December 2004 • Volume 114 • Number 6

Chronic rhinosinusitis: An enhanced immune response to ubiquitous airborne fungi

Shin SH, Ponikau JU, Sherris DA, Congdon D, Frigas E, Homburger HA, Swanson MC, Gleich GJ, Kita H.

Chronic rhinosinusitis: An enhanced immune response to ubiquitous airborne fungi.

J Allergy Clin Immunol. 2004 Dec;114(6):1369-75.
PMID: 15577837 [PubMed - in process]

SOURCES: Shin, S. *Journal of Allergy and Clinical Immunology*, online edition, Oct. 8, 2004. News release, Mayo Clinic.

Background Chronic rhinosinusitis (CRS) is one of the most common long-term illnesses in the United States. The etiology of CRS is unknown, and no effective treatment has been established.

Objective We investigated the hypothesis that abnormal immunologic responses to ubiquitous airborne fungi contribute to the pathogenesis of this disease.

Methods The proliferative and cytokine responses of PBMCs to extracts from 4 common airborne fungi—including *Alternaria*, *Aspergillus*, *Cladosporium*, and *Penicillium*—were examined by *in vitro* culture. Serum specimens were tested for specific IgE and IgG to these fungi.

Results PBMCs from approximately 90% of the patients with CRS, but not those from normal individuals, produced both IL-5 and IL-13 when exposed to *Alternaria*. Furthermore, PBMCs from patients with CRS produced significantly more IFN-γ than PBMCs from normal individuals in response to *Alternaria* (median, 553 pg/mL vs 98 pg/mL; $P < .01$). Levels of serum IgG antibodies to *Alternaria* and *Cladosporium* were clearly increased in patients with CRS compared with normal individuals ($P < .01$). Less than 30% of the patients with CRS had specific IgE antibodies to *Alternaria* or *Cladosporium*. The increased humoral (serum IgG) response strongly correlated with the increased cellular (IL-5 production) response to *Alternaria* (r=0.619; $P < .01$).

Conclusion Patients with CRS show exaggerated humoral and cellular responses, both T_H1 and T_H2 types, to common airborne fungi, particularly *Alternaria*. The anomalous immune and inflammatory responses to ubiquitous fungi may explain the chronicity of airway inflammation in CRS.

Abbreviations used	
AFS	Allergic fungal sinusitis
Con-A	Concanavalin A
CRS	Chronic rhinosinusitis
FEIA	Fluorescence enzyme immunoassay
MBP	Major basic protein

(Click on a term to search this journal for other articles containing that term.)

Key words **Chronic rhinosinusitis**, **immune response**, **cytokines**, **inflammation**, **fungus**, **Alternaria**

A recent survey reported that 14.1% of adults recalled a health professional's diagnosis of sinusitis.[1] As a common, long-term illness, chronic rhinosinusitis (CRS) is characterized by chronic inflammation of the nasal and paranasal sinus mucosa and is associated with mucosal alterations ranging from inflammatory thickening to gross nasal polyp formation.[2,3] Many patients with CRS have asthma.[4] The socioeconomic effect of CRS includes a direct cost of about $5.6 billion per year[5] and indirect costs, such as >70 million lost activity days per year and reduced physical and social functioning.[6] Antibiotics, antihistamines, and surgical treatment are usually ineffective in long-term treatment of CRS,[7] and systemic glucocorticoids often provide only temporary relief. Even with aggressive therapies, CRS persists in many patients.[7]

Although its etiology and pathogenesis are poorly understood, the histologic hallmark of CRS is a chronic inflammatory infiltrate of lymphocytes, plasma cells, and eosinophils,[8,9] similar to bronchial mucosa from patients with asthma. Patients with CRS have increased numbers of $CD4^+$ T lymphocytes positive for T_H1 and T_H2 cytokines, such as IL-5, IL-13, and IFN-γ, in their sinus mucosa,[10-12] suggesting an immunologic mechanism. However, >40% of patients with CRS are clinically nonatopic and lack specific IgE antibodies.[13] Therefore, a fundamental question exists: what drives the persistent and recurrent airway inflammation in CRS?

Fungi are ubiquitous in the environment, and as saprophytes or commensals, they coexist without effect with the host.[14] Airborne fungal spores, eg, *Aspergillus* and *Alternaria*, enter the upper and lower respiratory tract by inhalation, but are

rarely pathogenic in healthy individuals. Nonetheless, these airborne fungi may contribute to CRS pathogenesis. Previously, fungal organisms were thought to be important in allergic fungal sinusitis (AFS), and AFS was thought to be a rare subset of patients with CRS.[15] More refined methods detected certain diagnostic criteria of AFS, eg, airway eosinophilia and fungal organisms, in >90% of patients with CRS.[16,17] Further, approximately 70% of patients with CRS, who were refractory to conventional therapies, responded to topical nasal administration of antifungal agents with improved symptom scores and sinus computerized tomography and endoscopic findings.[18] However, the airborne organisms typically found in patients with CRS, namely *Alternaria*, *Aspergillus*, *Penicillium*, and *Cladosporium*, were also found in the upper airway secretions of normal healthy controls.[16,17] Therefore, we hypothesized that, as hosts, patients with CRS show an exaggerated immune response to common airborne environmental fungi.

