Innate immunity in the paranasal sinuses: a review of nasal host defenses.

Eng Hooi Ooi
Peter-John Wormald
Lor Wai Tan

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Innate immunity in the paranasal sinuses: A review of nasal host defenses

Eng Hooi Ooi, M.B.B.S., Peter-John Wormald, M.D., and Lor Wai Tan, Ph.D.

ABSTRACT

Background: Chronic rhinosinusitis (CRS) is a common inflammatory disorder of the paranasal sinuses. An abnormal host response to common bacterial or fungal pathogens is thought to be an important factor in the disease process. Host sinusal epithelium plays an important role in initially recognizing the presence of microbes and responding by increasing production of antimicrobial peptides and cytokines, with recruitment of phagocytes and lymphocytes of the adaptive immune system, to eliminate the infection. Recently, the innate immune system and its complex interplay with the adaptive immune system are increasingly being recognized as important in the pathogenesis of chronic inflammatory diseases such as asthma and CRS.

Methods: Review of recent findings on innate immunity in the pathogenesis of CRS.

Results: New areas of research into potentially novel therapies for CRS are highlighted in this review, with emphasis on toll-like receptors, antimicrobial peptides (cathelicidins and defensins), and surfactant proteins.

Conclusion: This review provides an overview of innate immunity in the sinonasal tract and discusses potential use of innate immune peptides as treatments against fungi, biofilms, and superantigens in CRS.


Key words: Cathelicidins, defensins, innate immunity, lactoferrin, sinusitis, surfactant proteins, toll-like receptors

The sinonasal tract plays an important role in airway immunity, because it is the first point of contact with inhaled pathogens. The innate immune system is the initial defense against infection and damage caused by microorganisms followed by activation of the adaptive immune system in response to the presence of pathogens. The key differences between the innate and adaptive responses are summarized in Table 1.

The sinonasal tract is lined by respiratory epithelium covered by a superficial layer of mucus and a deeper serous pericilliary layer. The respiratory epithelium now is known to be actively involved in innate immunity. It does this by mucociliary clearance allowing the physical removal of inhaled pathogens, recognition of microbial exposure by pattern recognition receptors expressed on epithelial cells, secretion of inflammatory mediators, antimicrobial peptides, and interaction with the adaptive immune response. The nasal innate immune system is illustrated in Fig. 1. Failure of the local innate defenses may result in microbial colonization leading to recurrent infections.

The aim of this review is to provide an overview of innate immunity, highlight the role of sinonasal epithelium in innate immunity and its links with the adaptive immune system, and to focus recent attention on antimicrobial peptides and surfactant proteins (SPs).

From the Department of Surgery—Otorhinolaryngology Head and Neck Surgery, The Queen Elizabeth Hospital, The University of Adelaide, South Australia, Australia Address correspondence and reprint requests to Peter-John Wormald, M.D., Department of Surgery—Otorhinolaryngology Head and Neck Surgery, The Queen Elizabeth Hospital, 28 Woodville Road, Woodville South, South Australia 5011, Australia E-mail address: peterj.wormald@adelaide.edu.au

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Table 1 **Innate and adaptive immune responses**

<table>
<thead>
<tr>
<th>Innate Immunity</th>
<th>Adaptive Immunity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Responses evolved early, present from birth</td>
<td>Responses acquired with exposure to pathogens</td>
</tr>
<tr>
<td>Immediate response</td>
<td>Slower response (3–5 days)</td>
</tr>
<tr>
<td>Germline encoded receptors (nonspecific, hundreds of receptors only)</td>
<td>T- and B-cell receptors specific to the antigen ($10^{14}$–$10^{18}$ receptors)</td>
</tr>
<tr>
<td>Not dependent on prior exposure</td>
<td>Has memory</td>
</tr>
<tr>
<td>Immune response the same regardless of exposure to antigen</td>
<td>More effective immune response on subsequent encounter with the antigen</td>
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LPS, and Gram-positive bacteria all have LTA. This property of PAMP's allows a limited number of germline encoded pattern recognition receptors to recognize a vast variety of pathogens. The receptors of the innate immune system are expressed on many effector cells of the innate immune response such as macrophages, dendritic cells (DCs), B cells, and respiratory epithelial cells. Mature dendritic cells (mDCs) also are important antigen-presenting cells whereby pattern recognition receptors on their surface recognize distinctive PAMP’s on the surface of organisms inducing activation of T cells.

