

Fungi in Chronic Hyperplastic Eosinophilic Sinusitis

Reasonable Doubt

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Abstract

Chronic hyperplastic eosinophilic sinusitis (CHES) is a T-helper (Th)2-like, lymphocyte-initiated, eosinophil-rich inflammatory disease. The complex immune interactions required to orchestrate these processes begin with the presentation of antigen by mature dendritic cells to Th lymphocytes that display the appropriate antigen-specific T-cell receptor. The objective of sinus research must be to identify and target that antigen; this will lead to the cure for this condition. This article reviews numerous models that may be responsible for the pathophysiology of this disorder, including putative roles for allergens, bacteria, and bacterial-derived superantigens, as well as recent interest in fungal-derived antigens. Additionally, we speculate that whatever the inciting cause of CHES may be, it is plausible that once initiated, cellular differentiation pathways may lead to the development of an antigen-independent permanent phase. More than one of these may be valid in different subjects and, furthermore, this list almost assuredly does not explain all cases of CHES. The concept that fungal antigens colonizing the sinuses are responsible for CHES represents an intriguing, but unproven, hypothesis. Presently, the case for the fungus remains circumstantial. The case for fungi will be proved only with definitive proof that T-cells within the sinuses are actively responding to fungal antigens present in the sinus and with the further demonstration that removal of those fungal antigens ameliorates the disease.

Index Entries

Chronic sinusitis; allergic inflammation; fungal colonization; bacterial superantigen; eosinophil.

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Introduction

Chronic sinusitis (CS) is increasingly recognized to encompass several disorders with infectious, inflammatory, and hypersensitivity etiologies. A recent summary statement comprising expert opinion in this field argued that CS consists of two disorders, one which is characterized by the presence of a chronic inflammatory infiltrate with or without neutrophils and generally by the absence of nasal polyps (NP), and a hyperplastic disorder (termed here chronic hyperplastic eosinophilic sinusitis [CHES]) that is characterized by eosinophils and generally by the presence of NPs (1). Chronic inflammatory disorders may reflect a chronic bacterial infection or, more likely, noninfectious processes such as the tissue remodeling and other long-term changes resulting from protracted bouts of recurrent acute sinusitis. However, this article focuses on the pathophysiology of CHES, the more prevalent form of CS. The prominent expression of eosinophils in this disorder has recently led to speculation that this is universally a T-helper (Th)2-like lymphocyte-mediated immune response to fungi colonizing the sinuses. Although evidence in support of the fungal hypothesis is potentially valid in some patients, we argue that it remains at best circumstantial and fails to recognize other mechanisms likely to be relevant to the etiology of this disorder.

CHES with or without NP is an inflammatory disease characterized by the accumulation of eosinophils, fibroblasts, goblet cells, and Th lymphocytes (2,3). However, the prominent accumulation of eosinophils is the diagnostic feature of this condition (2,4–6). Eosinophilic inflammation is a complex process (which is only superficially summarized here) reflecting the need to synthesize these cells, recruit them into the sinus tissue, and activate them to release the toxic cationic granule proteins and other mediators responsible for sinus inflammation. Eosinophilopoiesis primarily reflects the biological activity of the cytokine interleukin

(IL)-5. Other cytokines, including IL-3, and granulocyte macrophage-colony-stimulating factor (GM-CSF), as well as the cysteinyl leukotrienes (CysLTs), synergize with IL-5 in this process.

Recruitment of eosinophils into the sinus tissue reflects the synergistic influences of cellular adhesion and chemotaxis. The induction of P-selectin on endothelium by cytokines and CysLTs and vascular cell adhesion molecule-1 by IL-4, IL-13, and tumor necrosis factor- α are important to adhesion. Important chemotactic factors include CCL11 (eotaxin-1) and other eotaxins (7), platelet-activating factor (PAF), and the CysLTs. Newly synthesized eosinophils display a minimal ability to degranulate in response to inflammatory stimuli. For this to occur, the cells need to be “primed,” an effect mediated primarily by IL-3, -4, and -5 and GM-CSF. Within the sinuses, eosinophils are activated by many compounds, including CCL11, CCL5 (RANTES), IL-1, IL-3, IL-5, GM-CSF, tumor necrosis factor- α , PAF, and the CysLTs (8–13). Finally, although they are normally short-lived cells, many of these factors (including IL-3, IL-5, and GM-CSF) inhibit eosinophil apoptosis and permit cell survival for days or even weeks within the inflammatory milieu.

