

INTRODUCTION

Chronic rhinosinusitis (CRS) is a clinical syndrome associated with persistent inflammation of the mucosa of the nose and paranasal sinuses. The definition of CRS is intentionally inclusive, encompassing, for example, both the polypoid (CRSwNP) and nonpolypoid (CRSsNP) forms of the disease but does not address causation or etiology (*Tan et al., 2010*).

Research over the last 30 years suggests that the etiology of CRS is complex and multifactorial and the concept of chronic mucosal inflammation has supplanted infection to describe the disorder, but this shift still neglects the issue of etiology (*Kern et al., 2008*).

In a minority of CRS cases, distinct host genetic or systemic disorders such as Kartagener's syndrome, cystic fibrosis (CF), Wegener's granulomatosis and sarcoidosis are identified as the cause of sinonasal inflammation. (The overwhelming majority of CRS cases, however, are idiopathic and specific proposed mechanisms for persistent inflammation include obstruction of the osteomeatal complex, impaired mucociliary clearance, osteitis, atopy and microbial resistance, including biofilm formation) (*Van Cauwenberge et al., 2006*).

These potential pathogenic mechanisms form the underpinnings of most current therapies for CRS, which include antimicrobials, antihistamines, leukotriene antagonists, topical and systemic corticosteroids, and

endoscopic sinus surgery for restoring mucociliary clearance and drainage through the osteomeatal complex (*Gillespie and Osguthorpe 2004*). Although these therapies are effective at helping the majority of patients, significant variability remains in both the efficacy and durability of the clinical response, highlighting current limitations in our understanding of this disorder (*Tan et al., 2010*).

The human immune response is composed of two overlapping components, innate and acquired, both of which play critical roles in host defense. The innate immune system is that part of the immune system that does not depend on previous exposures for optimal function. Its main functions are largely to act as a physical and chemical barrier, to recruit immune competent cells to the site of infection, to activate the adaptive immune system, and to activate the complement system (*Van Drunen., 2012*).

Research on the innate immune system over the years had focused on a number of larger topics. The first topic was the diverse role of (airway) epithelium in the innate defense. As the epithelium forms the outer layer of an organism, it is well-positioned to detect and respond to changes in the environment. The second topic was the activity of different receptors by which cells can detect the outside environment, and the third was the action of (secreted) mediators that fight off potential threats (*Vroling et al., 2008*).

The relationship between innate immunity and CRS has always been special. In cystic fibrosis, there is an

absence of mucociliary clearance and a high prevalence of CRS. The presence of biofilms in CRS patients and the postulated roles for *Staphylococcus aureus*, viruses, and fungi in the pathogenesis of CRS over the years have all been based on a disrupted ability to fight off these microorganisms. Given that it is not clear to what extent the adaptive immune system is involved in the complex inflammation of CRS it seems logical that the innate immune reactions are so carefully considered in the pathogenesis of CRS (*Van Crombruggen et al., 2011*).

The innate immune system comprises cells and their associated mechanisms that provide the first line of defense against pathogens through genetically encoded pathways with limited specificity for molecular structures. In addition to the physical barrier and pathogen clearing effects of the mucociliary clearance system, sinonasal mucosa has been shown to express a vast arsenal of antimicrobial molecules (*Avila and Scheimer 2008*).

In general, earlier studies of CRS did not demonstrate consistent alterations in the expression of these antimicrobial molecules, with increased levels reported in some molecules, whereas others are reportedly decreased (*Psaltis et al., 2007*).

A recent observation in laboratory however, suggested that the S100 proteins might play a significant role in mediating some forms of CRS. The S100 proteins have multiple effects on cell differentiation and barrier function and several members (e.g. S100A7, S100A8 and

S100A9) act as classic antimicrobial proteins with direct antibacterial and antifungal effects, recruit neutrophils and lymphocytes, and also aid in wound healing (*Meyer et al., 2008*).

Also, S100A7, S100A8 and S100A9 are reported substantially reduced in CRS when compared with controls. Taken together, these studies suggest that diminished S100 proteins in sinonasal epithelial cells may predispose to development of CRS, possibly through increased microbial colonization or diminished wound healing (*Tieu et al., 2009*).

In addition to expressing secretory antimicrobial proteins, sinonasal epithelial cells express pattern-recognition receptors (PRRs) that recognize pathogen-associated molecular patterns (PAMPs). Prominent among the PRRs are the Toll-like receptors (TLRs) that, when activated, trigger a pro-inflammatory response through the activation of nuclear transcription factors such as nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B), activator protein (AP-1) and interferon regulatory factor 3 (IRF3) (*Vroling et al., 2008*).

