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## Perspectives on the etiology of chronic rhinosinusitis: An immune barrier hypothesis

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### Abstract

**Background**—Chronic rhinosinusitis (CRS) has been defined as persistent symptomatic inflammation of the nasal and sinus mucosa resulting from the interaction of multiple host and environmental factors. Recent studies have implicated *Alternaria fungi or toxigenic Staphylococcus aureus* as critical agents in CRS pathogenesis. The emphasis on environmental agents in CRS etiology has focused interest toward elimination of those agents as the prime mechanism of therapy. This viewpoint is in marked contrast to the current perspective on some other chronic inflammatory epithelial disorders that afflict the skin, lungs, and gut, wherein host factors are believed to predispose to disease expression in the presence of ubiquitous environmental agents.

**Methods**—The current review evaluates CRS etiology from this perspective and considers that CRS develops, in part, as an outcome of a dysfunctional host response. Specifically, evidence from our laboratory and others will be reviewed indicating that CRS is associated with a failure of the mechanical and immunologic barriers across the nasal mucosa. The hypothesis would further propose that genetic and epigenetic variation predisposes susceptible individuals to barrier failure in the presence of environmental stress leading to CRS.

**Results**—From this unifying perspective, bacteria and fungi are seen as disease modifiers rather than primary etiologic agents.

**Conclusion**—The goal is to place concepts of CRS pathophysiology in a framework consistent with a current understanding of chronic inflammation in general and epithelial disease in particular.

### Keywords

Adaptive immune system; chronic rhinosinusitis; fungal hypothesis; immune barrier hypothesis; innate immune system; nasal polyps; superantigen hypothesis

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The sinonasal tract is the site of interface with the external environment where foreign particulates, antigens, and potential pathogens are encountered and typically cleared with minimal tissue reaction. In a significant percentage of the population, however, a chronic inflammatory infiltrate in the mucosa is apparent, resulting in the symptoms, physical findings,

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and radiographic changes associated with chronic rhinosinusitis (CRS).<sup>1</sup> The overwhelming majority of cases are idiopathic although a small percentage can be associated with established genetic diseases such as cystic fibrosis (CF). Idiopathic CRS has been divided into CRS with nasal polyps (CRSwNP) and a tendency toward T-helper type 2 (T<sub>H2</sub>) cytokine polarization and CRS without nasal polyps (CRSsNP) associated with T<sub>H1</sub> cytokine polarization; it remains unclear whether these represent a continuum of severity or distinct pathophysiological entities.<sup>2,3</sup>

Medical treatments for CRS—most commonly antimicrobials and corticosteroids—are prescribed to decrease the amount of antigenic stimulation and/or decrease the inflammatory host response. Endoscopic sinus surgery addresses mechanical factors that accentuate the symptoms of CRS, typically providing significant quality-of-life improvement for patients that have failed medical therapy.<sup>4,5</sup> Although success rates with endoscopic sinus surgery appear to be similar in CRSwNP and CRSsNP, surgery does not directly address mucosal inflammation, resulting in symptom persistence or recurrence in a recalcitrant minority.<sup>6</sup> Progress has been hampered by a lack of understanding of the nonmechanical factors that foster this mucosal inflammation—essentially the etiology of CRS.

The etiology of idiopathic CRS remains a matter of vigorous debate and multiple host and environmental factors have been implicated.<sup>1,2</sup> Nevertheless, interest has centered on presumed microbial agents that set the inflammatory cascade in motion. This stems from the preantibiotic era wherein surgical sinonasal disease most commonly consisted of intervention for acute processes, a T<sub>H1</sub> host response, and undeniable attribution to invasive, infectious pathogens.<sup>1,7</sup> In the modern era, surgical sinonasal pathology is typically characterized by a chronic, noninvasive inflammation with a mixed T<sub>H1</sub>/T<sub>H2</sub> cytokine response.<sup>3</sup> Although the role of infectious agents in the development of CRS remains unclear, recent studies have implicated *Alternaria* fungi or toxigenic *Staphylococcus aureus* in CRS pathogenesis.

## FUNGAL HYPOTHESIS

The fungal hypothesis proposes that patients with CRS mount an eosinophilic response to fungi, with initial evidence showing some degree of fungi and eosinophilic mucin in all patients with CRS.<sup>8-11</sup> Follow up *in vitro* studies exposed peripheral blood mononuclear cells (PBMCs) to *Alternaria* fungal extracts; cells from CRS patients generated a mixed T<sub>H1</sub>/T<sub>H2</sub> cytokine profile while cells from normal patients did not respond.<sup>10</sup> Subsequent efficacy studies showed improvement in CRS patients using topical antifungal nasal rinses.<sup>12</sup> A 60-kDa component of the *Alternaria* fungus was shown to trigger degranulation of eosinophils from CRS patients by acting on protease-activated receptors (PARs).<sup>13</sup> Collectively, these data were interpreted to be consistent with a T-cell-driven, non-IgE-mediated hypersensitivity response that culminated in the attraction and specific targeting of eosinophils against colonized fungi in the nasal lumen of CRS patients with subsequent degranulation and mucosal damage. In this hypothesis CRSwNP and CRSsNP are viewed as differing forms of one disease resulting from a single pathogenic mechanism of variable intensity.

The mechanistic implications of the fungal hypothesis are twofold: first, *Alternaria* proteins are apparently recognized by antigen-presenting cells (APCs) and presented to T cells with a T<sub>H1</sub>/T<sub>H2</sub> cytokine response that attracts and activates eosinophils. Second, *Alternaria* is hypothesized to trigger the intraluminal targeting and degranulation of eosinophils by a protease-dependent mechanism. Data thus far presented, however, fails to show any specific T-cell receptor (TCR) responses to fungal antigens that are unique in CRS.<sup>10</sup> The obvious question is raised as to whether the cytokines induced in this study were the result of nonspecific protease effects of the fungal extract on PBMCs already activated by the concurrent asthma rather than fungal antigen presentation and T-cell responses.<sup>10</sup> Fungal extracts have

established non-specific protease effects distinct from any hypothetical immunologic interactions with TCRs and the amount of extract used in these *in vitro* studies may be far higher than would occur at the sinus mucosal surface *in vivo*.<sup>13-16</sup>

In summary, current data supporting the fungal hypothesis of CRS suggests that high levels of *Alternaria* can trigger effects on PBMCs and eosinophils obtained from patients with CRS, although it is not clear that this is a disease-specific response. The clinical extrapolation of these findings suggests that intranasal fungi in a patient with CRS would probably exacerbate the disease process through protease effects on nasal epithelial cells as well as activated eosinophils and lymphocytes present in the nose. It is unclear whether *Alternaria* has any relevance to the establishment of CRS in the first place, however. Furthermore, in contrast to initial promising results, subsequent trials using topical amphotericin failed to improve the clinical signs and symptoms in CRS patients.<sup>17</sup> Given these issues, it is reasonable to conclude that the role of fungi in CRS etiology remains unclear.