Methods

TOP

Patients

We tested blood specimens from 18 patients with CRS and 15 normal individuals for cellular and humoral immune responses to common airborne fungi. We tested nasal secretions from 9 patients with CRS and 9 normal individuals for fungal proteins and inflammatory mediators. Patients with CRS, who were seeking medical care at Mayo Clinic Rochester, were enrolled randomly between September 13, 1999, and April 12, 2000. Their CRS symptoms persisted for >3 months, including long-term nasal congestion, thick mucus production, loss of sense of smell, and intermittent acute exacerbations.[19] To confirm objectively the CRS diagnoses, a positive computerized tomography of paranasal sinuses (>5 mm thickening in 2 or more sinuses) and nasal endoscopy showed inflammatory mucosal thickening. Normal individuals (no history of allergic diseases, asthma, or CRS) were recruited by advertisement and enrolled consecutively.

Table I shows their clinical and demographic characteristics. Sixteen of the 18 patients with CRS and all of the normal controls resided in the midwestern United States. Age, sex distribution, and serum total IgE levels did not differ between the normal controls and patients. No one smoked, and no one received systemic glucocorticoids in the preceding 2 months. The Institutional Review Board at Mayo Clinic Rochester approved the study. All participants provided informed consent.

Table I. Clinical and demographic characteristics of normal controls and patients with CRS[*]

	Normals (n=15)	Patients with CRS (n=18)
Sex		
Men	10	11
Women	5	7
Age (y)	39.7 (34-48)	48.6 (27-78)
CRS duration (y)	0	11.2 (1-38)
Sinus surgery (n)	0	2.5 (0-8)
Aspirin sensitivity	0 (0%)	5 (28%)
Bronchial asthma[‡]	0 (0%)	14 (78%)
FEV_1 (% predicted)	Not determined	82.3 (56-105)
Total IgE (IU/mL)	133.9 (9-527)	164.8 (3-767)
IgE antibodies to common aeroallergens[‡]	0 (0%)	11 (61%)

[*]Data are number or mean (proportion or range).

[†]Demonstrated by reversible airflow limitation (20% percent variability in FEV_1) or by increased airway responsiveness to methacholine (more than 20% decrease in FEV_1 after inhalation of 20 mg/mL methacholine), or both.

[‡]Detectable serum IgE antibodies to cat, dog, *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, June grass, oak pollen, birch pollen, or short ragweed pollen by the Pharmacia ImmunoCAP FEIA system.

Serum levels of specific IgE and IgG antibodies to fungal antigens

Serum levels of IgE (IU/mL) and IgG (μg/mL) to common airborne fungi, including *Alternaria alternata*, *Aspergillus fumigatus*, *Cladosporium herbarum*, and *Penicillium notatum*, were assessed by the Pharmacia ImmunoCAP fluorescence enzyme immunoassay (FEIA) system (Pharmacia Diagnostics, Kalamazoo, Mich).

Culture, proliferation, and cytokine production

PBMCs were cultured with fungal extracts to study cell proliferation and production of T_H1 and T_H2 cytokines. PBMCs were isolated from heparinized blood by density centrifugation over Histopaque (Sigma, St Louis, Mo) and resuspended at 2×10^6 cells/mL in RPMI-1640 medium (Gibco, Rockville, Md) supplemented with 10% calf serum, 2 μmol/L glutamine and 50 μmol/L 2-mercaptoethanol. Aliquots of the cell suspension (0.2 mL) were cultured with extracts of 4 common airborne fungi, *A alternata, A fumigatus, C herbarum,* and

P notatum (Greer Laboratories, Lenoir, NC). These fungi are frequently isolated from nasal cavities of patients with CRS and normal individuals.[16,17] The fungal extracts were negative for endotoxins by IL-8 production from PBMCs; the limulus amebocyte lysate assay was unusable because of false-positive reactions of fungal β-glucan.[20] Phorbol myristate acetate (50 ng/mL) plus ionomycin (1 μmol/L; cell proliferation) or concanavalin A (Con-A; 5 μg/mL; cytokine production) were used as positive controls. Preliminary studies optimized fungal extract concentration at 50 μg/mL and examined the kinetics of cell proliferation and cytokine production. Cells were cultured at 37°C and 5% CO_2 for 72 hours, and cell proliferation was measured with the CellTiter-96 aqueous cell proliferation assay kit (Promega, Madison, Wis) according to the manufacturer's directions. For cytokine production, cell-free supernatants were collected and stored at –20°C. The levels of IL-4, IL-5, IL-13, and IFN-γ in the sample supernatants were determined by matched-pair antibody ELISA kits (Endogen, Woburn, Mass) according to the manufacturer's directions. The detection limit was 2 pg/mL.