In CHES, the sinuses contain eosinophil-specific stem cells (14), allowing the autonomous perpetuation of these processes. Because eosinophils are a prominent source of many of these cytokines and lipid mediators, CHES/NP may be a disease of unregulated inflammation in which eosinophils provide the growth factors necessary for their further recruitment, proliferation, and activation once they are recruited (15).

The critical issue deals with defining the immune basis for the development of this complex inflammatory process. Clearly, an initial immune insult is required to orchestrate this recruitment and activation of eosinophils. Current understanding of immune mechanisms suggests that this represents a coordinated response

of dendritic cells and Th-lymphocytes. Dendritic cells mistakenly determine that an infectious (and perhaps parasitic) assault on the sinuses occurs and that the usual state of immune quiescence or "ignorance" needs to be terminated and Th cells need to be activated. Th cells are responsible for targeting the ensuing immune response in an antigen-specific fashion through their specific T-cell receptor and (through their cytokine repertoire) determining that the ensuing inflammation is eosinophilic. The latter response reflects the development of a Th2-like cytokine repertoire. The challenge of sinusitis is determining the immune insult responsible for generating mature dendritic cells capable of recruiting and activating these Th cells and elucidating the antigenic target of these Th cells. Because T-cells can only be activated via engagement of their specific receptor, the most important objective for sinusitis research is determining this T-cell target. The identification of this ligand is the basis for the current debate.

Allergic Mechanisms in CHES

The similarities between the inflammatory processes observed in CHES and those of allergic rhinitis and asthma have led to speculation that CHES might represent an allergic process. To support this finding, CS has been linked to the increased expression of allergies. Allergic rhinitis was observed in 56% of 200 consecutive patients with CS (16). Similarly, 50% of children with "recalcitrant" sinusitis had positive skin tests, and 78% of patients with "severe" sinus disease also had positive skin tests (16). In a slightly contrasting study, slightly less than half of patients with CHES/NP had allergies (17). The significance of these observations is unclear because the prevalence of allergic rhinitis or positive skin tests in relevant, well-matched control populations was not reported and can be highly variable, which is partly reflective of operator variables in the methods by which skin testing is performed. The recogni-

tion that a positive skin test in a patient with CHES cannot be interpreted as signifying an allergic etiology is more problematic.

One argument provided against a role for allergy in CHES is the lack of evidence that inhaled allergens access the sinus cavities (arguably with the exception of fungal allergens). In one study, radiolabeled allergen did not access the sinuses (18). Using a much smaller radioisotope placed on the nasal mucosa, researchers showed that isotope could access the sinuses with nose blowing but not with nasal breathing, sneezing, or coughing (19). These studies were performed in healthy volunteers, and even this limited potential access of allergens into the sinuses is likely to be reduced with the advent of sinus disease and the concomitant occlusion of the sinus ostia. Ironically, this natural protection of sinus tissue from inhaled allergens is likely to be reversed by sinus surgery.

Despite the unlikelihood that allergens directly access the sinuses, intriguing evidence exists to support a plausible role for allergens in CHES. Therefore, insufflation of ragweed pollen in sensitive subjects was associated with increased hyperemia and metabolic activity in the ipsilateral maxillary sinus, and similar changes were observed during the ragweed season that became inactive postseasonally (20,21). In a different study, 37 subjects with CS underwent 73 nasal provocation challenges. A total of 32 positive challenges were associated with either radiographical changes in the maxillary sinuses (increased mucosal edema or opacification) or sinus symptoms such as increased pressure, headache, or otalgia (22). The strongest evidence that inhaled allergens could have a role in CHES is derived from studies in which catheters were inserted into both maxillary sinuses. Nasal challenges were performed with grass or ragweed extracts instilled into one nostril, after which bilateral sinus lavages were performed. In these studies, nasal allergen challenges triggered eosino-

