

Letter to the Editor

Allergic fungal rhinosinusitis—more than a fungal disease?

To the Editor:

Allergic fungal rhinosinusitis (AFRS) is a noninvasive form of fungal disease mostly caused by *Aspergillus* species.¹ Patients with AFRS commonly present with chronic rhinosinusitis with nasal polyps (CRSwNP), inhalant atopy with specific IgE antibodies present in serum against *Aspergillus* species, elevated total serum IgE, and sinus-obstructing viscoelastic eosinophil-rich material called “allergic mucin,” containing sparse numbers of fungal hyphae.¹ This constellation of findings is shared with allergic bronchopulmonary aspergillosis. Characteristic computed tomography findings including complete opacification of at least 1 paranasal sinus, a typical heterogeneity of the signal within involved sinuses, and the expansion and attenuation/erosion of the bone with displacement of adjacent anatomic compartments can be seen in AFRS (see Fig E1 in this article’s Online Repository at www.jacionline.org).¹

There is no doubt that fungi can function as allergens and induce an allergic IgE-mediated reaction; allergic disease should generally be considered a superimposed problem that contributes with a variable but relatively mild impact on the inflammation seen in most patients with CRS.² In that line of thinking, *Aspergillus* species may induce the formation of specific IgE antibodies to fungal allergens; however, the exorbitant elevations in serum total IgE level that is typical for AFRS cannot be explained. We therefore hypothesized that another stimulus with superantigenic properties is involved in aspergillus-related airway disease with high IgE production.³

Superantigens from *Staphylococcus aureus* are possible candidates for such an effect and have been demonstrated to be involved in upper and lower airway disease, preferentially amplifying T_H2-biased inflammatory reactions in CRS with nasal polyps and asthma, and inducing a local mucosal production of high concentrations of polyclonal IgE and IgG antibodies.⁴ The

mechanisms in AFRS could be similar, with *Aspergillus* preparing the ground for the impact of superantigens by breaking the epithelial barrier and initiating a T_H2 bias. *S aureus* could profit from the disturbed barrier and the weakened innate response of the sinus mucosa, partially created by this T_H2-bias, leading to the alternative activation of macrophages with a decrease in phagocytotic and intracellular killing properties for the germs, and symbioses with *Aspergillus* species to elicit a polyclonal B- and T-cell activation.⁵ A recent retrospective review showed that *S aureus* was significantly more prevalent in the AFRS group compared with other subsets of CRSwNP.⁶

Here we investigated serum samples of AFRS (n = 17), CRSwNP (n = 13), and healthy control (n = 12) patients, sampled in Riyadh, Saudi Arabia, with AFRS being abundant in this region. AFRS was diagnosed according to the clinical criteria of Schubert.⁷ *Aspergillus fumigatus* was found in the nasal samples of patients with AFRS, but not in CRSwNP subjects or controls; concentrations of serum total IgE, specific IgE antibodies to the classical *S aureus* superantigens (SE-IgE), specific IgE antibodies to *Aspergillus* species (m3-IgE), and eosinophil cationic protein were measured by using the CAP system (Phadia, Uppsala, Sweden) as detailed earlier.⁶ We furthermore studied formalin-fixed tissue removed from the sinuses in 3 subjects with AFRS by *in situ* hybridization (PNA-FISH) by using panfungal and *S aureus*-specific probes as detailed before.⁸

In 13 of 17 sera from subjects with AFRS, but not in control or CRSwNP subjects, specific IgE to *A fumigatus* (m3-IgE) could be detected (median and interquartile range, 8.7 kU/L [2.3-12.9 kU/L]); total IgE level was strongly elevated above normal values in patients with AFRS (median [interquartile range], 1220 [226-3287] kU/L). Sixteen of 17 subjects with AFRS also expressed SE-IgE in serum (3.5 [0.6-7.2] kU/L). There was a significant and strong correlation of SE-IgE with total IgE ($r = 0.78$; $P < .002$), whereas m3-IgE did not correlate with total IgE ($r = 0.31$; $P = .37$). However, the co-expression of *A fumigatus*-specific IgE antibodies in AFRS did amplify the total IgE

TABLE I. Serum total and specific IgE concentrations (median = IQR) of patients with AFRS versus non-AFRS CRSwNP and control subjects

	CRSwNP	AFRS	Control	P value	
				AFRS vs CRSwNP	AFRS vs control
Samples, n	13	17	12		
IgE (kU/L)					
Median	220.0	1220.0	36.7	<.005	<.0001
IQR	96.6-448.0	226.8-3287.5	17.7-63.0		
ECP (μg/L)				NS	NS
Median	10.2	21.7	9.3		
IQR	6.1-29.4	9.9-49.0	4.9-31.8		
SE-IgE (kU _A /L)				NS	<.004
n	10/13	16/17	3/12		
Median	0.4	3.5	0.5		
IQR	0.3-0.72	0.64-7.2	0.24-0.93		
m3-IgE (kU _A /L)				<.0001	<.0001
n	0/13	13/17	0/12		
Median		8.7			
IQR		2.3-12.9			

ECP, Eosinophil cationic protein; IQR, interquartile range; NS, not significant.

