Supplementary Methods

Patients

The research protocol for this study was approved by the Institutional Review Board of The University of Texas Health Science Center and Baylor College of Medicine, Houston, TX. Patients scheduled to undergo endoscopic sinus surgery who met inclusion and exclusion criteria (see **Table S1 in Supplementary Appendix**) were consented for the collection of peripheral blood and sinus lavage fluid. Peripheral blood was collected at the time of surgery after initiation of general anesthesia. Sinus lavage fluid was collected after the maxillary or sphenoid sinus was opened in patients undergoing endoscopic sinus surgery. Specimens from 118 patients were de-identified prior to laboratory processing.

Definitions of CRSwNP and CRSsNP were based on the criteria outlined by The Rhinosinusitis Initiative[26]. Allergic fungal rhinosinusitis (AFRS) is a specific clinical subtype of CRSwNP and was based on the criteria outline by Meltzer et al[12]. All CRS patients had 2 or more sinonasal symptoms. Patients with cystic fibrosis or aspirin exacerbated respiratory disease were excluded from this study. Control samples were taken from patients with no history of allergic rhinitis, asthma or CRS symptoms undergoing surgery for either repair of skull base defects or resection of non-malignant pituitary tumors. Patient characteristics are summarized in **Table S3 in the Supplementary Appendix**.

Collection of sinus lavage fluid

Sinus lavage fluid was collected mainly from the maxillary sinuses. However, in some patients in which completing a maxillary antrostomy was not medically indicated, lavage fluid was collected from the sphenoid sinus. Using an endoscope for direct visualization, approximately 10 cc of sterile saline was carefully infused into the sinus cavity. Then, the saline was suctioned directly from the sinus cavity into a sterile trap, taking care to minimize collection of fluid outside of the sinus cavity. This process was repeated several times until approximately 30 cc of sinus lavage fluid had been collected.

Fungal Culture of Sinus Lavage Fluid

The collected sinus fluid was processed separately in the lab by treating with ACK red blood lysis and, if necessary, trypsin digestion to disaggregate tissue and finally 50 mM dithiothreitol to digest the mucus as previously described[13]. Select common, mature fungal colonies were isolated for microscopic identification at the Fungus Testing Laboratory at The University of Texas Health Science Center (San Antonio, Texas, USA).

Fungal Antigen Preparation and ELISpot Assay

Fungal allergens were prepared from pure cultured hyphal mats using colloidal grinding in a planetary ball mill (Retsch) with 2 mm zirconium oxide balls in PBS. Grinding was performed in three 5 minute stages at 650 rpm with intermittent cooling of the grinding vessel to prevent overheating. Insoluble material was removed by centrifugation and the supernatant was then passed through a 0.22 micron filter. Protein concentration was determined by BCA analysis (Thermo Scientific, Waltham, MA).

Peripheral blood mononuclear cells (PBMCs) were isolated by ficoll-hypaque gradient centrifugation. Dilutions of PBMCs starting at 4 x10^5 cells were prepared in a 96-well 4HBX Immulon plate (Thermo Scientific) coated with human anti-IL-4 (BD Pharmingen, San Jose, CA) and blocked with 2% I-Block (Life Technologies, Grand island, NY). The dilutions were exposed overnight (20-24 h) to media alone, or media containing 5 μg of the fungal allergen, individually, prepared from *Aspergillus niger, Aspergillus fumigatus, Aspergillus flavus, Alternaria alternata, Curvularia lunata, Penicillium oxalicum, Paecilomyces variotii, Candida albicans*, and *Bipolaris spicifera* or from fungal allergens prepared from unidentifiable (non-maturing) fungal colonies isolated specifically from some control and CRSsNP patients. The plate was washed and captured IL-4 detected using sequential steps of biotinylated-anti-IL-4 antibody (BD Pharmingen, San Jose, CA), streptavidin conjugated alkaline phosphatase, and Bromo-4-chloro-3-indolyl phosphate substrate (Sigma-Aldrich, St. Louis, MO) embedded in a 0.6% agarose matrix. Spots were enumerated within 24 hours of substrate addition. As addressed in our results, a cutoff of 50 induced cells per million provided an optimal specificity (100%) and sensitivity (75%) between patients with and with allergic airway disease.

ELISA Fungal Specific IgE Assay

HBX Immunolon plates were coated with 20 ug fungal allergen from *A. niger*, *A. fumigatus*, or *A. alternata* for plasma sample analysis or poly clonal anti-human IgE (ICL Labs, Stuart, Florida) for standard curve preparation. The plates were blocked with 5% nonfat Carnation dry milk (Nestle, Vevey, Switzerland). Subject plasma samples and standards made from IgE calibrator serum (Bethyl Labs, Montgomery, TX) were diluted in PBST supplemented with 1% BSA and incubated in the coated plates 18 to 20 h at 4C. Plates were washed and bound IgE detected using sequential steps of a secondary polyclonal anti-human IgE antibody conjugated to HRP (ICL Labs) and use of 1-Step Ultra TMB-ELISA (Thermo Scientific) substrate kit. Lower limit of detection for the assay was 6kIU/L IgE.