## SUPERANTIGEN (SAg) HYPOTHESIS

The SAg hypothesis proposes that *S. aureus*, perhaps protected by biofilms or sequestered within epithelial cells, secrete SAg toxins that result in a generalized stimulation of T cells, cytokine release, and a local polyclonal IgE response, all of which stimulate eosinophil recruitment and the clinical and histopathological changes associated with CRSwNP (Fig. 1).<sup>18-20</sup>

In support of the SAg hypothesis, studies have shown an association between the presence of staphylococcus by nasal culture and nasal polyposis.<sup>21</sup> Specific IgE directed against the toxins in polyp tissue has been established in ~50% of CRSwNP patients.<sup>18</sup> Nasal tissue from CRSsNP and normal control patients had a comparatively low level of toxin-specific IgE.<sup>20</sup> Evidence suggests that SAg stimulates local immunoglobulin production in CRSwNP patients, possibly through direct effects on B cells in the nasal mucosa.<sup>22</sup> Skewing of the TCR V $\beta$  domains of polyp-dwelling lymphocytes has also been shown in ~50% of CRSwNP patients, which are changes consistent with local exposure to SAg toxins.<sup>23,24</sup> In addition, staphylococcal SAg toxins themselves have been detected in a portion of CRSwNP patients but were absent from controls.<sup>25</sup> Most recently, *in vitro* studies have indicated that staphylococcal SAg favor T<sub>H</sub>2 cytokine release from nasal mucosa, a pattern that is particularly skewed in nasal polyp samples.<sup>26</sup> These same studies also showed that another staphylococcal protein A (SpA) induces mast cell degranulation in nasal mucosa, further linking this organism with the pathogenesis of nasal polyposis.<sup>26</sup> In comparison, data supporting an SAg effect in CRSsNP is thus far lacking, implying that CRSwNP and CRSsNP are diseases with distinct etiologies.<sup>21,25</sup>

In summary, multiple lines of evidence indicate that perhaps one-half of CRSwNP patients show evidence of SAg exposure. Nevertheless, given the relatively ubiquitous nature of toxigenic staphylococci, it remains unclear why only a fraction of exposed individuals develop polyps. Conversely, at least one-half of the CRSwNP cases have no evidence of SAg responses, despite presenting with a similar phenotypic picture. CF patients have a known susceptibility to both staphylococcal colonization and polyp formation, but little evidence of demonstrable SAg effects and a strikingly distinct histology.<sup>27</sup> Given the absence of a unique histological or molecular phenotype, it is our view that SAg are best considered to be disease modifiers in CRSwNP at this point in time. The association of staphylococcal SAg with other epithelial diseases such as atopic dermatitis (AD), asthma, and ulcerative colitis (UC) provides indirect support for this view.<sup>28-31</sup>

## MECHANICAL AND IMMUNOLOGIC BARRIER OF THE NASAL MUCOSA

CRS occurs at the interface of the nasal mucosa with the external environment; it is therefore tempting to speculate that the patient manifests a response to foreign material in the nose that results in a persistent cellular inflammatory infiltrate triggering clinical disease. As discussed previously, current prevailing theories have focused interest on the identification of the predominant, presumably microbial, agents inciting CRS rather than searching for a putative defect(s) in the host response. Data confirming either fungi or staphylococci as the primary antigenic/etiologic agent triggering CRS are limited, however, and clinical success with either antifungals or antibiotics has been unimpressive. Furthermore, these two classes of organisms can be identified in the nasal lumen of a high percentage of normal people without CRS, indicating that disease expression will manifest only in susceptible individuals. From this perspective, CRS may be viewed as analogous to inflammatory bowel disease, wherein the tolerance mechanisms toward commensal organisms are impaired.<sup>32</sup> In this setting, it would appear worthwhile to search for defects in the immune response in CRS patients, in addition to attempting to identify unique environmental agents.

The degree of bacterial colonization in the gastrointestinal tract is far greater than in the airways, but the upper respiratory tract is not sterile, and the mechanical and immunologic barrier of the nasal mucosa is designed to expeditiously manage the constant load of foreign material with minimal collateral damage. Structurally, the nasal mucosa consists of an epithelial layer of ciliated, pseudostratified, columnar cells joined by tight junctions, interspersed with goblet cells. Beneath the epithelium reside lymphocytes, plasma cells, macrophages, dendritic cells (DCs), vascular arcades, and glands. Ciliary motility and the structural integrity of the epithelium serve as mechanical factors limiting antigenic stimulation. Allergens, fungi, and bacteria often contain proteolytic activity, which may diminish epithelial integrity, while viruses often have the capacity to lyse epithelial cells; all of these agents expose the underlying tissue to foreign stimulation. Despite these exposures, epithelial integrity is usually maintained and, when injury does occur, repair processes restore the mechanical barrier.

Thus, mechanical barriers, effective mucociliary clearance, and optimal healing limit the degree of antigenic stimulation of immune cells residing in the mucosa. Despite this impressive barrier function, animate and inanimate matter will stimulate the mucosal immune system, which must distinguish between commensal organisms and potential invading pathogens without excessive tissue damage. Two distinct but integrated immune responses to microbial entities and foreign proteins have been described: innate and acquired. The innate immune system refers to inborn resistance that is present before the first exposure to a pathogen. Innate responses are initiated by membrane-bound and cytoplasmic pattern recognition receptors (PRRs) that recognize pathogen-associated molecular patterns (PAMPs) found in parasites, viruses, bacteria, yeast, and mycobacteria.<sup>33</sup> PAMPs are conserved molecular patterns that are common among significant numbers of pathogens; recognition of PAMPs by PRRs serves as a “danger” signal to the host immune system.<sup>34</sup> PRRs also identify cellular damage through detection of debris from necrotic cells and the combined recognition of danger and damage signals sets in motion a response consisting of endogenous antimicrobial, antiviral, and antiprotease products designed to aid pathogen clearance and preserve the epithelial barrier.<sup>35</sup> In addition to the release of innate protective agents, PRR activation triggers the release of chemokines and cytokines mediating the inflammatory response that attracts innate cellular defenses such as neutrophils (Fig. 2). The stimulation of PRR also sets in motion and ultimately determines the nature of the acquired immune response.<sup>36</sup>

The two best-characterized classes of PRRs are the toll-like receptor (TLR) family and the NOD-like receptor family.<sup>34-35</sup> TLRs are transmembrane receptors expressed on multiple cell

types including respiratory epithelial cells.<sup>37-39</sup> TLR2 plays a prominent role in responses to Gram-positive bacteria (including *Staphylococcus*) as well as many fungal PAMPs. TLR3 responds to viral replication products, TLR4 recognizes endotoxin and TLR5 responds to components of flagellin.<sup>34</sup> The NOD-like receptor family includes NOD1 and -2, which are important in the recognition of bacterial cell wall products including staphylococci.<sup>40</sup>

The innate immune response in the sinonasal tract includes antimicrobial factors that can directly interact with potential pathogens.<sup>41-43</sup> The integration of the innate and acquired immune responses in the sinonasal tract has not been extensively studied but likely begins with the recognition of PAMPs and cellular damage by multiple cell types that respond by secreting immune activating factors including cytokines that stimulate APCs and chemokines that attract the cellular components of the immune response. Damage to the epithelium likely exposes more PRRs to PAMPs, amplifying the immune response; if the PAMP stimulus is sufficiently strong, an acquired immune response will result.