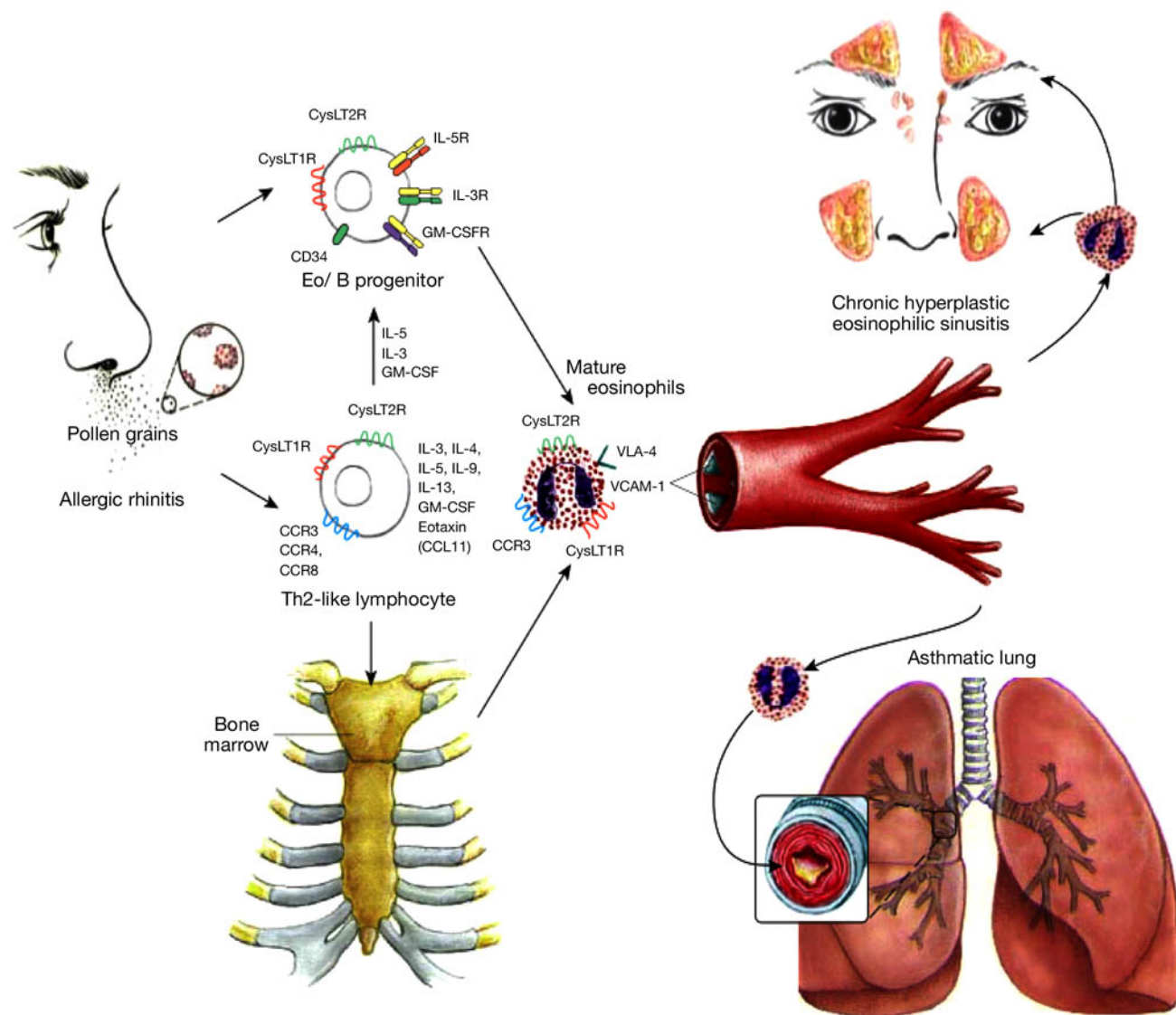


Fig. 1. Model displaying humoral mechanisms of allergic rhinitis exacerbation of CHES (and asthma). See text for details. (Adapted from ref. 25.)

phil influx into the ipsilateral—but more impressively, in the contralateral—maxillary sinus (23).

These data support a role for inhalant allergens in the pathophysiology of CHES but suggest that this does not necessarily require direct allergen access into the sinuses. This suggests development of a systemic inflammatory mechanism as summarized in Fig. 1. Allergen inhaled into the nares activates cellular immune responses involving prominently Th2-like lymphocytes. Lymphocytes recirculate systemically, and it is their ability to access the bone

marrow that is responsible for the delivery of IL-5 and other signals responsible for orchestrating an allergic immune response, such as the maturation of eosinophils as well as basophils and mast cells. Additionally, eosinophil/basophil precursors present in the nasal tissue may also be released into the circulation, where they mature into effector cells (14,24,25).

