Host-microbial interactions in patients with chronic rhinosinusitis

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There has been considerable investigation of host-microbial interactions in patients with chronic rhinosinusitis (CRS) in hopes of elucidating mechanisms of disease and better treatment. Most attention has been paid to bacterial infection and potential underlying defects in innate immunity. Bacterial biofilm is present in most patients with CRS undergoing surgical intervention, and its presence is associated with more severe disease and worse surgical outcomes. A role for viral or fungal infection in patients with CRS is less clear. There is no evidence for a primary defect in mucociliary clearance in most patients with CRS. Decreased levels of certain antimicrobial proteins, most notably lactoferrin, have been found in sinus secretions, whereas levels of other antimicrobial proteins have been found to be normal. No primary defects in Toll-like receptors have been found in patients with CRS, although a 50% reduced expression of Toll-like receptor 9 was reported in patients with recalcitrant nasal polyps. A polymorphism in a bitter taste receptor was recently associated with refractory CRS and persistent *Pseudomonas aeruginosa* infection. A downregulation of innate immunity by maladaptive T_H2 tissue inflammation has also been described in patients with recalcitrant nasal polyps, suggesting a link to persistent infection. To date, an effective means of restoring host-microbial balance and mitigating disease in patients with CRS remains elusive. (J Allergy Clin Immunol 2014;133:640-53.)

Key words: *Chronic rhinosinusitis, host, microbial, biofilm, innate, immunity, antimicrobial*

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Abbreviations used

AFRS:	Allergic fungal rhinosinusitis
BPI:	Bactericidal/permeability-increasing protein
CD:	Crohn disease
Cp110:	Centrosomal protein 110
CRS:	Chronic rhinosinusitis
CRSsNP:	Chronic rhinosinusitis without nasal polyps
CRSwNP:	Chronic rhinosinusitis with nasal polyposis
CSLM:	Confocal scanning laser microscopy
DMBT1:	Deleted in malignant brain tumor 1
EMCRS:	Eosinophilic mucin chronic rhinosinusitis
FISH:	Fluorescence *in situ* hybridization
hBD:	Human β-defensin
HC:	Healthy control subject
IBD:	Inflammatory bowel disease
IESA:	Intraepithelial *Staphylococcus aureus*
IL-22R:	IL-22 receptor
LBP:	LPS-binding protein
MBL:	Mannose-binding lectin
NO:	Nitric oxide
NOD:	Nucleotide-binding oligomerization domain
NP:	Nasal polyp
PCD:	Primary ciliary dyskinesia
PLUNC:	Palate lung and nasal epithelium clone
PNEC:	Cultured primary nasal epithelial cell
SEB:	Staphylococcal enterotoxin B
SEM:	Scanning electron microscopy
SLPI:	Secretory leukocyte proteinase inhibitor
SP-A:	Surfactant protein A
SP-D:	Surfactant protein D
TEM:	Transmission electron microscopy
TLR:	Toll-like receptor

Host-microbial interactions play a critical role in CRS disease initiation and perpetuation. This article aims to summarize knowledge of host-microbial interactions elucidated in relation to normal sinus physiology and pathology of patients with chronic rhinosinusitis (CRS), including the subsets regarded as chronic rhinosinusitis without nasal polyps (CRSsNP), chronic rhinosinusitis with nasal polyposis (CRSwNP), and allergic fungal rhinosinusitis (AFRS).[1]

Most studies of innate immunity and host-microbial interactions in patients with CRS have focused on patients with "refractory" or "recalcitrant" disease. Refractory CRS has been defined on the basis of failure to stabilize after surgery, antibiotics, saline rinses, and topical steroid treatment.[2] Somewhat differently, "recalcitrant CRS" has been defined based on recurrence of nasal polyps (NPs) after polyp surgery.[3] These definitions are noteworthy because patients with refractory polyposis, for example, might have more evidence of infection, whereas patients with recalcitrant polyposis might have little or no evidence of infection but more evidence for maladaptive T_H2-biased mucosal inflammation.

MICROBIOLOGY OF CRS

Role of viruses

Viral upper respiratory tract infections are potentially highly relevant to CRS. The average healthy adult person experiences 1 to 3 common colds per year (http://www.niaid.nih.gov/topics/commoncold/pages/overview.aspx). In healthy subjects the onset and time course of cold symptoms and levels of viral mRNA detectable in nasal secretions over 21 days have been

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Disclosure of potential conflict of interest: D. L. Hamilos has consultant arrangements with and has received grants from Merck and received royalties from UpToDate.

Received for publication January 14, 2013; revised June 25, 2013; accepted for publication June 27, 2013.

Available online November 28, 2013.

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0091-6749/$36.00

http://dx.doi.org/10.1016/j.jaci.2013.06.049

mapped out after experimental rhinovirus infection.[4,5] Patients with asthma or chronic obstructive pulmonary disease manifest a significantly higher peak rhinovirus 16 viral load and duration of symptoms. Asthmatic patients also manifest a corresponding 10-fold decreased induction of type I (β) and type III (λ1 and λ2/3) interferons.[6] Given the similarities between asthma and CRS at the tissue level and the fact that many CRS exacerbations occur during the viral season,[7] it is plausible that a similar defect exists in patients with CRS. However, experimental rhinovirus infection has not been studied in patients with CRS. The innate antiviral response to rhinovirus infection involves activation of type I interferons through interferon-regulatory factor 1 gene activation, an increase in nitric oxide (NO) production, and epithelial production of human β-defensin (hBD) 2, IL-8, and RANTES.[8,9] IL-17A was found to augment production of hBD-2 and IL-8 but downregulate production of RANTES in this model. The chemokine CXCL10 (interferon-inducible protein 10) is also induced.[10]