Collection and analyses of nasal secretions for levels of *Alternaria* allergens, eosinophil major basic protein, and IL-5

To investigate the local inflammatory responses, we collected nasal secretions from 9 additional patients with CRS and 9 normal individuals. Nasal secretions were obtained from the nasal cavities (the floor of nose, the septum, and the middle turbinate of both nostrils) by using a sterile sinus secretion collector (Xomed Surgical Produces, Jacksonville, Fla). The mean volume of secretions was 138 ± 35 μL (mean ± SEM; n=18). Each secretion specimen was extracted by adding twice the volume of 0.9% NaCl, vortexing, and centrifugation. The supernatants were stored at –20 °C. An *Alternaria* antigen, Alt a 1, was measured by using a specific ELISA kit (INDOOR Biotechnologies, Charlottesville, Va) according to the manufacturer's procedures with a slightly modified standard curve. Total *Alternaria* proteins were measured immunochemically by inhibition as previously described.[21] Briefly, samples and a standard extract from *A alternata* (Greer Laboratories) competed with solid-phase *A alternata* proteins bound on the ELISA plates for rabbit anti-*Alternaria* IgG antibodies (ALK-Abello, Round Rock, Tex). Radiolabeled, affinity-purified goat antirabbit IgG was used for detection. The results are expressed as mass protein per milliliter nasal secretion supernatants; the detection limits were 40 pg/mL and 12 ng/mL for Alt a 1 and total *Alternaria* proteins, respectively. The eosinophil major basic protein (MBP) was quantitated by radioimmunoassay by using monoclonal antibodies to MBP as described previously[22]; the detection limit was 45 ng/mL. IL-5 was measured by an ELISA kit (Endogen) as described.

Statistical analyses

The nonparametric Mann-Whitney *U* test was used to compare cytokine protein, specific IgE, and specific IgG. Differences (2-sided) were significant with $P \leq .05$.

Fisher exact tests were used to analyze the link between the IL-5 production by PBMC and the diagnosis of CRS. Associations between specific IgE or IgG and IL-5 production were assessed with Spearman rank. All analyses used Instat (version 2.03 for Macintosh) software (GraphPad Software, San Diego, Calif).

Results

TOP

Humoral immune response to common airborne fungi

IgE antibodies to *Alternaria*, *Aspergillus*, *Cladosporium*, and *Penicillium* were detectable in <30% of patients with CRS (Table II). IgG antibodies to these fungi were detectable in all specimens, but patients with CRS showed increased IgG levels compared with normal individuals (P=.003-.038; Table II). The median serum IgG antibody level to *Alternaria* was about 5-fold higher for patients with CRS.

Table II. Serum levels of IgE and IgG antibodies to fungi in normal individuals and patients with CRS[*]

	Normals (n=15)	**Patients with CRS (n=18)**	***P***
IgE antibodies (IU/mL)			
Alternaria	<0.3 (<0.3-<0.3, 0%)	<0.3 (<0.3-2.31, 28%)	NS
Aspergillus	<0.3 (<0.3-<0.3, 0%)	<0.3 (<0.3-<0.3, 17%)	NS
Cladosporium	<0.3 (<0.3-<0.3, 0%)	<0.3 (<0.3-0.4, 22%)	NS
Penicillium	<0.3 (<0.3-<0.3, 0%)	<0.3 (<0.3-<0.3, 17%)	NS
IgG antibodies (μg/mL)			
Alternaria	4.1 (3.2-9.1, 100%)	19.2 (7.5-24.2, 100%)	.003
Aspergillus	12.2 (8.3-16.5, 100%)	21.3 (12.8-34.6, 100%)	.029
Cladosporium	9.7 (7.4-15.4, 100%)	22.3 (13.8-29.1, 100%)	.007
Penicillium	11.3 (6.7-16.1, 100%)	15.4 (10.2-25.3, 100%)	.038

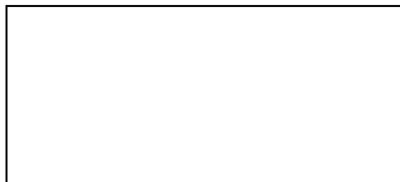
[*]Serum samples were assayed for specific IgE and IgG antibodies by the Pharmacia ImmunoCAP FEIA system. Data are medians (25% to 75% percentile, proportion of subjects with detectable antibodies). The detection limits

of the assays are 0.3 IU/mL for IgE and 0.2 μg/mL for IgG antibodies. Serum from 1 patient with CRS was not available for measurement of IgG.

Cellular immune responses to common airborne fungi

We exposed isolated PBMC from patients with CRS and normal individuals to common airborne fungal extracts. Proliferation did not differ significantly between groups (data not shown), but the cytokine responses were strikingly different. When cultured with *Alternaria* extract, PBMCs from patients with CRS produced significantly more IL-5 (median, 50.2 pg/mL) than the undetectable amounts produced by PBMCs from normal individuals (median, <2.0 pg/mL; $P < .0001$; Fig 1). With *Aspergillus* or *Cladosporium* extracts, PBMCs from patients with CRS produced more IL-5 than PBMCs from normal controls. More IL-5 was produced with *Alternaria* than with *Aspergillus* or *Cladosporium* (P=.0002 and .0025, respectively). With *Penicillium* extract or medium alone, negligible IL-5 was produced. When PBMCs were cultured with the nonspecific stimulus, Con-A, IL-5 production was not different (P=.57). When cultured with different preparations of fungal extracts, specifically fungal culture supernatants, extracts of fungal hyphae, or supernatants of an *Alternaria* culture isolated from nasal secretions of a patient with CRS, PBMCs from CRS patients produced IL-5, but PBMCs from normal individuals did not (data not shown).