Newly generated eosinophils (and other inflammatory cells) generated through these mechanisms express adhesion molecules (“addressins”) that direct their migration and adher-

ence to inflamed tissue that displays relevant counterligands. For example, very late antigen-4 on eosinophils interact and adhere to endothelial cells that express the counterligand vascular cell adhesion molecule-1. This leads to the further influx of eosinophils into the nares. However, in the presence of established inflammation in the sinuses (or in the lungs), vascular endothelium in these organs also expresses the relevant counterligands that lead to inflammatory cell adherence. These inflamed organs are also rich in chemotaxins such as CCL11, PAF, and CysLTs, which drive the diapedesis and chemotaxis of these cells into the inflamed tissue. Through these systemic humoral mechanisms, allergic inflammation in the nares can produce increased inflammation in the sinuses and lungs, including the reported eosinophil influx (25), but only in the presence of pre-existing inflammation in those organs. Although evidence exists to support this model, it remains only a model. For example, no convincing studies have been performed to show that allergen avoidance or immunotherapy has any beneficial effects in CHES.

Bacterial Antigens/Bacterial Superantigens in CHES

An alternative proposal is that bacterial colonization may be the pathogenic agent that drives CHES—specifically, superantigens produced by *Staphylococcus aureus* (26). Superantigens have been implicated in the pathogenesis of other chronic inflammatory diseases such as atopic dermatitis, Kawasaki disease, and rheumatoid arthritis (27). The *S. aureus*-derived enterotoxins (SAEs) are a group of superantigens that activate the immune system by crosslinking the V_β-chain of the T-cell receptor with major histocompatibility complex class II molecules on accessory cells. Binding occurs outside of the peptide-binding groove and can lead to activation of all T-cells that express the target V_β-chains (up to 30% of all T-cells) (28).

The role of bacterial colonization in subjects with CS (with and without nasal polyps) was compared with a healthy control group (29). There was no difference in the colonization rates in the control group compared to the CS group (33.3 and 27.3%, respectively). However, in the group of subjects with CS and NPs, the colonization rate was 66.7%, and this rate jumped to 87.5% if a subgroup of aspirin-sensitive subjects was examined. IgE production can occur locally, because secondary lymphoid tissue has been found in the polyp tissue that contains polyclonal IgE (30). The occurrence of IgE antibodies to SAEs in tissue homogenates paralleled colonization rates and there was a sevenfold increase in the likelihood of finding IgE to SAEs in NPs if the subjects also had a diagnosis of asthma (29). Previous work by this group demonstrated an association of SAEs to eosinophilic inflammation in nasal polyposis (31).

Although these studies suggest a role for SAEs in CHES, the data remain correlative and many issues need to be addressed, such as what factors determine whether an individual becomes a carrier and why most carriers largely remain symptom free. Presumably, at some point a balance is tipped and subjects with colonization develop CHES and, eventually, NPs. Superantigens may require the existence of pre-existing CS and the availability of T-cells on which the SAEs can act. The critical proof of a role for SAEs in CHES could be provided by immunological data demonstrating overexpression of relevant V_β-chains specific to the SAEs in the sinus tissue and that elimination of the bacteria and responding T-cells ameliorates the disease.

Fungi and CHES

Recently, much speculation has been generated regarding the putative role of fungal antigens in the pathophysiology of CHES. Arguments supporting this concept are provided elsewhere in this issue. A Th2-like immune

response to fungi colonizing the sinuses could explain the development of an eosinophil-enriched inflammatory process. Intriguing preliminary data have been generated to support this hypothesis. Evidence that fungi have a role in CHES remains circumstantial.

The key to proving an immune basis for fungi in CHES is providing categorical proof that there are activated T-cells *in the sinuses* responding to the fungi *colonizing the sinuses*. The authors of the fungal hypothesis initially identified fungal antigens in the sinuses of patients suffering from CHES. Historically, the investigation of the role of fungi in respiratory disorders has been stymied by the difficulties in culturing fungi out of mucus. Therefore, the presence of *Aspergillus* ironically was always viewed as a minor criterion for the diagnosis of allergic bronchopulmonary aspergillosis, despite the need for *Aspergillus* to be present, by definition. Similarly, allergic fungal sinusitis is primarily defined by the appearances of the allergic "mucin" with histological evidence of fungi, relatively specific changes observed on radiography, and the presence of allergic sensitization to the fungus but not by the culturing of the causative fungus itself. The fungal hypothesis of CHES was generated partly through the development of techniques to culture fungi out of mucus (32) or to visualize fungal hyphal elements (33). Not surprisingly, because of the prevalence of airborne fungal spores (at least in humid environments), fungi were universally identified at similar frequencies in both CHES and healthy controls when these studies were performed. Therefore, the presence of fungal antigens cannot be (and has not been) used to diagnose CHES. Rather, the fungal hypothesis states that it is an immune, Th2-like lymphocyte-mediated response to these fungal antigens that is responsible for CHES.

