance of the genetic association discovered. Future work will attempt to identify the mechanism by which these SNPs affect ST2L and/or sST2 expression.

In summary, this study presents evidence in human participants for a relationship of epithelial ST2L expression to severe, particularly T_H2-high asthma. Further studies examining the functional significance of SNPs in *IL1RL1* as well as the counter-regulatory role of sST2 may provide more insight into whether this pathway is critical to the initiation, maintenance, and/or augmentation of T_H2-inflammation in asthma. Ultimately, targeting this pathway therapeutically, likely primarily in those with evidence for T_H2-like inflammation, will be required to determine its importance in human asthma.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations used

AEC	3-Amino-9-ethylcarbazole
ALI	Air-liquid interface
ANCOVA	Analysis of co-variance
ATS	American Thoracic Society
BAL	Bronchoalveolar lavage
BMI	Body mass index
CLCA1	Calcium-activated chloride channel regulator 1
F_{ENO}	Exhaled nitric oxide
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
ICS	Inhaled corticosteroids
LTRA	Leukotriene receptor antagonist
OCS	Oral corticosteroids
SARP	Severe Asthma Research Program
SNP	Single nucleotide polymorphism

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Key messages

- ST2L is increased in severe asthma, particularly in those with features of T_H2-like inflammation, suggesting that targeting this pathway may be beneficial therapeutically.
- Single nucleotide polymorphisms in *IL1RL1* are associated with ST2L and sST2 expression.
- IFN- γ appears to be a key regulator of sST2 expression.

FIG 1.

qRT-PCR of epithelial ST2L mRNA expression by asthma severity. RNA was extracted from fresh epithelial brushings, and cDNA was prepared and quantified by qRT-PCR, with ST2L expression normalized to GAPDH. Data represented as geometric mean $\pm$ standard error of the mean.

FIG 2. Epithelial ST2L protein expression in endobronchial biopsies by immunohistochemistry. Tissue biopsies were stained with anti-ST2 antibody, with representative images shown for healthy controls (A), participants with mild/moderate asthma (B), and those with severe asthma (C). Quantification of all staining is also shown (D).

FIG 3.

Epithelial ST2L mRNA expression by T_H2 -like score. T_H2 -like score was calculated, as described in the text, based on median splits of F_{ENO} and epithelial CLCA1 and eotaxin-3 expression and elevated eosinophils, using a cutoff of 300 eosinophils/ mm^3 . Data are represented as a geometric mean $\pm$ 95% CI.

FIG 4.

Regulation of sST2 mRNA (A) and protein (B) levels by IFN- γ in air-liquid interface culture of epithelial cells. Cells were stimulated with 10 ng/mL of IFN- γ for 8 days. sST2 mRNA levels were determined by qRT-PCR. sST2 protein levels were determined by ELISA. Each symbol represents an independent experiment.

TABLE I

Baseline subject demographic characteristics

Subject group (n)	Healthy controls (22)	Mild/moderate (25)	Severe asthma (35)	Overall difference (<i>P</i> value)
Demographic				
Age (y)	26.5 (23.3–33.5)	24.6 (20.9–38.4)	44.6 (34.4–55.2)	.0002*
Age at onset	n/a	6 (2–13.5)	5 (2–24)	.98
Gender (male/female)	12/10	6/19	10/25	.06
Race (white/AA/other)	14/4/4	14/8/3	28/4/3	.17
BMI (kg/m ²)	23.4 (22.1–28.0)	26.3 (23.1–29.8)	31.7 (25.6–36.2)	.0002*
Serum IgE (kU/L)	30 (15–60)	179.5 (68.3–439.5)	150 (27.3–458.5)	.004*
Baseline FEV ₁ (%p)	99 (92.8–105.3)	89 (81.5–97.5)	52 (39–68)	<.0001*
Blood eosinophils (cells/mm ³)	100 (100–100)	200 (100–300)	200 (100–500)	.03*
Exhaled nitric oxide (ppb)	20 (16–32)	31 (21–58.25)	38 (19–58.3)	.045*
LTRA use (yes/no)	n/a	3/22	16/19	.0001*
Anti-IgE therapy (yes/no)	n/a	0/25	9/26	.008*
ICS use	n/a	16/25	35/35	<.0001*

Discrete data were analyzed using the Pearson χ^2 test or Fisher exact test, and continuous data were analyzed using the Kruskal-Wallis test.

AA, African American; *n/a*, not applicable; %*p*, percent predicted; *ppb*, parts per billion.

* *P* < .05.

TABLE II

Relationship of epithelial ST2L mRNA to markers of TH2-like inflammation

TH2-like marker group					
Low			High		
	n	Relative ST2L mRNA, Geometric mean (95% CI)	n	Relative ST2L mRNA, Geometric mean (95% CI)	P value
Blood eosinophils	50	0.05 (0.03–0.1)	27	0.29 (0.14–0.62)	.001*
BALF eosinophils	38	0.08 (0.04–0.17)	33	0.12 (0.06–0.24)	.45
Sputum eosinophils	32	0.1 (0.05–0.21)	18	0.05 (0.02–0.12)	.21
Exhaled nitric oxide	43	0.05 (0.03–0.1)	33	0.21 (0.10–0.42)	.003*
CLCA1 epithelial mRNA	33	0.04 (0.02–0.07)	39	0.32 (0.16–0.62)	<.0001*
Eotaxin-3 epithelial mRNA	35	0.05 (0.03–0.09)	41	0.24 (0.12–0.47)	.001*

Blood eosinophils cutoff was 300 cells/mm³.