Fig 1. Production of IL-5 by PBMCs from normal individuals *(Normal)* and patients with CRS *(CRS)* cultured with extracts of common environmental fungi. PBMCs isolated from normal individuals (n=15) or patients with CRS (n=18) were cultured with medium alone *(None)*, extracts of various fungi, or Con-A *(Con-A)* for 72 hours. Amounts of IL-5 in the supernatants were measured by ELISA. *Horizontal bars* and *boxes* indicate median and 25% to 75% percentile. *Error bars* indicate the range. Statistical analyses show results between the patient and normal groups or between different fungi for the patients with CRS.



Click on Image to view full size

Table III summarizes IL-5 production. With medium alone, PBMCs from patients with CRS and normal individuals produced <11.0 pg/mL IL-5 (mean+2SD; n=33); this is the baseline. With *Alternaria* extract, 16 of 18 patients with CRS (89%), but 0 of 15 normal individuals, produced significant IL-5. The odds of showing a positive IL-5 response to *Alternaria* and to *Cladosporium* were significantly increased in the patients with CRS. The odds of positive IL-5 responses to *Alternaria* for the atopic (ie, detectable IgE to ≥1 common aeroallergen; Table I) patients with CRS (11/18) and nonatopic patients with CRS (7/18) were each increased compared with the 15 normal individuals (713 [13-38,700] $P < .0001$; 68 [3-1655] $P=.0008$, respectively). However, the prevalence of a positive response to *Aspergillus* was not significant, and no one responded to *Penicillium*. Thus, PBMCs from patients with CRS, but not normal individuals, react to certain airborne fungi, such as *Alternaria* and *Cladosporium*, and produce a T_H2 cytokine, IL-5.

Table III. Frequency of elevated IL-5 production by PBMCs incubated with fungal extracts in normal individuals and patients with CRS

Fungi to which subjects' PBMCs responded	Number of patients with positive PBMC response to fungi[*]			
	Normals	**Patients with CRS**	***P* values**	**Odds ratio (95% CI)**
Alternaria	0/15 (0%)	16/18 (89%)	<.0001	205 (9-4611)
Aspergillus	0/15 (0%)	4/18 (22%)	.108	10 (0.5-195)
Cladosporium	0/15 (0%)	6/18 (33%)	.021	16 (0.8-315)
Penicillium	0/15 (0%)	0/18 (0%)	—	—

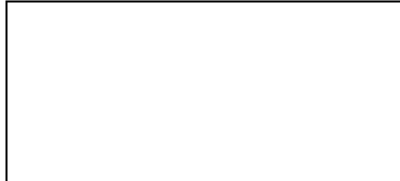
[*]Elevated IL-5 production by PBMCs in response to fungi was defined as 2 SDs above the mean value in the presence of medium alone (>11.0 pg/mL; mean+2SD from 33 subjects).

Characterization of cytokine response

To characterize the cytokine profile, 2 other T_H2 cytokines (IL-4, IL-13) and a T_H1 cytokine (IFN-γ)[23] were measured in the PBMC supernatants after culture with *Alternaria* extract (Fig 2). PBMCs from all of the patients with CRS produced IL-13, but PBMCs from the normal individuals did not. With *Aspergillus* or *Cladosporium*, PBMCs from patients with CRS, but not PBMCs from normal individuals, produced IL-13, although the amounts were less than with *Alternaria*

(data not shown). With Con-A, comparable quantities of IL-13 were produced by PBMCs from both patients with CRS and normal individuals.

Fig 2. Production of IL-13 **(A)** or IFN-γ **(B)** by PBMCs from normal individuals *(Normal)* and patients with CRS *(CRS)* cultured with *Alternaria* extract or Con-A *(Con-A)*. PBMCs isolated from normal individuals (n=15) or patients with CRS (n=18) were cultured with an extract of *Alternaria* or Con-A for 72 hours. Amounts of IL-13 or IFN-γ in the supernatants were measured by ELISA. Results presented as described in Fig 1.