There has not been a study of the incidence of rhinovirus infection in patients with CRS. A study by Jang et al[11] reported that 21% of patients with CRS have detectable rhinovirus infection. This study examined nasal lavage fluid and turbinate epithelial cells (collected with a Rhino-probe mucosal curette; Rhino-Probe, Arlington Scientific, Arlington, Tex) from 39 patients with CRS and 27 healthy control subjects (HCs). Using an RT-PCR–based assay, they found that lavage fluid from all patients with CRS and HCs and turbinate epithelial cells from HCs were negative for picornavirus, whereas 8 (21%) of 39 epithelial cell samples from patients with CRS were positive. Further examination revealed that all 8 patients with CRS with positive results had positive results for rhinovirus. It is unclear whether these represented subclinical infections because patients were studied at only 1 time point.

In an *in vitro* experiment Wang et al[12] infected NPs and nasal turbinate epithelial cells from 16 patients with CRSwNP and sphenoid sinus and turbinate epithelial cells from 19 HCs with rhinovirus (rhinovirus 16). No significant differences in rates of infection or induction of IL-6 or IL-8 were found.[12] Our group found that cultured airway epithelial cells from patients with CRSsNP had an exaggerated response to stimulation with the combination of double-stranded RNA (a Toll-like receptor [TLR] 3 agonist and surrogate for viral infection) plus cigarette smoke extract, with exaggerated production of RANTES and hBD-2.[13]

Finally, although studies are quite limited, there is a lack of evidence for persistence of viral infection in patients with CRS. Again using PCR methodology, Wood et al[14] found no evidence for common respiratory tract viruses, including parainfluenza 1, 2, and 3; respiratory syncytial virus; human metapneumovirus; adenovirus; rhinovirus; coronavirus; bocavirus; cytomegalovirus; or influenza A or B virus in sinus mucosal samples from 13 patients with CRS.[14]

Whether upper respiratory tract viruses could contribute causally to the inception of CRS analogous to their hypothesized role in asthma pathogenesis remains unexplored.[15]

Bacterial involvement in patients with CRS

Bacteriology of CRS determined by using conventional culture techniques. Studies with conventional culture techniques in children with CRS cultured in the absence of antibiotic treatment reported positive cultures in roughly 60% of cases, with the most common pathogens being *Haemophilus influenzae*, *Streptococcus pneumoniae*, and *Moraxella catarrhalis* (reviewed by Meltzer et al[1]). Studies by Brook et al[16,17] using special techniques to optimize recovery of anaerobic bacteria identified these bacteria in roughly 80% of children with CRS.

Prospective studies in adults identified a positive bacterial culture in a variable percentage of patients with CRS (reviewed by Meltzer et al[1]). Coagulase-negative *Staphylococcus* species was the most common aerobic isolate in several studies, often accompanied by *Staphylococcus aureus* and viridians streptococci. Organisms associated with acute bacterial rhinosinusitis were cultured in some cases. In several studies gram-negative enteric rods, including *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Enterobacter* species, and *Escherichia coli* were also isolated. These organisms are rarely found in middle meatus cultures from healthy subjects. More recent studies of intraoperative sinus cultures with simultaneous analysis of cultures and biofilm reported positive cultures in 72.6% to 80% of cases, with a predominance of *S aureus* and *P aeruginosa* in the isolates.[18-20]

The frequency with which anaerobic organisms have been recovered from adults with CRS has varied widely, with anaerobes found mainly by investigators using special techniques to optimize their recovery.[21,22] Several species, including pigmented *Prevotella*, *Fusobacterium*, *Bacteroides*, and *Peptostreptococcus* species, were isolated. In support of a role for anaerobic bacteria in chronic maxillary sinusitis, Finegold et al[22] found recurrence of signs and symptoms to be twice as frequent when cultures yielding anaerobic bacterial counts of greater than 103 cfu/mL. Further supportive evidence came from the detection of IgG antibodies to anaerobic organisms commonly recovered from sinus aspirates, namely *Fusobacterium nucleatum* and *Prevotella intermedia*. Antibody levels to these organisms decreased in the patients who responded to therapy but did not decrease in those in whom therapy failed.[23] Recent studies with molecular techniques have shed new light on the potential role of anaerobes in patients with CRS (see below).

Role of atypical bacterial infection in patients with CRS. Studies with conventional culture techniques or molecular techniques to overcome issues of detection of nonculturable bacteria suggest that atypical mycobacterial infection is rare in patients with CRS but should be sought in patients with refractory CRS (see additional text in this article's Online Repository at www.jacionline.org).

Bacterial biofilm in patients with CRS. Biofilm formation is an important survival mechanism for microorganisms through attachment to surfaces.[24] Formation of biofilm is a complex process controlled by different genetic pathways depending on growth conditions and exposure to membrane-targeting antibiotics.[25] Furthermore, biofilm-associated bacteria are known to have enhanced resistance to antimicrobial agents relative to floating (planktonic) bacteria.[26] Biofilm formation on sinonasal mucosal surfaces was first described in 2004[27] and later in several other studies.[28-33]

Multiple techniques for biofilm detection have been described and are discussed in the additional text in this article's Online Repository. Table E1 in this article's Online Repository at www.jacionline.org summarizes the results of several studies of biofilm (including fungal biofilm) in patients with CRS, including the techniques used for biofilm identification. Most studies have not