Functionally, pattern recognition receptors can be divided into three classes: secreted, endoctic, and signaling. Secreted pattern recognition receptors function as opsonins for phagocytosis or complement activation. The best-characterized receptors of this class are mannann-binding lectin and SP-A and -D. Endoctic pattern recognition receptors occur on the surface of phagocytes. These receptors mediate the engulfment of pathogens by phagocytes and DCs where it can be processed and presented to lymphocytes. An example is the macrophage mannose receptor. Signaling receptors trigger signaling pathways that result in the transcription of a variety of inflammatory cytokines and antimicrobial peptides.

The recently described Toll-like receptors (TLRs) are signaling transmembrane receptors that contain an extracellular leucine-rich repeat domain and a cytoplasmic signaling domain. TLRs are homologues of the fly Drosophila toll and are key players in innate immunity and activation of adaptive immunity. Studies of gene knockout mice have identified the roles for some of the TLRs. TLR4 has been identified as the key receptor for recognizing LPS in Gram-negative bacteria. TLR2 is involved in recognizing LTA and peptidoglycan from Gram-positive bacteria. Infections with *Candida albicans* and *Aspergillus fumigatus* involve TLR2 and TLR4 activation. TLR2 and TLR4 expression has been shown in nasal biopsies by real-time reverse transcription polymerase chain reaction (RT-PCR). Expression of mRNA for all TLRs has been detected by RT-PCR in human sinonasal tissue from chronic rhinosinusitis (CRS) patients. TLR2 expression is increased in CRS patients compared with normal controls in a recent study.

Nasal epithelial cells also can serve as antigen-presenting cells and amplify the inflammatory response at the local tissue level. Primary nasal epithelial cells from the middle meatus were grown at the air-liquid interface and expression of mRNA for Human Leukocyte Antigen (HLA)-B, HLA-DR, HLA-B7 to 1 (CD80); and HLB7 to 2 (CD86), B7-H2, B7-F, and B7-DC1 genes associated with antigen-presenting functions was detected by RT-PCR.

**Figure 1. Schematic of the host nasal innate immune system.** (1) Mucociliary clearance removes debris and microorganisms. (2) Multiple substances with antimicrobial and immunomodulatory actions are secreted by the epithelial cells, local inflammatory cells, and submucosal glands resulting in killing of the pathogen. (3) Effector cells are represented by the neutrophil, macrophage, eosinophil, NK cell, basophil, and mast cells. (4) The adaptive immune response with its T and B cells are triggered by the innate immune mechanisms. (5) The innate immune system can subsequently inhibit (−) or activate (+) the adaptive immune response by secretion of effector substances or through the TLRs.

**CELLS INVOLVED IN INNATE IMMUNE RESPONSES**

The phagocytes in the body include the polymorphonuclear neutrophil, macrophage, eosinophil, and basophil. Opsonization of the organism with antibodies, complement, and collectins enhances binding to phagocytes. Killing of the engulfed microorganism occurs by oxygen-dependent and -independent mechanisms. The neutrophil contains preformed bactericidal peptides (lysozyme, lactoferrin, bacterial permeability protein, and cathepsin G), various proteases, and cationic antimicrobial peptides (cathelicidins and defensins). Basophils and mast cells release preformed inflammatory mediators such as histamine, prostaglandin, and leukotrienes. Eosinophils release major basic protein, eosinophil-derived neurotoxin, leukotrienes, and various cyto-
kines.\(^{18}\) Immunohistochemical staining of sinus mucosa from allergic fungal sinusitis patients indicated strong reactivity for the eosinophil mediators major basic protein, eosinophil-derived neurotoxin, and neutrophil elastase.\(^{19}\) Natural killer cells (NKC) can target IgG-coated cells and kill them by a process called antibody-dependent cellular cytotoxicity.\(^{2}\) A second method involves killer-activating receptors that recognize a number of molecules present on the surface of normal cells, and in the absence of an inhibitory signal from killer-inhibitory receptors the killer-activating receptors signal the NKC to attack and kill the cell. The NKC inserts the pore-forming molecule perforin into the cell membrane of the target cell and releases cytolytic granzymes into the cell.\(^2\)