Tissue DCs are particularly important in generation of the acquired immune response, acting as APCs. After stimulation by PRRs through PAMP recognition, DCs become activated, cease phagocytic activity, and acquire chemokine receptors that lead them to migrate to lymph nodes where they present antigen to T<sub>H</sub> cells. IL-6 has been proposed to be a key cytokine mediating the transition between the innate and acquired immune responses, helping to shut down many components of the innate response and promoting the acquired response.<sup>44</sup> The subsequent T<sub>H</sub> responses have classically been divided into T<sub>H1</sub> and T<sub>H2</sub> based on cytokine profiles. T<sub>H1</sub> responses (IL-12 and IFN- $\gamma$ ) facilitate defense against intracellular pathogens. T<sub>H2</sub> responses (IL-4, IL-5, and IL-13) are of primary importance in parasitic immunity and are associated with allergy and asthma. The type, duration, and intensity of the PAMP stimulus shape the cytokine milieu and are believed to be critical in determining the T<sub>H</sub> profile. Additional T<sub>H</sub> subsets besides T<sub>H1</sub> and T<sub>H2</sub> have recently been recognized, including T<sub>H17</sub> and T<sub>reg</sub> cells.<sup>45</sup> T<sub>H17</sub> responses are thought to play a role in defense against extracellular bacteria and T<sub>reg</sub> cells mediate immunosuppression and immune tolerance. Several cytokines, including IL-6, TGF- $\beta$ 1, and IL-23, appear to be key factors in fostering a T<sub>H17</sub> response. TGF- $\beta$ 1 also promotes T<sub>reg</sub> differentiation, except in the presence of high IL-6, in which case this response is suppressed. T<sub>H1</sub> and T<sub>H2</sub> responses reciprocally inhibit one another and both suppress T<sub>H17</sub> responses.<sup>45</sup> T<sub>reg</sub> cells appear to suppress T<sub>H1</sub>, T<sub>H2</sub>, and T<sub>H17</sub> responses, acting to limit excessive immune responses.<sup>46</sup> T<sub>reg</sub> responses are inactivated *in situ* by strong PRR stimulation, most prominently TLR2.<sup>47</sup> These permit active protective responses to be mediated at the sites of strong PAMP stimulation while suppressing excessive or inappropriate immune responses.

The maturation of T<sub>H</sub> subsets has been studied extensively *in vitro* and in mice, but the conditions necessary for *in vivo* polarization of the acquired effector immune responses in health and disease in the human nose are unknown. As mentioned earlier, however, T<sub>H1</sub> and T<sub>H2</sub> inflammation patterns have been associated with CRSsNP and CRSwNP, respectively.<sup>3</sup> With regard to the T<sub>H17</sub> subset, increased IL-17<sup>+</sup> cells have been detected in CRSwNP by *in situ* hybridization.<sup>48</sup> Immunohistochemistry has also suggested increased expression of IL-17 and its receptor in polyp mucosa in comparison with inferior turbinate.<sup>49</sup> On the other hand, ELISA studies done in our laboratory on nasal tissue extracts from both CRSsNP and CRSwNP patients have failed to establish elevated expression of IL-17A, IL-17B, or IL-17E (Carr T and Suh L, unpublished observations, 2008). With regard the T<sub>reg</sub> subset, recent evidence has emerged suggesting reduced numbers of T<sub>reg</sub> cells in allergic rhinitis and CRSwNP.<sup>50,51</sup>

The cell types of the innate and acquired nasal immune responses, including epithelial cells, neutrophils, eosinophils, mast cells, and lymphocytes, all express PARs on their surface membranes.<sup>16</sup> Although not classically considered host defense molecules, these receptors are

activated by environmental proteases present in bacteria, fungi, and allergens.<sup>52</sup> PAR receptors use many of the same intracellular signaling pathways (*e.g.*, NFκB) triggered by PRR stimulation.<sup>53</sup> In consequence, at the nasal epithelial interface *in vivo*, PAR activation likely modulates both the innate and the acquired immune responses to animate and inanimate foreign material.<sup>16,54</sup>

In summary, the mechanical and innate immune barriers across the nasal mucosa serve to appropriately repel the constant load of exogenous stimulation and limit activation of the acquired immune response. Genetic and/or acquired defects in this complex process may at least theoretically lead to the development of chronic inflammation seen in CRS.<sup>55-57</sup>

## GENETIC FACTORS IN THE DEVELOPMENT OF CRS

CF is the prototypic example of genetic CRS and dysfunction of the mechanical and innate immune barrier presumably mediated through CFTR gene mutations has been shown.<sup>27,58, 59</sup> Genetic factors have long been suspected to influence the development of idiopathic CRS as well, based on familial patterns of disease expression. Interestingly, individuals that are heterozygous for CFTR mutations are found at a higher frequency among patients with CRS than in normal populations.<sup>60</sup> As discussed previously, the nasal immune response is quite complex and the potential genetic derangements in addition to CFTR mutations that could trigger CRS would appear to be numerous. Clinical experience, however, indicates that CRS patients do not typically appear to have systemic immune defects because the chronic inflammation is restricted to the nasal mucosa or, at most, the respiratory tract mucosa, in the vast majority of cases. This suggests that narrowing the focus to genes that regulate the immunobiology of the nasal mucosa will be of primary importance in understanding CRS. Support for this idea comes from genetic studies on other chronic inflammatory mucosal disorders such as asthma, Crohn's disease (CD), UC, psoriasis, and AD. In these disorders abnormalities have been identified in genes that maintain the mechanical and innate immune barrier at the site of interface between self and nonself, as opposed to primary alterations in the acquired immune system.<sup>56,61-63</sup> In overview, this perspective suggests three broad areas of research into the etiology of idiopathic CRS: (1) defects in the mechanical barrier, (2) defects in the innate immune barrier, and (3) defects in the transition from the innate to the acquired immune response.