Click on Image to view full size

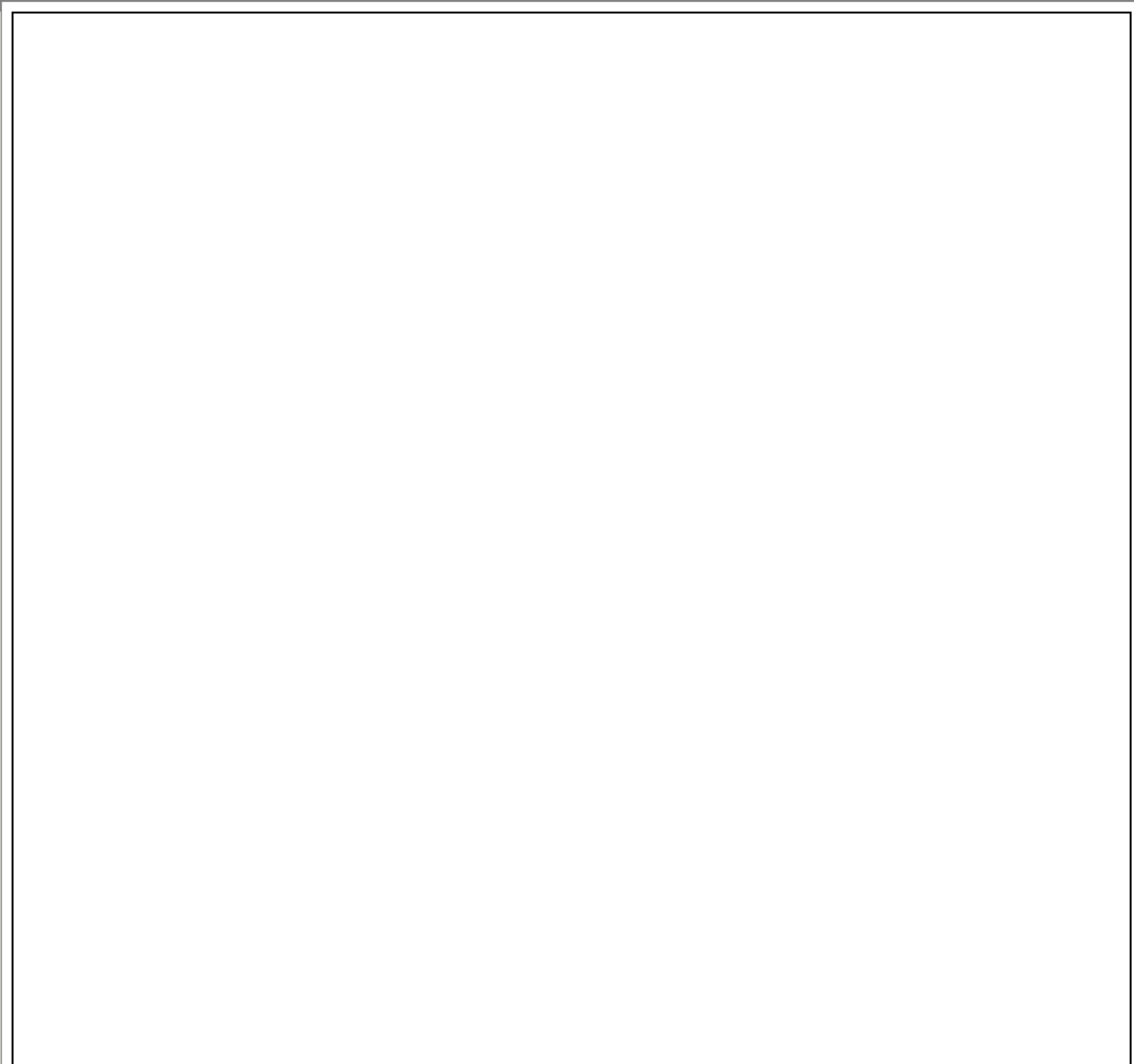
With *Alternaria*, PBMCs from patients with CRS produced approximately 5.5 times more IFN-γ than PBMCs from normal individuals (Fig 2). With Con-A, no difference in IFN-γ production by PBMCs was observed between groups. There was very little IL-4 in the PBMC supernatants from patients with CRS or normal individuals; this could be a result of the relatively long culture period (72 hours), optimized to detect IL-5 and IL-13. Thus, both T_H2 cytokines (IL-5 and IL-13) and a T_H1 cytokine (IFN-γ) were produced by PBMCs from patients with CRS during exposure to *Alternaria*. Only small amounts of IFN-γ were produced by PBMCs from normal individuals.

The humoral immune responses (IgE and IgG antibodies) to *Alternaria* were analyzed relative to the cellular immune response (PBMC IL-5 production) in the patients with CRS. The serum levels of IgE or IgG antibodies to *Alternaria* were plotted versus the IL-5 produced by PBMCs cultured with *Alternaria* (Fig 3). Serum IgE levels to *Alternaria* did not correlate with the PBMC production of IL-5 (Fig 3, *A*). Indeed, 11 of 18 patients with CRS without IgE antibody to *Alternaria* produced IL-5 levels comparable with the 5 of 18 patients with CRS with IgE, suggesting a dissociation between the IgE humoral response and the T_H2-like cellular response. Serum levels of IgG to *Alternaria* correlated strongly with IL-5 produced by PBMCs cultured with *Alternaria* (r=0.619; P=.0093; Fig 3, *B*). Furthermore, this IL-5 response by PBMCs from atopic CRS (11/18; median, 55.6 pg/mL) and nonatopic CRS (7/18; median, 39.2 pg/mL) patients did not differ (P=.25; Fig 4).

Fig 3. Correlation between *Alternaria*-specific IgE **(A)** or IgG **(B)** in sera and *Alternaria*-induced PBMC production of IL-5 in patients with CRS. The serum levels of IgE or IgG antibodies to *Alternaria* in each patient with CRS (n=18, n=17, respectively) are plotted versus the amount of IL-5 produced when their PBMCs were cultured in the presence of *Alternaria* extract. The *vertical bar (A)* indicates the lowest point on the standard curve for the specific IgE assay (0.35 IU/mL). The *solid line (B)* shows the linear regression line, and the *dashed lines* show the 95% CIs. Serum from 1 patient was not available for measurement of IgG.