Given that TLR2, TLR3, TLR4 and TLR9 are expressed in the airway it is likely they play an important role in mediating host inflammation, with potential derangements contributing to the development of CRS (*Kato and Schleimer, 2007*).

An important inducer of innate immune responses is the cytokine interleukin 22(IL-22) that is secreted by T

helper 17 (Th17) and T helper 1(Th1) cells and activates epithelial cells via the IL-22 receptors (IL22R) (*Wolk et al., 2004 and 2006*).

In CRSwNP, the levels of IL22R1 were found to be significantly decreased in nasal polyposis when compared with controls, suggesting a diminished IL-22 response in CRSwNP .This provides an interesting paradigm in which decreased IL-22R on nasal epithelial cells may impair innate immune responses in CRSwNP (*Ramanathan et al., 2007*).

Together these data suggest that important facets of the innate immune system and its regulatory mechanisms may be impaired in CRS. Whether this results in impaired pathogen clearance, commensal overgrowth and dysregulation of the host inflammatory response is under investigation; however, altered expression of the above-mentioned genes, and altered performance of the molecular networks they maintain, could, at least theoretically, predispose to CRS (*Tan et al., 2010*).

AIM OF THE STUDY

The aim of this study:

To review recent researches addressing immunity and changes related to chronic rhinosinusitis in order to identify a possible role in pathogenesis and its implication on treatment.

CHAPTER (I): INNATE IMMUNITY

The human immune response is composed of two overlapping components, innate and acquired, both of which play critical roles in host defense (Fig. 1). By definition, the innate immune system is that part of the immune system that does not depend on previous exposures for optimal function. Research into the workings of the innate immune system is slowly developing from the dark horse it once was into a frontrunner with the realization that the innate immune system is an integral part of immunity. (*Van Drunen, 2012*).

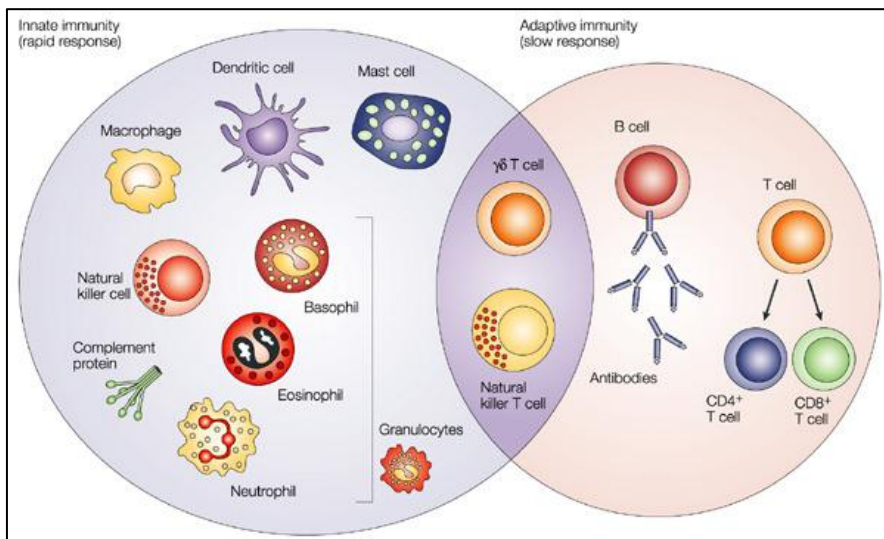


Fig. (1): Innate and adaptive immunity
(*Gleen Dranoff, 2004*)

Overview - Components of the Innate Immune System Include

- Physical barriers (tight junction in skin, epithelial and mucous membrane surfaces, mucus itself).
- Enzymes in epithelial and phagocytic cells (e.g., lysozyme).
- Inflammation-related serum proteins {e.g., complement, C-reactive protein, lectin (carbohydrate-binding proteins) such as mannose-binding lectin and ficolins}.
- Antimicrobial peptide (defensins, cathelicidine, and many more) on the surfaces of cells and within phagocyte granules.
- Cell receptors that sense microorganisms and signal a defensive response (eg, Toll-like receptors).
- Cells that release cytokines and inflammatory mediators (macrophage, mast cells, natural-killer cells).
- Phagocytes (neutrophils, monocyte, macrophages) (*Johnston et al., 2014*).