Evidence for mechanical and innate barrier defects in idiopathic CRS is thus far relatively scant.<sup>43,55</sup> To begin to address this question, a series of experiments was undertaken by our laboratory from three groups of subjects: normals, CRSsNP, and CRSwNP. Real-time quantitative reverse transcription polymerase chain reaction analysis of epithelial cell scrapings from these three groups of patients was performed to determine the expression of specific genes implicated in other chronic inflammatory mucosal disorders including asthma, psoriasis, AD, chronic obstructive pulmonary disease, CD, or UC.<sup>61</sup> Results indicate a statistically significant (5- to 10-fold) decrease in expression of the *S100* family of genes in both groups of CRS patients when compared with healthy controls.<sup>64</sup> These genes, part of the Epidermal Differentiation Complex, participate in epithelial defense and repair and are regulated by the T-cell cytokine IL-22 and its receptor (IL22R).<sup>65,66</sup> Recent studies have suggested that IL22R may be deficient in nasal polyps, suggesting that this may be one mechanism for the observed deficit in *S100* in CRS epithelial cells.<sup>67</sup> In addition to *S100*, a significant decrease in expression was also observed for the gene *SPINK5* in CRSwNP epithelial cells when compared with normal patients. Immunohistochemistry studies confirmed diminished SPINK5 protein expression in nasal polyps compared with normal mucosa.<sup>64</sup> SPINK5 is a secreted antiprotease that likely protects gap junctions from the attack of proteases derived from host sources as well as microbes and allergens.<sup>68</sup> The predictable effect of a loss of epithelial integrity through gap junction degradation is an increase in epithelial cell death with increased exposure of TLR

ligands to PAMPs and an accentuated inflammatory reaction.<sup>56</sup> Furthermore, SPINK5 could also serve to protect PAR receptors, present on multiple cell types in the nasal mucosa, from exogenous proteases.<sup>16</sup> In support of this dual protective effect, both human and animal studies have indicated that *SPINK5* mutations are associated with chronic inflammation at epithelial surfaces.<sup>61,68</sup> In the case of CRSsNP patients, results showed a strong trend for lower expression of *SPINK5* mRNA compared with normal but the difference was not statistically significant. Although preliminary, this report suggests that both forms of CRS may be associated with diminished expression of genes for epithelial repair and innate defense; CRSwNP may be associated with a deficient antiprotease activity, deficient innate immune defense, a more fragile mechanical barrier, and a heightened proinflammatory PAR response<sup>64</sup> (Fig. 3).

PRRs are pivotal in the maintenance of the immune barrier and it is important to better characterize their expression and function in CRS because abnormalities have already been associated with other chronic inflammatory diseases.<sup>61,69-71</sup> In CF, epithelial cells mount an excessive response to TLR2 stimulation mediated by high levels of this protein on the cell surface.<sup>59</sup> This suggested the hypothesis that abnormalities in PRR signaling may be critical in the development of idiopathic CRS as well, possibly TLR2, given its importance in recognition of both fungi and staphylococci. Initial studies using various methodologies revealed inconsistent results, some suggesting elevated and some reduced TLR2 expression in CRS.<sup>27,72,73</sup> A more recent study, however, showed little TLR2 and a minimal functional response to TLR2 ligands in nasal polyp epithelial cells but CRSsNP and controls were not studied.<sup>74</sup> More extensive studies were then undertaken by our group, thus far showing only a trend for diminished TLR2 protein in freshly obtained CRSsNP epithelial cells when compared with normal controls.<sup>75</sup> More significantly, however, epithelial cultures taken from CRS patients and normal controls indicated a decrease in some, but not all, functional responses to TLR2 ligands as assessed by release of cytokines after *in vitro* challenge.<sup>75</sup> These preliminary results show that epithelial cells from CRS patients have a poor spontaneous and TLR2-induced release of neutrophil attracting chemokines such as IL-8, extending previously reported observations, and suggest that there is an abnormality in TLR2 signaling in the nasal epithelium of CRS patients.<sup>76</sup> We tested CRS patients for the *R753Q* dysfunctional allele of *TLR2* but have not detected this rare variant in our patient samples, suggesting the possibility of a more subtle, downstream abnormality in TLR2 signaling.<sup>71,75</sup> In support of this hypothesis, other epithelial cytokines associated with TLR2 responses such as IL-6 were preserved and possibly enhanced in CRS; therefore, a global decrease in nasal epithelial TLR2 signaling was not observed.

A third potential mechanism for CRS development encompasses aberrant communication and/or signaling between the innate and acquired responses. IL-6 is a key cytokine mediating the transition from innate to acquired immunity, possibly acting by dampening the innate response and fostering the acquired response.<sup>44</sup> One key component of IL-6 action is that this cytokine frees helper and effector T cells from the suppressive effects of IL-10 secreted by T<sub>reg</sub>.<sup>77</sup> Studies of tissue extracts indicate the presence of significantly higher levels of IL-6 protein and the soluble IL-6 receptor protein in CRSwNP when compared with CRSsNP and controls, findings that support and extend an earlier study.<sup>78-80</sup> The association of elevated IL-6 and sIL-6 receptor with CRSwNP suggests that derangements of this signaling pathway may be significant in polyp formation; however, net increases in IL-6 signaling have not been indicated in the tissue at this point. The cellular sites of IL-6 production in the nasal mucosa are not known but a low level of secretion has been documented in epithelial cell cultures.<sup>76</sup> This suggests the hypothesis that local increases in IL-6, possibly mediated by TLR2 or PAR stimulation, are sufficient to inhibit local innate immune responses and may also dampen local adaptive immunosuppression mediated through T<sub>reg</sub> cells (Fig. 4). This finding also raises the possibility that the reduced secretion of IL-8 that we have observed in CRS epithelial cells (see

aforementioned data) may reflect the influence of prior exposure to IL-6 *in vivo*. Defects in genes that govern the pathways of production and regulation of IL-6 and its receptor in the nasal mucosa have not been identified although defects in STAT3, a signaling molecule activated by IL-6, have been associated with hyper-IgE syndrome. At least theoretically, however, the net effect of excess local IL-6 in the nasal mucosa would be to diminish the innate immune response and accentuate the acquired response, a pattern consistent with the CRS phenotype.

Another pathway whereby the epithelium helps guide the acquired immune response centers around the protein BAFF (B-cell activating factor of the TNF family), a secreted epithelial factor instrumental in fostering local immunoglobulin responses, in particular B-cell proliferation and class switch recombination.<sup>57</sup> In regard to CRS, BAFF protein and IgA are significantly elevated in CRSwNP but not CRSsNP patients.<sup>81</sup> These results suggest that dysfunction of the BAFF regulatory pathways may account for the excessive local immunoglobulin production described in CRSwNP.<sup>22,82</sup> Furthermore, IgA is a very potent stimulator of eosinophil degranulation. Although high local BAFF levels probably do not account for eosinophil migration, this protein may, through B-cell proliferation, class switch recombination, and production of IgA, indirectly influence mediator release from eosinophils and subsequent mucosal edema characteristic of nasal polyps. In summary, there is emerging evidence that CRS may be associated with changes in gene expression of proteins that regulate the immunobiology of the nasal epithelium including the mechanical and innate immune barriers as well as the subsequent acquired response.