Click on Image to view full size

Fig 4. Production of IL-5 in response to *Alternaria* by PBMCs from normal individuals *(Normal)* and patients with atopic and nonatopic CRS *(CRS)*. PBMCs isolated from normal individuals (n=15) or atopic (n=11, Table I) or nonatopic (n=7) patients with CRS were cultured with extracts of *A alternata* for 72 hours. Amounts of IL-5 in the supernatants were measured by ELISA. Each *dot* represents data from 1 individual, and *horizontal bars* indicate group medians. Statistical analyses show comparisons between the groups.



Click on Image to view full size

Concentrations of *Alternaria* proteins, eosinophil MBP, and IL-5 in nasal secretions

Perhaps the elevated humoral and cellular immune responses to *Alternaria* in patients with CRS could be explained by increased airway exposure to *Alternaria.* Nasal secretions from 9 other patients with CRS all contained markedly elevated levels of eosinophil MBP (4093 ± 878 ng/mL, mean ± SEM) and IL-5 (226 ± 69 pg/mL) compared with the undetectable levels in secretions from 9 other normal individuals (*P*=.0003 and .0092, respectively). However, both the *Alternaria* protein, Alt a 1, and the total proteins from *Alternaria* did not differ between groups (243 ± 50 vs 208 ± 60 pg/mL, CRS vs normal; *P*=.7076; and 231 ± 82 vs 135 ± 41 ng/mL, CRS vs normal; *P*=.4679, respectively).

Discussion TOP

We investigated the mechanism by which common airborne fungi could mediate persistent sinus inflammation in patients with CRS. The pathogenesis of CRS is unknown; the roles of viral or bacterial infections and allergic responses have been previously studied and debated. Although the histopathologic features of CRS, consisting of lymphocytes, plasma cells, and eosinophils,[2,7] are consistent with allergic inflammation, the role of type I hypersensitivity in CRS pathogenesis has been questioned.[4,13] We now report that PBMCs from most patients with CRS responded vigorously to common airborne fungi, particularly *Alternaria*, and produced IL-5, IL-13, and IFN-γ. These cytokines are consistent with those expressed in the airways of patients with CRS[10-12] and, considering their biological activities,[23] may explain marked tissue eosinophilia in patients. Although we did not examine the responses of PBMCs from atopic patients without CRS, the atopic status of the patients with CRS (28% with IgE to *Alternaria*, 61% with IgE to common allergen panel) did not significantly influence the responses of their PBMCs to fungi (Figs 3 and 4). Patients with CRS also had increased IgG directed toward *Alternaria* and *Cladosporium*. Although each participant's indoor and outdoor exposure to fungi is unmeasurable, *Alternaria* antigen and total *Alternaria* protein in the nasal secretions were not different for patients with CRS and normal controls. Overall, patients with CRS show vigorous cellular and humoral immune responses to airborne fungi, such as *Alternaria* and *Cladosporium*.

Airborne fungi and their spores are ubiquitous and constitute the largest proportion of airborne biological particles.[24] Most patients with CRS produced IL-5 in response to *Alternaria*, only 30% responded to *Aspergillus* or *Cladosporium*, and none responded to *Penicillium* (Fig 1). *Alternaria* also showed the most striking difference (~5-fold) in circulating IgG responses between CRS and normal individuals (Table II). These differences in response to various fungi may reflect the outdoor and indoor ubiquity of *Alternaria*,[25] the unique biochemical properties (eg, enzymes) of *Alternaria* antigens,[26] and the high rate of *Alternaria* spore germination and antigen release.[27] *Alternaria* spores are larger (20-200 μm × 7-18 μm) than other airborne fungal spores, making them readily deposited on the upper airway mucus. *Alternaria* is 1 of the most common fungal genera worldwide,[24] and sensitization to *Alternaria* is associated with asthma in various countries.[28] Moreover, exposure to *Alternaria* is a risk factor for respiratory arrest in patients with asthma.[29] Therefore, an abnormal immune response to *Alternaria* may be important in the pathophysiology of CRS and asthma.

To understand the pathogenesis of CRS, the non–IgE-mediated lymphocyte response must be considered. T_H1 lymphocytes produce IFN-γ and IL-2, which activate mechanisms important in host defense against microorganisms; in contrast, T_H2 lymphocytes produce IL-4, IL-5, IL-9, and IL-13, which activate mechanisms important in IgE production and allergic inflammation.[23] To mediate asthma and other allergic diseases, an increased T_H2 and decreased T_H1

imbalance has been hypothesized, but this hypothesis may be too simplistic.[30] Indeed, IFN-γ–producing T cells are increased in asthmatic airways, and IFN-γ levels correlated with asthma severity, bronchial hyperresponsiveness, and blood eosinophils.[31] Sinus tissue specimens from patients with CRS showed cells expressing IL-5 and IL-13,[10,11] and cells in sinus mucosa produce IFN-γ,[10,12] suggesting that T_H1 and T_H2 responses also coexist in CRS. Our patients with CRS show increases in both T_H1 (IFN-γ) and T_H2 (IL-5, IL-13) immune responses directed toward *Alternaria* and not a shift in the T_H1/T_H2 balance. In animal models, antigen-specific T_H1 cells reportedly potentiate, rather than prevent or protect, T_H2 cell-mediated airway inflammation.[32] Thus, the extensive inflammation and remodeling in the sinonasal airways of patients with CRS may reflect the collaborative effects of both T_H1 and T_H2 immune responses to fungi.