**SECRETED FACTORS IN INNATE IMMUNE RESPONSES**

Complement is a system of >30 proteins in plasma and on cell surfaces in which the main physiological activities\(^{20,21}\) include (i) host defense against infection by opsonization, chemotaxis, and activation of leukocytes cell lysis; (ii) linking innate and adaptive immunity (by augmentation of antibody responses and enhancement of immunologic memory); and (iii) disposing of immune complexes and products of inflammatory injury. For example C3b is a major opsonin; C3a, C4a, and C5a cause mast cells to degranulate; C5a is a powerful chemoattractant; C5b, C6, C7, C8, and C9 form the membrane attack complex, which causes cell lysis. C3 expression by real-time PCR and immunohistochemistry has been shown in sinonasal biopsy specimens.\(^{14}\)

IL-1β, IL-6, and IL-8 have been implicated as important factors in the innate immune response.\(^{22}\) Cytokines are low molecular weight proteins that regulate differentiation, cell growth, inflammation, immunity, proliferation, and function of the immune cells.\(^{23}\) Chemokines are a superfamily of small proteins that act as activators and chemoattractants for leukocytes. Infectious inflammation is associated with increased neutrophil influx and cytokine levels of IL-1β and IL-6 in sinus tissue by epithelial cells in response to bacteria or viruses.\(^{25}\)

**HUMAN NASAL ANTIMICROBIAL PEPTIDES**

Innate immune responses are constitutive and inducible. The two major families of cationic antimicrobial peptides involved in innate immunity at mucosal surfaces are cathelicidins and defensins.\(^{6}\) Human defensins (HDs) are members of the family of antimicrobial peptides, which can be divided further into two classes, α- and β-defensins based on structural characteristics. The defensins are small (29–40 amino acids) cationic peptides containing six cysteine residues linked by three disulfide bonds. The α-defensins are found in neutrophils (human neutrophil peptides [HNP] 1–4),\(^{24}\) Paneth cells of the small intestine (HD-5 and -6),\(^{25}\) and nasal epithelial cells.\(^{26}\) Human β-defensins (HBD-1 to -4) is found at epithelial surfaces such as the lungs, skin, and gut.\(^{27,28}\) The defensins are both constitutive and inducible, possessing broad-spectrum antimicrobial activity, and have roles in innate immunity and wound healing.\(^{27}\) Using RT-PCR and immunohistochemical staining, HD-1, -2, and -3, HBD-1 and -2 expression was up-regulated in maxillary sinus epithelium and nasal polyps, whereas HBD-2, HNP-5, and HNP-6 expression was not detected in normal controls.\(^{29,30}\) However, in one study Western blot detected HBD-1 and HBD-2 peptide in nasal lavage fluid, suggesting that HBDs were secreted from nasal mucosa.\(^{30}\)

Cathelicidins are synthesized as prepropeptides characterized by a highly conserved signal peptide (29–30 amino acids), an N-terminal prosesequence termed cathelin (~100 amino acids), and a highly heterogeneous C-terminal domain (~10–40 amino acids).\(^{31}\) Most cathelicidins undergo extracellular proteolytic cleavage that releases the C-terminal peptide containing the antimicrobial activity. Cathelicidins have been named by using acronyms (e.g., cathelicidin antimicrobial peptide [CRAMP]) or one-letter symbols of key amino acid residues present in the antimicrobial sequence followed by the number of residues (e.g., LL-37 and PR-39). The only known human cathelicidin hCAP18 (human cationic antimicrobial peptide, 18 kDa) or CAMP was initially identified in specific granules of human neutrophils.\(^{32}\) The free C-terminal peptide of hCAP18 is called LL-37 (37 amino acids; the two N-terminal amino acids are leucines). Expression of hCAP18 has been identified by RT-PCR, in situ hybridization, and immunohistochemistry in other immune cells.\(^{33–35}\) Cytolytic epithelial airway cells,\(^{36}\) serous and mucous cells of the submucosal glands,\(^{36}\) and nasal mucosa.\(^{37}\)