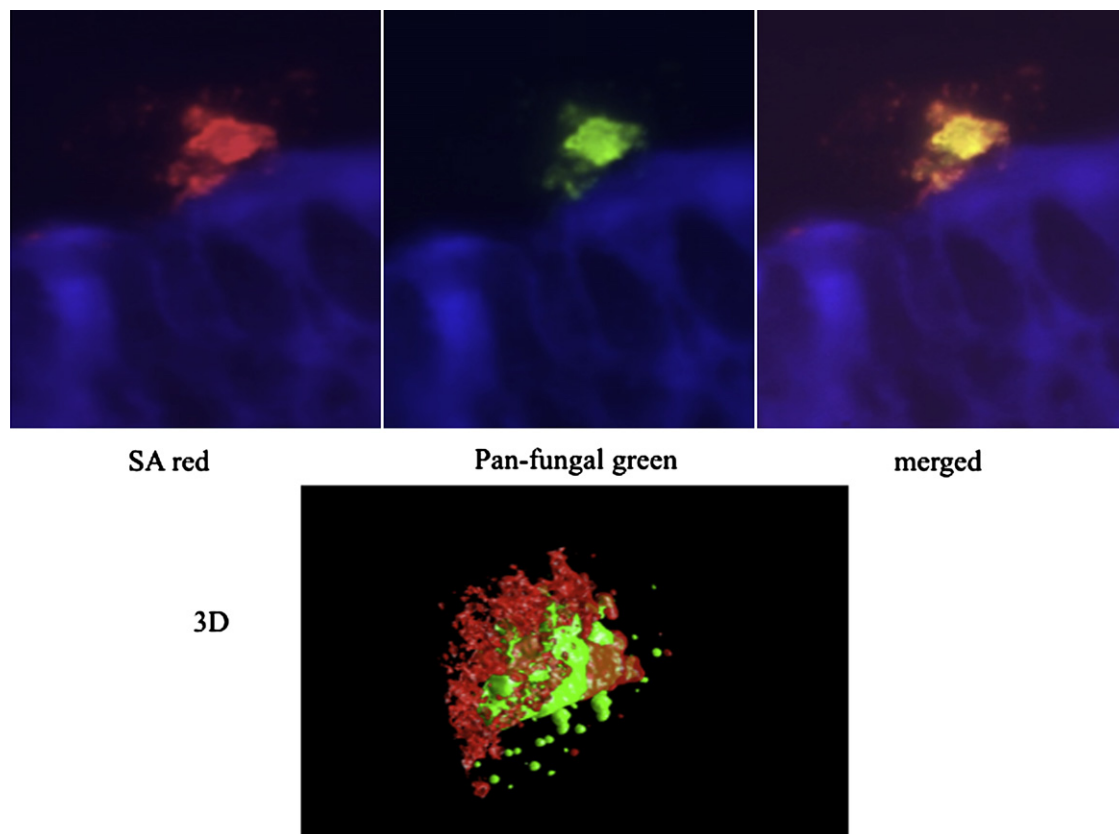


FIG 1. *In situ* hybridization (PNA-FISH) of a biofilm-like structure on the epithelium of paranasal sinus mucosa in a patient with AFRS. Red: *Staphylococcus aureus*; green: fungus (*Aspergillus fumigatus* according to culture). Confocal microscopy, magnification $\times 63$.

concentrations significantly versus CRSwNP. Serum eosinophil cationic protein tended to be higher in patients with AFRS than in control patients (21.7 vs 9.3 $\mu\text{g/L}$), but differences did not reach statistical significance. For all results, please see [Table I](#).

In all 3 tissue samples, we could discover biofilms adherent to the epithelium, carrying fungus and *S aureus* together. A representative confocal microscopy, showing the staining with the fungal probe (*Aspergillus* species has been demonstrated in the same patients by culture), the probe for *S aureus*, and the three-dimensional reconstruction of the combination of both is depicted in [Fig 1](#).

On the basis of our findings of *S aureus* coexisting with *Aspergillus* species within the sinuses, and the presence of SE-IgE in the sera of nearly all subjects with AFRS, significantly correlating with the total IgE (in contrast to m3-IgE, which does not correlate with total IgE), we hypothesize that *S aureus* may play a crucial role in AFRS. We estimate that *S aureus* synergizes with or makes use of *Aspergillus* species in creating a T_H2 tissue signature, and adds its superantigenic activities to the disease, resulting in the high total IgE concentrations typically found in AFRS.⁹ In this way, *Aspergillus* species and *S aureus* benefit from each other's potential to overcome the mucosal barrier, bias the immune system, and cause the fulminant characteristics of AFRS.

This hypothesis is supported by the published literature demonstrating a role for *S aureus* superantigens in upper airway disease, specifically in adult nasal polyp disease unrelated to

AFRS.⁴ The presence of SE-IgE in 10 of the 13 patients with CRSwNP and a total IgE concentration of 220 kU/L in this investigation is in line with former findings.^{3,10} Also, in CRSwNP, we reported a T_H2 signature of the mucosal inflammation, which is amplified by *S aureus* enterotoxins resulting in high total IgE tissue and serum concentrations and an increase in the degree of eosinophilic inflammation. The involvement of *S aureus* and its superantigenic activities in AFRS and possibly allergic bronchopulmonary aspergillosis does open a new insight into the pathophysiology of this disease, may have an impact on our diagnostic tools in aspergillus-related airway disease, and finally may result in new therapeutic options including anti-IgE strategies.¹⁰

The role of *S aureus* in the disease needs to be demonstrated by the local presence of *S aureus*-derived enterotoxins and *S aureus*-specific interventions in comparison to antifungal therapeutic approaches. The contribution of the polyclonal IgE antibodies in patients with AFRS needs to be studied by making use of anti-IgE strategies.

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FIG E1. Typical computed tomography scan of AFRS with complete opacification of the paranasal sinuses, typical heterogeneity of the mucosal signal, and displacement of adjacent anatomic structures.