In summary, patients with CRS likely show exaggerated cytokine and humoral immune responses to noninvasive and nonpathogenic fungi, particularly to *A alternata*. These responses to ubiquitous airborne fungi may explain both the chronic airway inflammation and the concomitant asthma symptoms in patients with CRS. The effects of intranasal administration of antifungal agents, such as amphotericin B and itraconazole, on the symptoms and clinical findings of patients with CRS are controversial.[18,33,34] Further elucidation of the underlying abnormalities in the immune cells of patients with CRS may reveal the molecular mechanisms involved in this dysregulated immune response and suggest ways to reverse it.

Acknowledgements TOP

We thank Ms Diane L. Squillace for measurements of IL-5 and MBP, Ms Cheryl R. Adolphson for editorial assistance, and Ms Debra D. Ward for secretarial help.

References TOP

1. Lethbridge-Çejku M, Schiller JS, Bernadel L. Summary health statistics for U.S. adults: National Health Interview Survey, 2002. National Center for Health Statistics Vital Health Stat 2004;10:222.

2. Kaliner MA, Osguthorpe JD, Fireman P, Anon J, Georgitis J, Davis ML. Sinusitis: bench to bedside: current findings, future directions Otolaryngol Head Neck Surg 1997;116:S1-S20.

MEDLINE

3. Spector SL, Bernstein IL, Li JT, Berger WE, Kaliner MA, Schuller DE. Parameters for the diagnosis and management of sinusitis J Allergy Clin Immunol 1998;102:S107-S144.

MEDLINE

4. Slavin RG. Sinusitis in adults and its relation to allergic rhinitis, asthma, and nasal polyps J Allergy Clin Immunol 1988;82:950-956.

MEDLINE

5. Ray NF, Baraniuk JN, Thamer M, Rinehart CS, Gergen PJ, Kaliner M. Healthcare expenditures for sinusitis in 1996: contributions of asthma, rhinitis, and other airway disorders J Allergy Clin Immunol 1999;103:408-414.

MEDLINE ABSTRACT FULL TEXT

6. Ware JE Jr, Sherbourne CD. The MOS 36-item short-form health survey (SF-36), I: conceptual framework and item selection Med Care 1992;30:473-483.

MEDLINE

7. Hamilos DL. Chronic sinusitis J Allergy Clin Immunol 2000;106:213-227.

MEDLINE CROSSREF ABSTRACT FULL TEXT

8. Harlin SL, Ansel DG, Lane SR, Myers J, Kephart GM, Gleich GJ. A clinical and pathologic study of chronic sinusitis: the role of the eosinophil J Allergy Clin Immunol 1988;81:867-876.

MEDLINE

9. Stoop AE, van der Heijden HA, Biewenga J, van der Baan S. Eosinophils in nasal polyps and nasal mucosa: an immunohistochemical study J Allergy Clin Immunol 1993;91:616-622.

MEDLINE

10. Hamilos DL, Leung DY, Wood R, Cunningham L, Bean DK, Yasruel Z. Evidence for distinct cytokine expression in allergic versus nonallergic chronic sinusitis J Allergy Clin Immunol 1995;96:537-544.

MEDLINE ABSTRACT FULL TEXT

11. Hamilos DL, Leung DY, Wood R, Beam DK, Song YL, Schotman E.

Eosinophil infiltration in nonallergic chronic hyperplastic sinusitis with nasal polyposis (CHS/NP) is associated with endothelial VCAM-1 upregulation and expression of TNF-alpha Am J Respir Cell Mol Biol 1996;15:443-450.

MEDLINE

12. Sanchez-Segura A, Brieva JA, Rodriguez C. T lymphocytes that infiltrate nasal polyps have a specialized phenotype and produce a mixed TH1/TH2 pattern of cytokines J Allergy Clin Immunol 1998;102:953-960.

MEDLINE ABSTRACT FULL TEXT

13. Newman LJ, Platts-Mills TA, Phillips CD, Hazen KC, Gross CW. Chronic sinusitis: relationship of computed tomographic findings to allergy, asthma, and eosinophilia JAMA 1994;271:363-367.

MEDLINE

14. van Burik JA, Magee PT. Aspects of fungal pathogenesis in humans Annu Rev Microbiol 2001;55:743-772.

MEDLINE CROSSREF

15. Katzenstein AL, Sale SR, Greenberger PA. Allergic Aspergillus sinusitis: a newly recognized form of sinusitis J Allergy Clin Immunol 1983;72:89-93.

MEDLINE

16. Ponikau JU, Sherris DA, Kern EB, Homburger HA, Frigas E, Gaffey TA. The diagnosis and incidence of allergic fungal sinusitis Mayo Clin Proc 1999;74:877-884.

MEDLINE

17. Braun H, Buzina W, Freudenschuss K, Beham A, Stammberger H. “Eosinophilic fungal rhinosinusitis”: a common disorder in Europe? Laryngoscope 2003;113:264-269.