The antimicrobial activity of LL-37 is mediated by binding to negatively charged bacterial surfaces and disrupting the cell membrane.\(^{38}\) In contrast HBDs, which are inactive at high salt concentrations seen in cystic fibrosis patients, LL-37 generally retains its antibacterial activity in vitro against *Pseudomonas aeruginosa*, *Salmonella typhimurium*, Escherichia coli, *Listeria monocytogenes*, *Staphylococcus epidermidis*, and *Staphylococcus aureus* at moderate to high salt concentrations.\(^{39–41}\) LL-37 is chemotactic for human neutrophils, monocytes, and T cells acting through the receptor formyl peptide receptor-like 1 (FPRL1), a Gi protein coupled receptor expressed on these cells.\(^{42}\) LL-37 induces degranulation and histamine release in mast cells.\(^{43}\)

Inferior turbinate tissue from 15 CRS patients compared with 6 controls was examined using immunohistochemistry and RT-PCR.\(^{37}\) The investigators showed immunostaining for LL-37 in the surface epithelia, serous and mucous cells of the submucosal glands, and in stromal inflammatory cells of the rhinitis (11/15) and control patients (2/6). RT-PCR detected LL-37 mRNA in all rhinitis patients and only in one-half of the control patients. Expression of LL-37 mRNA was increased in nasal polyps compared with normal nasal mucosa by semiquantitative RT-PCR analysis in another study.\(^{44}\) A study from our department showed up-regulation of LL-37 mRNA in CRS patients and this was significantly greater in the eosinophilic mucus CRS (EMCRS) subgroup.\(^{45}\) On further challenge with *Alternaria*, LL-37 mRNA and protein was increased in the CRS but not EMCRS subgroup, suggesting that the inducible response to increasing fungal load is affected in the EMCRS group.\(^{46}\)

Lactoferrin, lysozyme, and secretory leukoprotease inhibitor (SLPI) also are antimicrobial peptides that have been identified in nasal secretions and sinus mucosa.\(^{47}\) Lysozyme and lactoferrin are bacterialic peptides contained within neutrophil granules and epithelial cells.\(^{49}\) Lysozyme is a 14-kDa enzyme directed against the β1 → 4 glycosidic bond between N-acetylglucosamine and N-acetylmuramic acid residues that
make up peptidoglycan, a crucial component of bacterial cell membrane, causing cell lysis. SLPI is a 12-kDa nonglycosylated protein with modest antimicrobial and antifungal activity in vitro.\textsuperscript{50} Lactoferrin is an 80-kDa iron-binding protein that is highly abundant in the specific granules of human neutrophils. It inhibits microbial growth by sequestering iron essential for microbial respiration. It also can be microbiidal and block biofilm formation by \textit{P. aeruginosa}.\textsuperscript{51} Recently, our department has shown down-regulation of lactoferrin in the CRS patients compared with healthy controls providing further evidence of abnormal innate immune responses in CRS patients.\textsuperscript{52} These results suggest that CRS patients may be more susceptible to biofilm formation because of a defect in lactoferrin expression.