## GENE-ENVIRONMENT INTERACTIONS IN THE DEVELOPMENT OF CRS

Data recounted previously in this study suggest the hypothesis that defects in genes governing key pathways maintaining mechanical and innate immune epithelial barriers may underlie the development of chronic inflammatory epithelial diseases such as CRS. Unlike the simple Mendelian genetics associated with CF, however, idiopathic CRS likely has a much more complex pattern of inheritance involving multiple genes. In fact, even in CF only ~50% of patients exhibit nasal polyps and the range of disease severity is broad despite identical mutations in the CFTR gene.<sup>83</sup> The variation of clinical phenotype indicates that even in CF, the most straight-forward case of genetic CRS, multiple factors in an individual patient strongly determine disease expression.<sup>84</sup> Alterations in expression of genes other than CFTR, mediated *via* genetic variation or environmental effects, apparently combine to affect disease phenotype. Like asthma, CRS thus appears to be a disease of gene–environment interaction with complex immunobiology involving multiple genetic loci.<sup>85,86</sup>

The relative importance of genetic versus environmental influences on disease expression in CRS is completely unknown, but in asthma the concordance rate for disease expression in identical twins is only 50%.<sup>87</sup> From this perspective, alterations in a few genes dispersed within critical pathways create an inherited susceptibility to development of clinical disease that is heavily dependent on environmental exposures.<sup>86,88</sup> Although clear epidemiologic data are difficult to obtain, CRS is widely believed to be increasing in incidence and prevalence, similar to other chronic inflammatory diseases. In asthma and AD, the rate of increase is too rapid to be attributed to genetic mutation and is thus attributed to environmental effects, including changes in microbial exposure early in life (*i.e.*, the “hygiene hypothesis”).<sup>89,90</sup> The effects of changing environment on prevalence of CRS have not been directly studied but it is certainly reasonable to hypothesize that many of the same environmental factors that influence the prevalence of atopy also influence the prevalence of CRS.<sup>91</sup> Beyond the limited scope of atopy, chronic inflammatory disorders appear to be increasing in incidence at all of the sites of interface between self and nonself, including the gut (CD and UC), lungs (asthma and chronic obstructive pulmonary disease), and skin (AD and psoriasis). A mechanistic explanation of



precisely how the hygiene hypothesis promotes clinical disease incidence remains elusive; however, it has been proposed that environmental factors may directly alter gene expression *via* epigenetic mechanisms such as DNA methylation and histone acetylation.<sup>87,92</sup> These concepts would suggest that the absence of appropriate microbial stimulation in childhood may result in epigenetic variations that mediate durable changes in gene expression that later manifest as disease on subsequent challenge. In brief, the CRS phenotype most likely results from the combined effect of genetic variation and acquired epigenetic effects across critical pathways that control the immunobiology of the nasal mucosa. Epigenetic changes create *de facto* genetic changes by altering gene expression without directly altering the DNA sequence.

## IMMUNE BARRIER HYPOTHESIS OF CRS

The collective analysis of the observations noted previously suggests an immune barrier hypothesis of CRS positing that defects in the mechanical and innate immune protective barrier promote antigen passage and processing across the nasal epithelium leading to the generation of the chronic inflammatory infiltrate observed in CRS. These barrier defects may arise from genetic, epigenetic, or environmental (acquired) influences.

The complex interplay of multiple genetic loci and varied environmental exposures may account for the range of CRS disease severity and clinical course. T-cell differentiation patterns appear to correlate somewhat with phenotype, with CRSsNP skewing toward T<sub>H1</sub> and CRSwNP toward T<sub>H2</sub>.<sup>3</sup> From the standpoint of clinical symptoms, CRSwNP is most closely associated with smell loss and obstruction and CRSsNP is associated with pain and drainage.<sup>3</sup> Viewed in this light, CRS may best be viewed as a spectrum of disease with CRSsNP at one end and CRSwNP at the other (Fig. 5). Individual patients may present at different points along this continuum with varying degrees of tissue eosinophilia and clinical symptoms depending on particular genetic and epigenetic variations. Moreover, CRS can then be seen as a member of a family of chronic inflammatory disorders that occur at sites of interface with the outside world. Skewing of the chronic inflammation in the T<sub>H1</sub> or T<sub>H2</sub> directions creates variable disease phenotypes at each of the anatomic sites and it seems plausible that genetic variations may be shared by diseases, if those loci code for proteins that maintain more universal components of the mechanical and innate immune barrier.<sup>61-63,93</sup> The implications of this perspective are protean as the various environmental factors thus far implicated in CRS pathogenesis can now be seen as disease modifiers rather than discrete etiologic agents. Colonization with toxigenic *S. aureus* increases exposure to SAGs, potentially resulting in an enhanced T<sub>H2</sub> pattern with heightened eosinophil recruitment. Exposure to fungal proteases that degrade tight junctions may result in a generalized increase in antigen exposure to PRRs with more pronounced T<sub>H2</sub> responses in a fashion analogous to the effect from allergen proteases. Acquired mucociliary defects or the presence of biofilms may increase antigen exposure and enhance T<sub>H1</sub> responses.

Identifying key genetic polymorphisms and epigenetic variations and how they promote disease susceptibility in the individual patient will likely be essential for the development of future treatments for CRS. Targeting therapies to intervene in the particular defective pathway (s) present may be preferable to broad suppression of inflammation or extensive efforts to reduce microbial colonization with antibiotics. The corollary is that CRS is not one uniform disease despite broad similarities in clinical phenotype. In short, the genetic and epigenetic variations will be distinct between patients despite, *e.g.*, the common presence of eosinophilic polyps. This perspective is supported by the results of a recent trial of anti-IL-5 in CRSwNP, wherein efficacy was shown only in the fraction of patients with extremely high IL-5 levels.<sup>94</sup>

In summary, current studies on the etiology of most chronic inflammatory mucosal disorders have emphasized abnormalities in the expression or function of genes that maintain the mechanical and innate immune barrier as it interfaces with the external environment, as well as the environmental changes that appear to be accelerating disease expression. In the study of CRS etiology, most interest still centers on identification of putative inciting microbial agents, likely reflecting an earlier era when sinonasal disease was primarily infectious in nature. Comparatively few studies on CRS etiology have focused on host defects, despite recent acceptance that CRS is best considered an inflammatory disease. Although the evidence for the hypothesis that barrier function is compromised in CRS is currently very limited, it places the current controversies in rhinology in a framework consistent with modern concepts of complex genetic disorders and chronic mucosal inflammatory disease in general. Additional studies on host immune dysfunction in CRS will be necessary to generate a comprehensive understanding of the pathogenesis of this common disease and to make targeted therapies a reality.