It is proposed that despite the similar presence of colonizing fungi, healthy subjects remain healthy because they do not respond immuno-

logically. Although specific IgE is associated with a Th2-like immune response, it reportedly does not develop to the fungal antigens, and these patients have negative skin tests and negative IgE immunoassays (34). The concept that Th2-like immune responses can develop and can even include IL-4 and IL-13 but not generate specific IgE is certainly plausible; for example, this concept is exactly what is observed in nonallergic ("intrinsic") asthma. A difficulty created by the absence of an IgE-mediated immune response is the challenge it creates for proving the existence of an immune response to fungal antigens in CHES that distinguishes those patients from the healthy controls. As noted earlier, proof of the fungal hypothesis requires the categorical demonstration that there are activated Th-lymphocytes present in the sinuses or associated lymphatic tissue that specifically respond to the colonizing fungi. Those data do not exist. Identification of the activated T-cell target is easier in the mouse, where tools exist to identify and quantify antigen-specific T-cells (e.g., through the use of major histocompatibility complex/antigenic peptide "tetramers"). In humans, these approaches are not generally available.

In lieu of studies using sinus-derived T-cells, data have been generated showing that patients with CHES have peripheral blood T-cells that proliferate and produce IL-5 in response to *Alternaria* (34). Although interesting, it is not clear how relevant these studies are to the sinus disease. Circulating Th2-like cells could reflect the prevalence of *Alternaria* in the subject's environment and the secondary development of immune responses. These data need to be compared with those derived from healthy nonallergic, nonasthmatic controls, as well as controls with allergic rhinitis and asthma. Circulating *Alternaria*-specific T-cells may be a relatively common and nonspecific development in allergic rhinitis, asthma, and CHES. Sensitization to *Alternaria* was previously shown to be common and is a risk factor for the devel-

opment of asthma (35,36), and hypersensitivity to this fungus in asthma and CHES may reflect the ubiquitous nature of *Alternaria* and the presence of a robust Th2-like lymphocyte-mediated disease. With the proliferation of mature dendritic cells in the airways, immune responses to bystander allergens (such as those derived from *Alternaria*) are likely to be developed and do not necessarily suggest that these immune responses are responsible for disease diathesis.

Although studies with sinus-derived T-cells are ultimately required, when using peripheral blood-derived cells, it is incumbent to show specificity of these immune responses to the fungi present in the sinuses. In subjects whose sinuses contain *Alternaria*, there should be a high precursor frequency of circulating *Alternaria*-specific T-cells compared to other uninvolved fungi such as *Cladosporium* or *Aspergillus*. Similarly, in subjects in whom CHES is ascribed to *Aspergillus*, relevant Th cells should be enriched in their circulation, with few—if any—cells responding to *Alternaria* or other irrelevant fungi.

Enthusiasm for a fungal etiology of CHES has been derived from the visualized ability of CHES-derived eosinophils to engulf and destroy fungi. Although this finding produces dramatic images, it cannot be used as mechanistic evidence in CHES. Eosinophils presumably evolved with the purpose of destroying invasive fungi and parasites. Destruction of fungal hyphae may merely represent a nonspecific feature of the activated “primed” eosinophil. It is equally plausible that any “primed” eosinophil (e.g., those derived from eosinophilic esophagitis, Churg Strauss vasculitis, asthma, or idiopathic hypereosinophil syndrome) could function equally well in a fungicidal assay. Eosinophils lack antigen receptors. Therefore, their ability to destroy fungal hyphae represents a nonspecific innate immune response or opsonization of the fungus—in the absence of IgE—by IgG (or IgA). However, whether this is an innate

immune response or eosinophils responding in vitro to opsonized targets, the destruction by eosinophils of hyphae does not provide evidence that this is a fungal-mediated disorder.