\textbf{SURFACTANT PROTEINS}

Pulmonary surfactant is a mixture of phospholipids (90%) and proteins (10%). There are four SPs: SP-A, SP-B, SP-C, and SP-D. SP-A and -D are members of the family of collagens containing carbohydrate-binding proteins, involved in innate immunity, known as collectins because they have collagenous and lectin-binding domains.\textsuperscript{9} There is increasing evidence that the collectins are involved in innate immunity against various bacteria, fungi, and viruses.\textsuperscript{9} In humans, there are at least two SP-A genes expressed (SP-A1 and SP-A2) but the functional significance of this is unclear at present.\textsuperscript{53} Mice lacking a functional SP-A gene did not show any significant difference in lung function or surfactant lipid metabolism\textsuperscript{24} but were more susceptible to bacterial\textsuperscript{55} and viral\textsuperscript{56} infections. Similarly, SP-D-deficient mice developed emphysema and fibrosis but maintained normal respiratory function.\textsuperscript{57} Human SP-D shares 92% homology with the mouse SP-D protein and gene\textsuperscript{58} with a significant amount of SP-D (50–90%) in the form of a soluble protein.\textsuperscript{59}

Type II lung pneumocytes, Clara cells and nonciliated bronchial epithelial cells are known to produce SP-A and SP-D. The collectins are assembled as oligomers of trimeric units. Each subunit monomer consists of a C-terminal lectin carbohydrate recognition domain (CRD) that recognizes and binds carbohydrates on allergens proteins in a calcium-dependent manner, connected via a short neck to a type IV collagen-like domain and a cysteine containing N-terminal domain.\textsuperscript{60}

The collectins bind to carbohydrate moieties on the surface of bacteria and fungi through its CRD. The clustering of three CRDs in close proximity ensure binding with tight affinity to dense sugar arrays on the surface of microbes and also provide a large docking surface for effector molecules.\textsuperscript{60} Collectins represent a unique class of pattern recognition receptors similar to TLRs. SP-D binds to both Gram-positive and Gram-negative bacteria including \textit{Klebsiella pneumoniae},\textsuperscript{53} \textit{P. aeruginosa},\textsuperscript{52} \textit{Streptococcus pneumoniae}, and \textit{S. aureus}.\textsuperscript{63} SP-D recognizes and aggregates several viruses implicated in nasal and respiratory diseases including influenza A virus\textsuperscript{64} and respiratory syncytial virus.\textsuperscript{65} Collectins also recognize LPS on the outer membrane of Gram-negative bacteria and the mannann-like high-mannose structures on the surface of fungi.\textsuperscript{66,67} SP-D binds several fungal species including \textit{A. fumigatus},\textsuperscript{67} as well as the beech-fungus \textit{Cryptococcus neoformans},\textsuperscript{68} \textit{C. albicans},\textsuperscript{69} and \textit{Pneumocystis carinii}.\textsuperscript{70}

Collectins promote phagocytosis of various microorganisms, either directly after binding to microbes or indirectly through up-regulation of phagocytosis mediated by other phagocytic receptors. SP-D has numerous immunomodulatory functions on effector cells of the adaptive immune system. SP-D is a potent chemotactaractant for blood neutrophils and monocytes.\textsuperscript{71} Native human SP-D and recombinant SP-D inhibited phytohemagglutinin or \textit{D. p} allergen stimulated lymphocyte proliferation.\textsuperscript{72} SP-A and SP-D inhibition of CD3(+)CD4(+) lymphocyte proliferation in vitro supports a role for surfactant proteins in dampening the inflammatory response.\textsuperscript{73,74}

SP-A, SP-D, and recombinant SP-D bind \textit{A. fumigatus} conidia.\textsuperscript{67,75} Human SP-A and SP-D bound to 3-week culture filtrate allergens and purified glycosylated allergens gp45 and gp55 of \textit{A. fumigatus}.\textsuperscript{76} Both SP-A and SP-D were able to inhibit binding of allergen-specific IgE from aspergillusosis patients to these allergens and also inhibit the \textit{A. fumigatus} allergen-induced histamine release from sensitized basophils of allergic bronchopulmonary aspergillusosis (ABPA) patient's.\textsuperscript{76} Therefore, SP-D has the potential to inhibit histamine release in the early phase of allergen provocation and suppress lymphocyte proliferation in the late phase of allergen inflammation. Intranasal treatment with SP-D (3 μg/mouse), SP-D (1 μg/mouse), and recombinant SP-D (1 μg/mouse) in a mice model of ABPA decreased blood eosinophilia, pulmonary infiltration, and levels of allergen-specific IgG and IgE.\textsuperscript{75} The levels of IL-2, IL-4, and IL-5 decreased while IFN-γ increased in the splenic homogenates of the treated ABPA mice, indicating a marked shift from a Th2 to a Th1 cytokine response.\textsuperscript{75} Additional studies have shown that lack of SP-D in an allergen challenge is associated with a greater Th2-like response, suggesting that SP-D may dampen the allergic responses by promoting IFN-γ secretion (a Th1 response).\textsuperscript{77}