Sputum eosinophils cutoff was 2%.

BALF eosinophils, exhaled nitric oxide, CLCA1 mRNA and Eotaxin-3 mRNA were divided based on a median split.

BALF, Bronchoalveolar lavage fluid.

TABLE III

Relationship of epithelial ST2L mRNA to healthcare use

	Healthcare use					P value
	No		Yes			
	n	Relative ST2L mRNA, Geometric mean (95% CI)	n	Relative ST2L mRNA, Geometric mean (95% CI)		
ED/hospital visits in past year	32	0.08 (0.04–0.16)	28	0.24 (0.11–0.52)	.03*	
ICU/intubation	40	0.09 (0.05–0.17)	20	0.30 (0.12–0.73)	.03*	
3+ Oral steroid bursts in past year	26	0.07 (0.03–0.15)	34	0.22 (0.11–0.44)	.03*	
On oral steroids	34	0.10 (0.08–0.43)	26	0.19 (0.05–0.20)	.23	

ED, Emergency department; ICU, intensive care unit.

* $P < .05$.

TABLE IV

Expression quantitative trait loci in *IL1RL1* associated with ST2L mRNA levels

rsID	Location	MAF	n	Allele frequency			P value (odds ratio)		
				Genotype	HI (n)	LO (n)	Overall	Dominant model	Recessive model
12712135	Intron of ST2L mRNA	0.44	49	AA = 24.5%	6	6	.07	.25	.048 (5.2)
				GA = 53.1%	5	21			
				GG = 22.4%	1	10			
1041973	Missense A to E ST2L and sST2 position 78	0.288	52	AA = 7.7%	1	3	.02	.02 (.15)	1.00
				AC = 34.6%	1	17			
				CC = 57.7%	12	18			
10185897	Intron in ST2L mRNA	0.142	49	AA = 2.0%	0	1	.03	1.00	.01 (n/a)
				AC = 26.5%	0	13			
				CC = 71.4%	12	23			

Statistical analysis was performed using Pearson χ^2 test or Fisher exact test, as appropriate.

N/a indicates that an odds ratio could not be calculated due to a 0 value.

MAF, Minor allele frequency.

TABLE V

Expression quantitative trait loci in *IL1RL1* associated with sST2 BAL levels

rsID	Location	MAF	Allele frequency				P value (odds ratio)		
			n	Genotype	HI (n)	LO (n)	Overall	Dominant model	Recessive model
10178436	5' of <i>IL1RL1</i>	0.441	46	CC = 21.7%	1	10	<.0001	.0009 (n/a)	<.0001 (n/a)
				TC = 56.5%	15	11			
				TT = 21.7%	10	0			
11685424	5' of <i>IL1RL1</i>	0.458	46	AA = 21.7%	0	10	<.0001	<.0001 (n/a)	.0009 (n/a)
				GA = 56.5%	15	11			
				GG = 21.7%	10	0			
12712135	Intron of ST2L mRNA	0.44	36	AA = 22.2%	8	0	.0001	.001 (n/a)	.005 (n/a)
				GA = 58.3%	12	9			
				GG = 19.4%	0	7			
1420089	Intron of ST2L mRNA	0.102	46	AA = 78.2%	16	20	.02	.35	.01 (11.3)
				GA = 19.6%	8	1			
				GG = 2.2%	1	0			
1041973	Missense A to E ST2L and sST2 position 78	0.288	41	AA = 7.3%	0	3	.02	.02 (.18)	.06
				AC = 29.3%	5	3			
				CC = 63.4%	19	7			
1420101	Intron in both ST2L and sST2 mRNA	0.371	46	AA = 10.9%	0	5	.02	.14	.01 (n/a)
				GA = 47.8%	12	10			
				GG = 41.3%	13	6			
17696376	Intron in ST2L mRNA	0.074	36	CC = 80.6%	13	16	.01	1.0	.01 (n/a)
				CT = 16.7%	6	0			
				TT = 2.8%	1	0			
1921622	Intron in ST2L mRNA	0.411	46	AA = 10.9%	0	5	.03	.01 (n/a)	.36
				AG = 52.1%	14	10			

rsID	Location	MAF	n	Allele frequency			P value (odds ratio)		
				Genotype	HI (n)	LO (n)	Overall	Dominant model	Recessive model
				GG = 37.0	11	6			
10185897	Intron in ST2L mRNA	0.142	36	AA = 2.8%	0	1	.04	.44	.03 (.18)
				AC = 27.8%	3	7			
				CC = 69.4%	17	8			
13015714	3' of IL1RL1	0.27	46	GG = 6.5%	3	0	.01	.24	.02 (5.1)
				GT = 52.2%	16	8			
				TT = 41.3%	6	13			

N/a indicates that an odds ratio could not be calculated due to a 0 value.

MAF, Minor allele frequency