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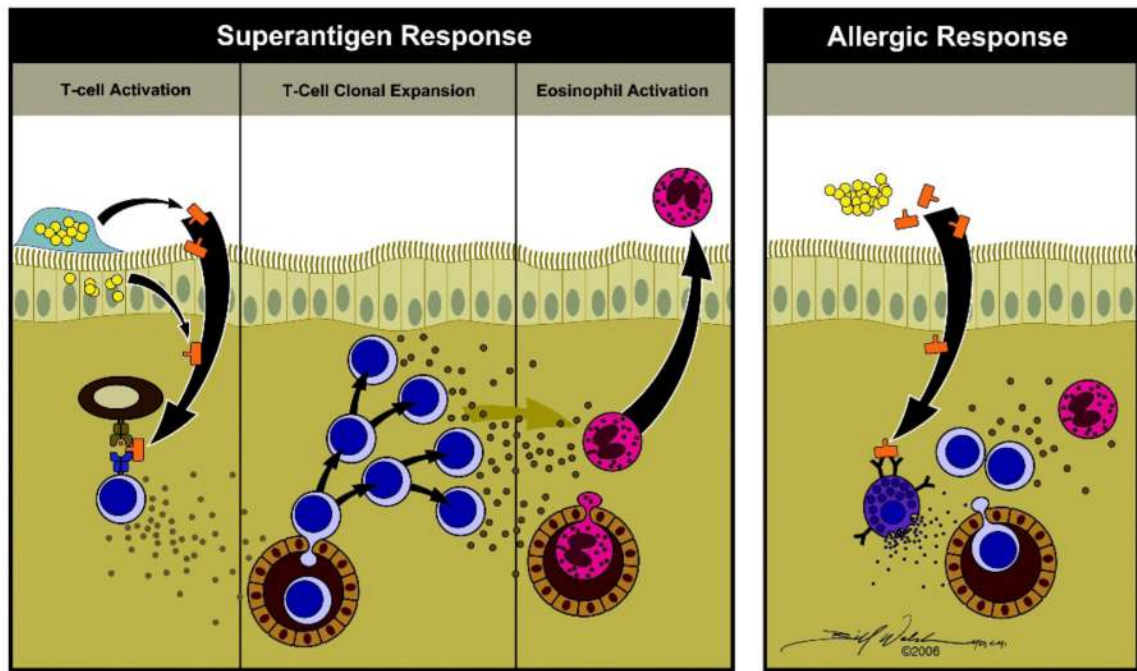
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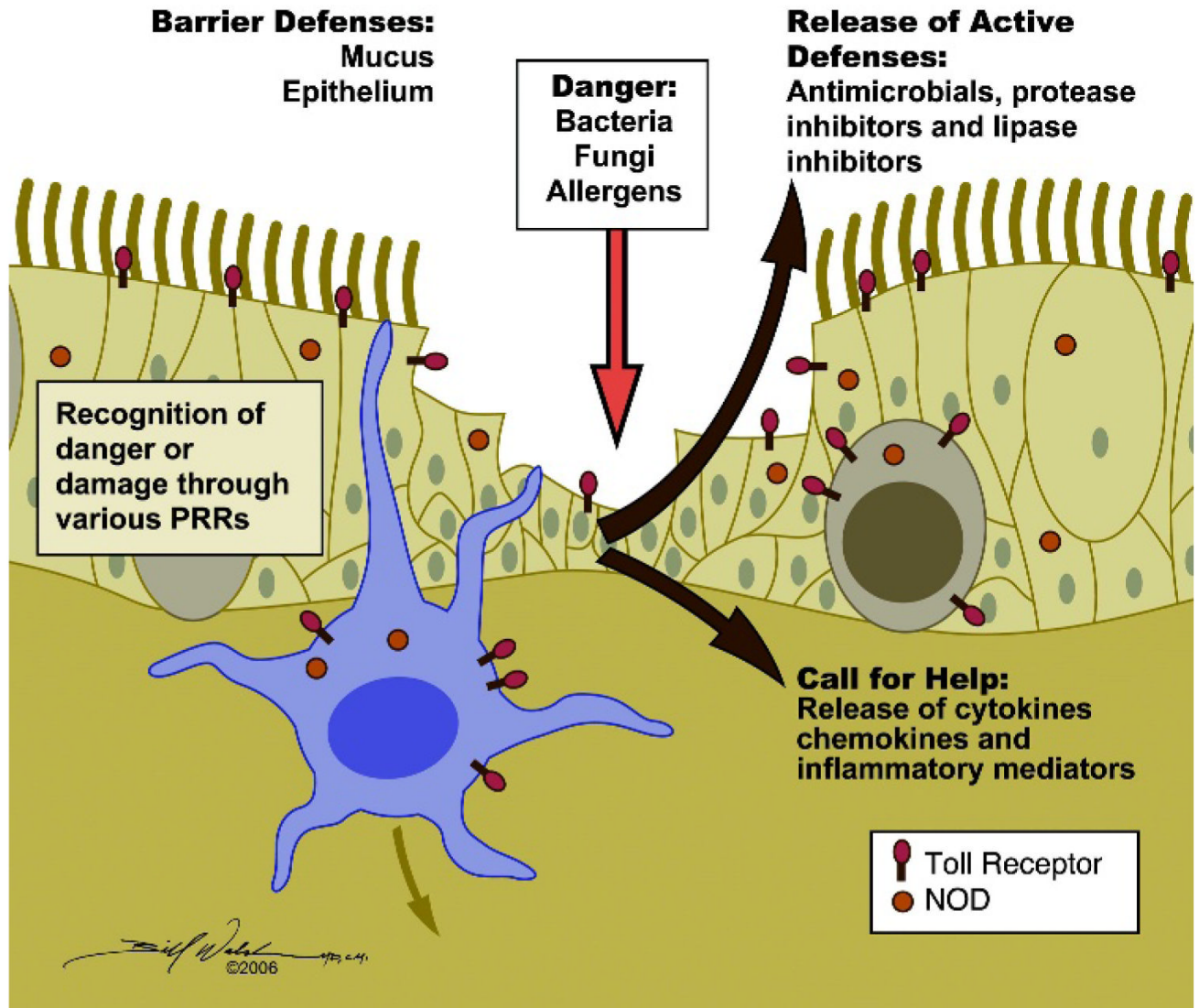
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**Figure 1.**