MEDLINE CROSSREF

18. Ponikau JU, Sherris DA, Kita H, Kern EB. Intranasal antifungal treatment in 51 patients with chronic rhinosinusitis J Allergy Clin Immunol 2002;110:862-866.

MEDLINE CROSSREF ABSTRACT FULL

TEXT

19. Lanza DC, Kennedy DW. Adult rhinosinusitis defined Otolaryngol Head Neck Surg 1997;117:S1-S7.

MEDLINE

20. Vassallo R, Limper AH. Fungal beta-glucan can yield false-positive results with the limulus amebocyte lysate endotoxin assay Chest 1999;116:583-584.

MEDLINE CROSSREF

21. Swanson MC, Bubak ME, Hunt LW, Yunginger JW, Warner MA, Reed CE. Quantification of occupational latex aeroallergens in a medical center J Allergy Clin Immunol 1994;94:445-451.

MEDLINE ABSTRACT FULL TEXT

22. Wagner JM, Bartemes K, Vernof KK, Dunnette S, Offord KP, Checkel JL. Analysis of pregnancy-associated major basic protein levels throughout gestation Placenta 1993;14:671-681.

MEDLINE

23. Romagnani S. The Th1/Th2 paradigm Immunol Today 1997;18:263-266.

MEDLINE CROSSREF

24. D'Amato G, Spieksma FT. Aerobiologic and clinical aspects of mould allergy in Europe Allergy 1995;50:870-877.

MEDLINE

25. Solomon WR. Assessing fungus prevalence in domestic interiors J Allergy Clin Immunol 1975;56:235-242.

MEDLINE

26. Kauffman HF, Tomee JF, van de Riet MA, Timmerman AJ, Borger P. Protease-dependent activation of epithelial cells by fungal allergens leads to morphologic changes and cytokine production J Allergy Clin Immunol 2000;105:1185-1193.

MEDLINE CROSSREF ABSTRACT FULL

TEXT

27. Mitakakis TZ, Barnes C, Tovey ER. Spore germination increases allergen release from Alternaria J Allergy Clin Immunol 2001;107:388-390.

MEDLINE CROSSREF ABSTRACT FULL TEXT

28. Zureik M, Neukirch C, Leynaert B, Liard R, Bousquet J, Neukirch F. European Community Respiratory Health Survey. Sensitisation to airborne moulds and severity of asthma: cross sectional study from European Community respiratory health survey BMJ 2002;325:411-414.

MEDLINE CROSSREF

29. O'Hollaren MT, Yunginger JW, Offord KP, Somers MJ, O'Connell EJ, Ballard DJ. Exposure to an aeroallergen as a possible precipitating factor in respiratory arrest in young patients with asthma N Engl J Med 1991;324:359-363.

MEDLINE

30. Salvi SS, Babu KS, Holgate ST. Is asthma really due to a polarized T cell response toward a helper T cell type 2 phenotype? Am J Respir Crit Care Med 2001;164:1343-1346.

MEDLINE

31. Magnan AO, Mely LG, Camilla CA, Badier MM, Montero-Julian FA, Guillot CM. Assessment of the Th1/Th2 paradigm in whole blood in atopy and asthma: increased IFN-gamma-producing CD8(+) T cells in asthma Am J Respir Crit Care Med 2000;161:1790-1796.

MEDLINE

32. Hansen G, Berry G, DeKruyff RH, Umetsu DT. Allergen-specific Th1 cells fail to counterbalance Th2 cell-induced airway hyperreactivity but cause severe airway inflammation J Clin Invest 1999;103:175-183.

MEDLINE

33. Ricchetti A, Landis BN, Maffioli A, Giger R, Zeng C, Lacroix JS. Effect of anti-fungal nasal lavage with amphotericin B on nasal polyposis J Laryngol Otol 2002;116:261-263.

MEDLINE CROSSREF

34. Weschta M, Rimek D, Formanek M, Polzehl D, Podbieski A, Riechelmann H. Topical antifungal treatment of chronic rhinosinusitis with nasal polyps: a randomized, double-blind clinical trial J Allergy Clin Immunol 2004;113:1122-1128.

MEDLINE CROSSREF ABSTRACT FULL TEXT

Publishing and Reprint Information

TOP

- [a]*From the Division of Allergic Diseases and Department of Internal Medicine and*
- [b]*the Department of Otorhinolaryngology, Mayo Clinic Rochester;*
- [c]*the Department of Otorhinolaryngology, University at Buffalo, The State University of New York;*
- [d]*the Department of Laboratory Medicine and Pathology, Mayo Clinic Rochester; and*
- [e]*the Departments of Dermatology and Internal Medicine, University of Utah.*
- *Supported by a grant from the National Institutes of Health (AI49235) and by the Mayo Foundation.*
- [*]*Reprint requests: Hirohito Kita, MD, Mayo Clinic Rochester, Guggenheim Bldg 401A, 200 First Street SW, Rochester, MN 55905.*
- *Email address:* kita.hirohito@mayo.edu (Hirohito Kita)
- *Submitted May 13, 2004.*
- *Accepted August 9, 2004.*
- *Copyright © by American Academy of Allergy, Asthma and Immunology*
- *doi: 10.1016/j.jaci.2004.08.012*