Finally, the best proof for an etiological role of fungi in CHES can be derived from observations that elimination of the fungal-derived antigenic load ameliorates the disease (in the absence of a nonspecific anti-inflammatory effect that can be ascribed to the antifungal agent). Despite initial evidence of benefit in an uncontrolled study (37), two subsequent studies either failed to show clinical benefit (38) or showed, at best, only modest benefit (39). The disappointment of these latter results may reflect the failure of the investigators to prove that their interventions actually decreased fungal antigen load. Therefore, it remains plausible that intervention with better, perhaps longer acting, and more stable agents might provide the missing therapeutic evidence. Presently, in the absence of convincing immunological data demonstrating sensitization with Th2-like cells specific for the colonizing fungi present in CHES and evidence for clinical improvement with antifungal agents, the case against the fungus remains unproven.

Auto-Immune/Homeostatic Breakdown/Postviral Restructuring

An alternative hypothesis to all of those previously discussed is that CHES could develop—or at least could persist—in the absence of the need for any external antigen. Several concepts, which are not mutually exclusive, could permit development. As noted previously, once established, the primary source for IL-5 and other cytokines responsible for eosinophilic inflammation in CHES may be the eosinophil (11). CHES (and asthma) could reflect a cellular differentiation process. Inflammatory cytokines are generally tightly regulated and are only transiently expressed by newly activated immune cells. However, it is plausible

that with persistent inflammation, a differentiation process occurs in which these cytokines become constitutively expressed independently of antigen or other activation signal and are refractory to the usual signals that act to terminate inflammation. Through chromatin remodeling and other epigenetic mechanisms, the expression of this inflammatory phenotype persists in daughter cells after cellular division. This is certainly consistent with the results observed in “extrinsic” asthma, which similarly to CHES, is irreversible once established. In “extrinsic” asthma, although allergen avoidance can prevent exacerbations and reduce bronchial hyperreactivity, the underlying asthma is never completely abolished. Similar processes may be extant in CHES.

One mechanism for the development of prolonged inflammatory cellular differentiation after an acute immune insult is the mechanism observed after viral infections. For example, in established asthma, constitutive expression of the interferon-dependent signaling protein, stat1, is observed (40). Infection of respiratory epithelium by respiratory viruses may be associated with prolonged expression of the stat1 and other signaling and differentiating factors long after the virus is cleared and persists in daughter cells after replication (41). It is reasonable to suggest a model in which viral infection of the sinuses, perhaps occurring in synergy with concomitant allergic inflammation and in a genetically susceptible host, could create the framework for the development of CHES.

A final mechanism for CHES in the absence of need for persistent exposure to any external immune insult is the possibility that a “self”-antigen drives a persistent Th2-like response. This has not been explored but is certainly consistent with the persistence of this disease once established. As noted earlier, none of these mechanisms are mutually exclusive. CHES may be initiated as a viral response, after which the loss of homeostasis and increased expression of mature dendritic cells in the sinuses

Table 1
Pathophysiological Mechanisms in CHES

Allergens	Inhalant allergens
Bacterial colonization	Bacteria as sources of antigens: <i>Staphylococcus</i> Bacterial superantigens Bacterial biofilms
Fungal colonization	<i>Alternaria</i>
Antigen-independent mechanisms	Postviral gene modulation Cellular differentiation Auto-immunity

could lead to the loss of immune ignorance to self-antigens and the persistence of this disorder as an auto-immune disease.

Conclusions

CHES is a Th2-like lymphocyte-initiated eosinophil-rich inflammatory disease. The complex immune interactions required to orchestrate these processes must begin with the presentation of antigen by mature dendritic cells to Th-lymphocytes that display the appropriate antigen-specific T-cell receptor. The overarching goal of sinus research must be to identify and target that antigen; this will lead to the cure for this condition. This article has reviewed numerous models that may be responsible for the pathophysiology of this disorder (summarized in Table 1). This list is assuredly incomplete and does not explain all cases of CHES. Different mechanisms are likely relevant in different individuals, and these are not mutually exclusive; therefore, more than one pathway may be relevant in an individual CHES sufferer. For example, Staphylococcal-derived superantigens can only act on pre-existing Th-cells that display the correct T-cell receptor target, and the natural ligand for that T-cell represents the primary immune insult. The concept that fungal antigens colonizing the sinuses are responsible for CHES represents an intriguing but unproven hypothesis. Presently, the case for the fungus remains circumstantial.

Only with definitive proof that T-cells within the sinuses are actively responding to fungal antigens present in the sinus and only with the further demonstration that removal of those fungal antigens ameliorates the disease can the case against fungi be proved.

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