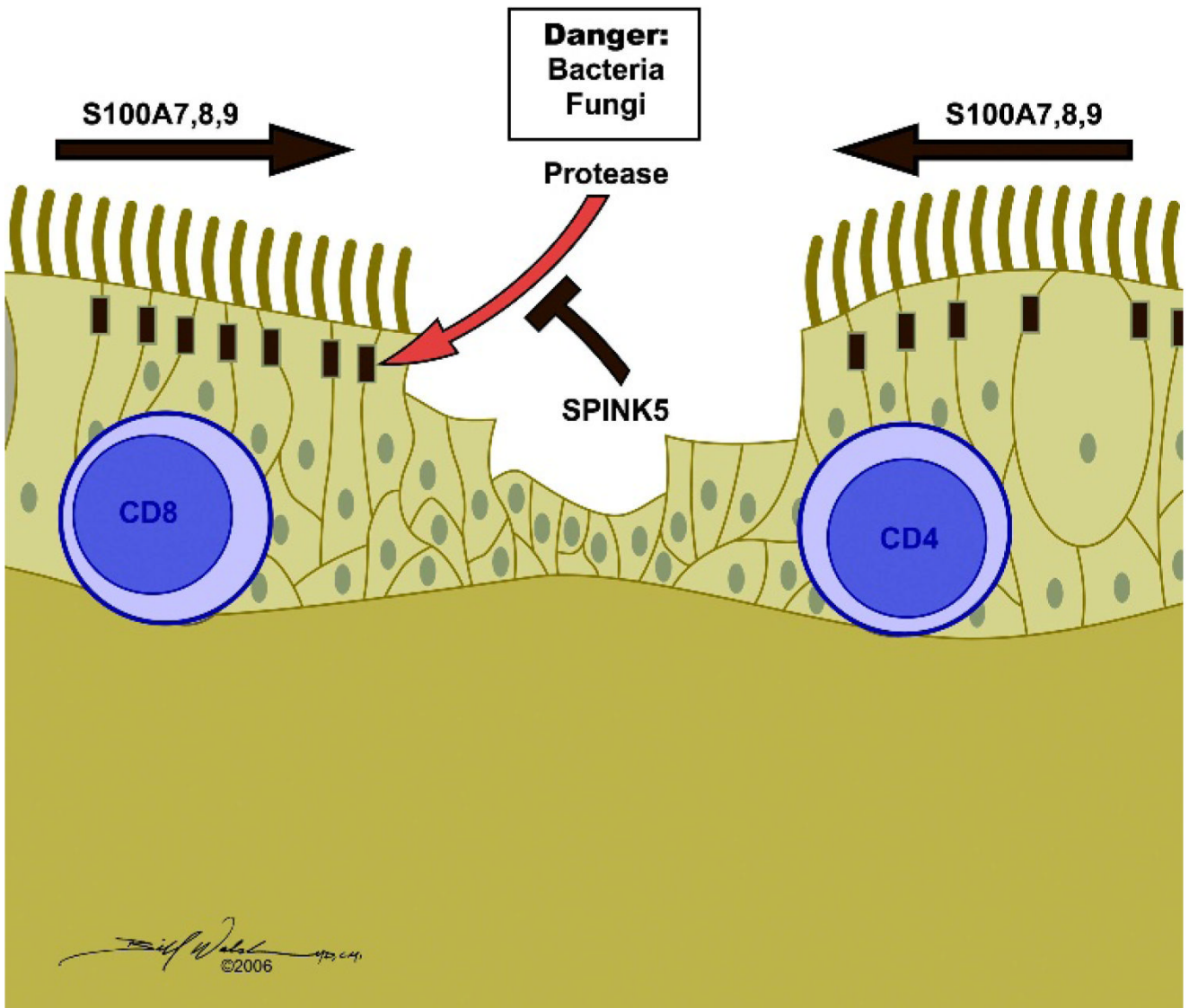
*The superantigen hypothesis proposes that Staphylococcus aureus, perhaps protected by biofilms or sequestered within epithelial cells, secretes toxins that result in a generalized stimulation of T cells with cytokine release as well as a local polyclonal IgE response. (Illustration by William E. Walsh, MD, CMI, 2008 William Walsh; used with permission.)*



**Figure 2.**

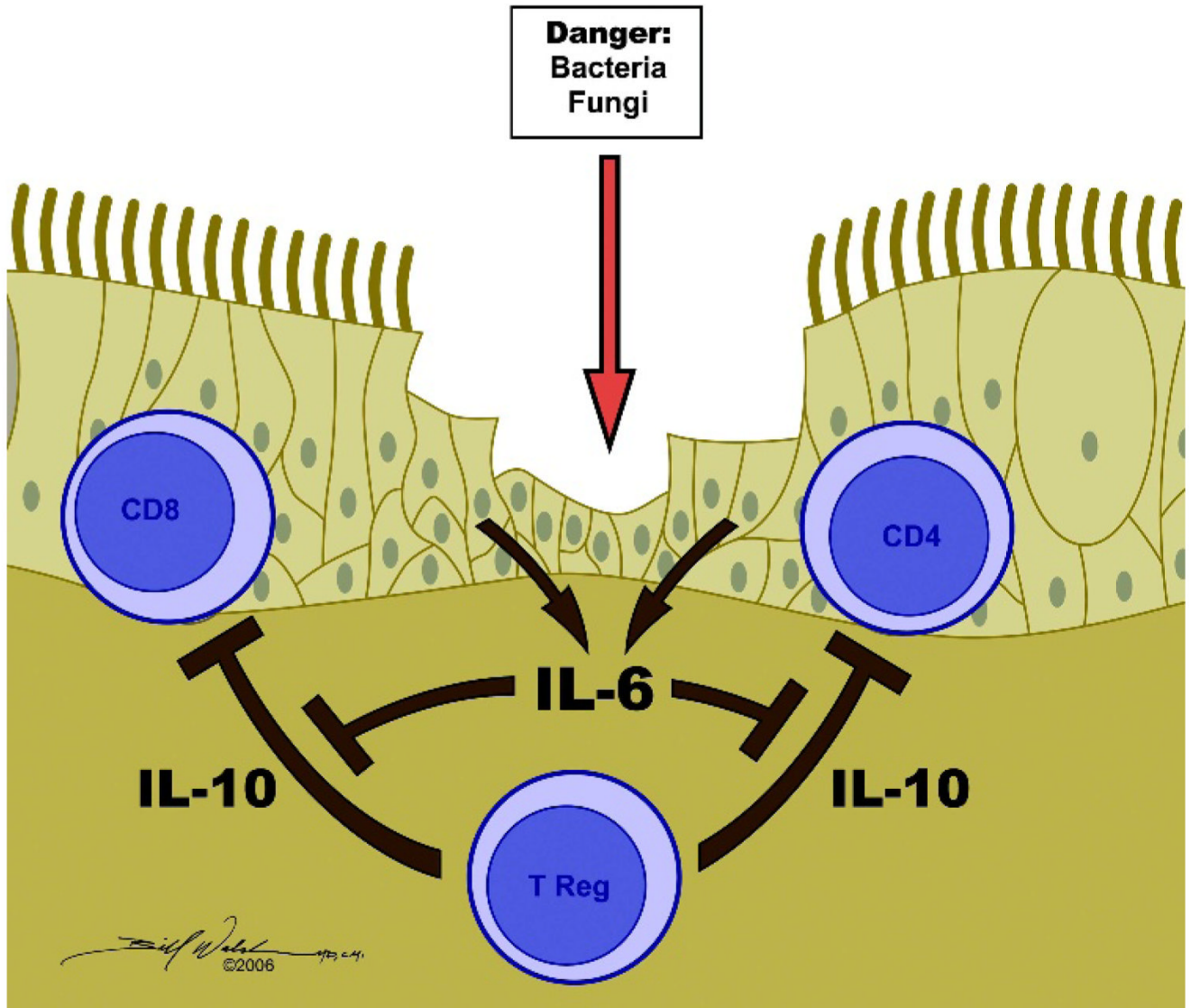
Epithelial defense in the nose first consists of mechanical barriers including mucociliary flow and tight junctions between respiratory epithelial cells that limit stimulation of pattern recognition receptors (PRRs) through diminished exposure and access. PRRs recognize pathogen-associated molecular patterns (PAMPs), which are conserved molecular patterns common among pathogens; recognition of PAMPs by PRRs serves as a “danger” signal to the host immune system. PRRs also identify cellular “damage” through detection of debris from necrotic cells and the combined recognition of danger and damage signals sets in motion a response consisting of endogenous antimicrobial, antiviral, and antiprotease products designed to aid pathogen clearance. PRR activation also triggers the release of chemokines and cytokines mediating the inflammatory response that attracts innate cellular defenses such as neutrophils. If sufficiently strong, PRR stimulation also sets in motion and ultimately determines the nature of the acquired immune response. Although not considered host defense molecules, PAR receptors (not depicted) are also present on many of the cell types present in the nasal mucosa. (Illustration by William E. Walsh, MD, CMI, 2008 William Walsh; used with permission.)