Statistics

Summary statistics are presented as proportions, means (with standard error of the mean (SEM)). Bivariate associations were examined using Mann-Whitney and Fisher's exact tests, as appropriate. The discriminatory ability of IgE E and IL-4 ELISpot were characterized by the area under the receiver operating characteristic (ROC) curve. All analyses were performed using Prism v6.0 (GraphPad Software, San Diego, CA) or Stata v12.0 software (StataCorp, College Station, TX). All *P* values are two-sided, with $P<0.05$ considered statistically significant. When pairwise comparisons were made, the *P* value threshold was lowered to 0.01 for appropriate interpretations that took into account multiple comparisons.

Supplementary Figure Legend

Figure S1 Fungal-specific IgE responses to *A. niger*, *A. fumigatus*, or *A. alternata* are elevated in AFRS patients and highly concordant with the species isolated. (A-C) IgE positivity data analyses based on various patient groupings. (D-F) IgE ELISA data limited to patients who grew *A. niger*, *A. fumigatus*, or *A. alternata* from their airways. * $p<0.05$. [§]Control is a subset of all non-asthmatics.

Supplementary Appendix

Table S1. Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
• All patients aged 16 to 85 years undergoing medically-indicated sinus surgery presenting at the ENT surgical clinic at the University of Texas Health Sciences Center	• Pregnant or breast-feeding women • History of smoking in the last month • History of significant medical disease that would affect immune system such as malignancy, uncontrolled diabetes, or HIV • History of any lung disease other than asthma including cystic fibrosis and aspirin exacerbated respiratory disease

Table S2. Clinical definitions

- Healthy controls were defined as having no history of sinonasal symptoms
- CRSsNP was defined as having $\geq$2 following symptoms (mucopurulent drainage, nasal obstruction, facial pain/pressure/fullness or decreased sense smell) for $\geq$12 weeks, nasal endoscopic exam showing no nasal polyps, and nasal endoscopic exam and/or sinus CT scan consistent with sinus inflammation
- CRSwNP was defined as having $\geq$2 following symptoms (mucopurulent drainage, nasal obstruction, facial pain/pressure/fullness, or decreased sense smell) for $\geq$12 weeks, nasal endoscopic exam positive for nasal polyps, and nasal endoscopic exam and CT sinus consistent with sinus mucosal inflammation
- AFRS defined as having $\geq$2 following symptoms (mucopurulent drainage, nasal obstruction, decreased sense smell, facial pain/pressure or decreased sense smell) for $\geq$12 weeks, nasal endoscopic exam positive for nasal polyps and mucin, CT sinus consistent with eosinophilic mucin, evidence of fungal hypersensitivity, and evidence of elevated total serum IgE levels
- Asthma was assigned as a diagnosis based on prior documentation in the medical record.

Table S3. Patient demographics

Characteristic	**Healthy Control**	**CRSsNP**	**CRSwNP**	**AFRS**
Sex – no.				
Male	7	12	27	20
Female	10	12	17	13
Age – yr				
Median (interquartile range)	53 (42 – 56)	51.5 (42.5 – 63)	47 (38 – 56)	25 (19 – 47)
Range	22 - 72	20 - 76	19 - 82	16 - 64
Asthma	0	7	28	9

Table S4. Fungal growth in 99 patients based on CRS disease, asthma status and combined Th2-associated airway disease

Fungal Growth	**Healthy Control**	CRSsNP	CRSwNP*	CRSwNP AFRS*
	3/15	4/21	25/37	23/26
No Asthma 28/60	3/15	2/16	6/9	17/20
Asthma* 27/39	N/A	2/5	19/28	6/6
No Th2-associated airway disease ->				5/31
*Combined Th2-associated airway disease ->				50/68

Table S5. Fungal specific PBMC reactivity in 48 Patients based on CRS disease, asthma status and combined Th2-associated airway disease

Fungal IL-4 Reactivity	**Healthy Control**	CRSsNP	CRSwNP*	CRSwNP AFRS*
	0/11	1/8	8/14	13/15
No Asthma 9/32	0/11	0/7	2/6	7/8
Asthma* 13/16	N/A	1/1	6/8	6/7
No Th2-associated airway disease ->				0/18
*Combined Th2-associated airway disease ->				22/30

Table S6. Concordance between fungal species identified by fungal growth within sinus cavity and fungal species inciting fungal specific PBMCs reactivity in 13 patients based on CRS disease, asthma status and combined Th2-associated airway disease

Growth / IL-4 Concordance	**Healthy Control**	CRSsNP	CRSwNP*	CRSwNP AFRS*
	0/3	1/2	2/3	5/5
No Asthma 1/5	0/3	0/1	No Data	1/1
Asthma* 7/8	N/A	1/1	2/3	4/4
No Th2-associated airway disease ->				0/4
*Combined Th2-associated airway disease ->				8/9

Table S7. Fungal species identified*

Alternaria alternata
Aspergillus flavus
Aspergillus fumigatus
Aspergillus niger
Aspergillus species
Aspergillus versicolor
Aspergillus wentii
Aureobasidia pullans
Basidiomycete species
Bipolaris species
Bipolaris Spicifera
Candida albicans
Candida species
Cladosporium cladosporioides
Cladosporium species
Cochliobous spicifer
Curvularia lunata
Curvularia species
Paecilomycetes variotii
Penicillium miczynskii
Penicillium species
Trichoderma harzarium
Trichosporon species

*does not include several species isolated only once