Recently, SP-A mRNA was detected to increase in CRS patients without nasal polyps by RT-PCR compared with normal controls.\textsuperscript{78} Immunohistochemical staining showed SP-A and SP-D in the epithelial cells and submucosal glands of sinus mucosa in control and CRS patients.\textsuperscript{78,79} A study in our department recently found SP-D transcripts and protein expression in human nasal tissue by immunohistochemistry, ELISA, and real-time RT-PCR.\textsuperscript{80} SP-D immunostaining was strongest in the submucosal glands of CRS but was absent in APS patients. We surmised that absence of significant SP-D gene up-regulation and depletion of SP-D protein reserves during the progression to a chronic inflammatory state in the APS group may result in failure to clear the pathogen from the sinuses and lead to a chronic inflammatory state from bacterial or fungal reinfestation. We subsequently showed increased SP-D expression in CRS patients, but down-regulation of SP-D in the EMCRS subgroup, challenged with fungal allergens in a nasal tissue explant model.\textsuperscript{80} We postulated that lack of up-regulation of SP-D mRNA levels may contribute to chronic infection as seen in animal studies.\textsuperscript{81}

\textbf{CLINICAL IMPLICATIONS OF SPs AND ANTIMICROBIAL PEPTIDES}

Reduced expression or production of these innate immune peptides may lead to recurrent infections whereas their overactivity may lead to persistent and potentially damaging inflammation. The abnormal host response to the pathogen
rather than the pathogen itself is likely to be an important differen-
tiating factor between healthy individuals and CRS patients. Numerous studies have shown increased expres-
sion of various antimicrobial peptides in CRS patients. This may be the “driving force” behind the inflammatory process in a bid to eliminate the bacterial or fungal pathogen, result-
ing in overactivation of the adaptive immune response. A Th2-type profile has been implicated as a dominant path-
genic process in eosinophilic CRS with stimulation of the adaptive immune response. Corticosteroids are used fre-
frequently in CRS to reduce the inflammatory adaptive immune response (suppressing cytokine production) but with signifi-
cant side effects. However, corticosteroids generally spare innate immune responses, and, in fact, induce SP expres-
sion. Novel therapies targeting adaptive immune response are now emerging from recent research on innate immunity. SPs have been shown to inhibit pulmonary inflammation limiting lung damage by producing a shift from a pathogenic Th2 to a protective Th1 cytokine profile. Potentially recombinant SPs may be useful therapy in CRS because of its immunomodulating effects (Fig. 1) allowing a more directed effect without the side effects of corticosteroids. Another form of immunotherapy are CpG deoxyribonucleotides, which prevent development of Th2-mediated cytokine response in a murine model of allergic rhinosinusitis. These CpG motifs coupled to bacterial DNA stimulate TLRs to preferentially induce a Th1-mediated response that is considered beneficial in CRS. Biofilms have been implicated in the pathogenesis of CRS. Recombinant lactoferrin with its antibiotic also may be potentially therapeutic in CRS patients. Finally, there are few reports of bacterial resistance to antimicrobial peptides, making them potentially useful as a form of “naturally occurring antibiotic” in infectious sinusitis.

CONCLUSIONS

New findings on antimicrobial peptides may lead to novel therapies against fungi, biofilms, and superantigens, which have all been implicated in CRS pathogenesis. The innate immune system is an active participant at the nasal tissue level and changes in its responses and effects to pathogens affects the adaptive immune system. This complex interplay between the innate and adaptive immune systems is only now being recognized with further research into innate immune responses required to understand its role in CRS.

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