**Figure 3.**

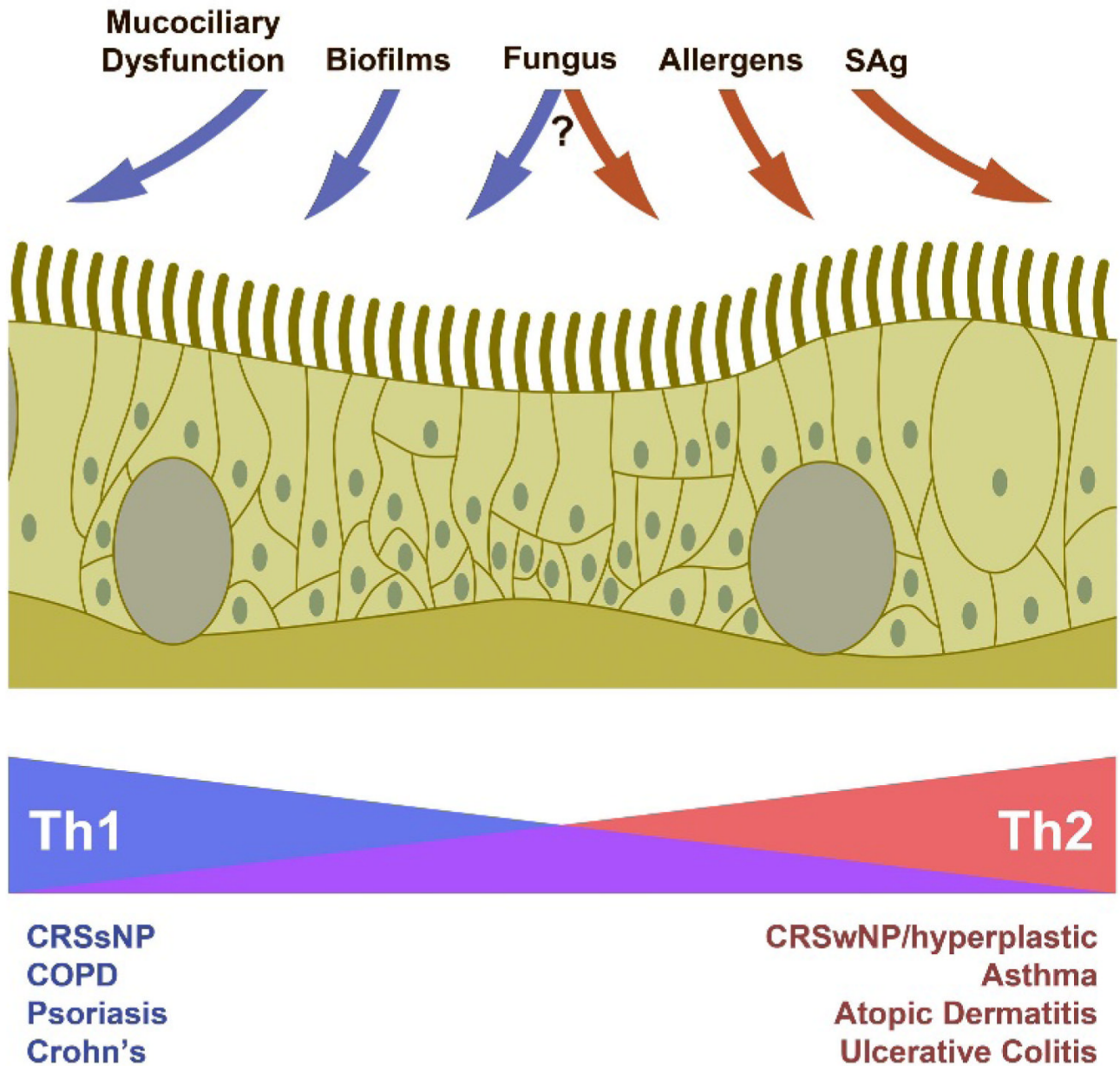
Expression of the S100 family of genes, which participate in epithelial repair and defense, is decreased in chronic rhinosinusitis (CRS). This suggests that CRS is associated with diminished innate defenses and a diminished capacity for epithelial repair after damage (i.e., viral injury). SPINK5, a secreted polyvalent antiprotease, is reduced in CRS with nasal polyps (CRSwNP) patients. SPINK5 protects epithelia from the attack of endogenous and exogenous proteases suggesting that CRSwNP may be associated with a more fragile mechanical barrier. (Illustration by William E. Walsh, MD, CMI, 2008 William Walsh; used with permission.)



**Figure 4.**

IL-6 action frees helper and effector T cells from the suppressive effects of IL-10 secreted by T<sub>reg</sub> cells. High levels of IL-6 protein and its soluble receptor in CRS with nasal polyps suggest the hypothesis that local increases in IL-6 activity may dampen local immunosuppression fostering polyp formation. (Illustration by William E. Walsh, MD, CMI, 2008 William Walsh; used with permission.)

## Spectrum of CRS



**Figure 5.**

Heterogeneity of chronic rhinosinusitis (CRS). According to this model, variations in the expression of genes that govern critical host epithelial pathways may increase the susceptibility to CRS. Environmental factors, rather than discrete etiologic agents, can be seen as disease modifiers that skew the clinical presentation in an individual patient. Fungi may accentuate both T-helper type 1 ( $T_{H1}$ ) and  $T_{H2}$  cytokine expression. Chronic inflammatory disorders occur at other sites of interface with the outside world, including the skin, gut, and lungs. (Illustration by William E. Walsh, MD, CMI, 2008 William Walsh; used with permission.)