The main functions of innate immune system are largely to act as a physical and chemical barrier, to recruit immune competent cells to the site of infection, to activate the adaptive immune system, and to activate the complement system. In this light, the innate immune system seems to buy time for the adaptive immune system to come to the rescue. However, it can also acts as a fully accomplished eradicating defense system (*Vroling et al., 2008*).

Innate Immunity in Paranasal Sinuses

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 (*Medzhitov and Janeway, 2000*). The key differences between the innate and adaptive responses are summarized in Table 1 (*Delves and Roitt, 2000*).

Table (1): Innate and adaptive immune responses (*Delves and Roitt, 2000*)

Innate immunity	Adaptive immunity
<ul style="list-style-type: none"> • Responses evolved early, present from birth • Immediate response • Germline encoded receptors (nonspecific, hundreds of receptors only) • Not dependent on prior exposure • Immune response is the same regardless of exposure to antigen 	<ul style="list-style-type: none"> • Responses acquired with exposure to pathogens • Slower response (3-5 days) • T- and B-cell receptors specific to the antigen (10^{14} - 10^{18} receptors) • Has memory • More effective immune response on subsequent encounter with the antigen

The sinonasal tract is lined by respiratory epithelium covered by a superficial layer of mucus and deeper serous pericilliary layer. The respiratory epithelium recently is known to be actively involved in innate immunity (*Kaliner, 1992*).

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, pseudo stratified, 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 (*Kern et al., 2008*).

Recognition of Pathogens by Sinonasal Cells:

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 (*Janeway and Medzhitov, 2002*). 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 (*Akira et al., 2006*). 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 antiproteinase products designed to aid pathogen clearance and preserve the epithelial barrier (*Meylan et al., 2006*).

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 (*Jwasaki and Medzhitov, 2004*).

The two best-characterized classes of PRRs are the toll-like receptor (TLR) family and the nucleotide-binding oligomerization domain receptors (NOD-like receptor) family (*Akira et al., 2006; Meylan et al., 2006*). TLRs are transmembrane receptors expressed on multiple cell types including respiratory epithelial cells (*Diamond et al., 2000; Lane et al., 2006a*). 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 (*Akira et al., 2006*). The NOD-like receptor family includes NOD1 and -2, which are important in the recognition of bacterial cell wall products including staphylococci (*Fournier and Philpott, 2005*).

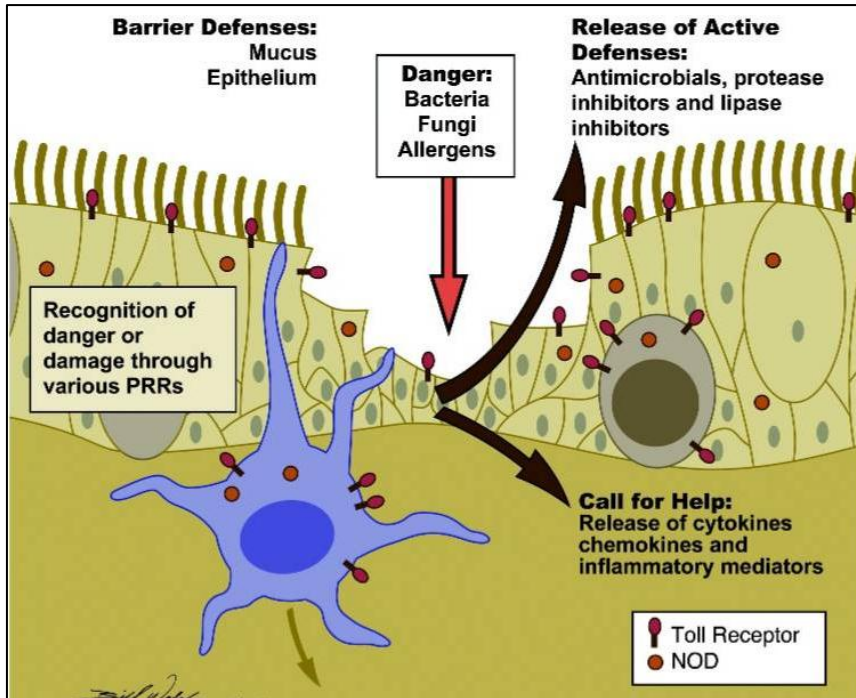


Fig. (2): The mechanical and immunologic barrier of nasal mucosa
(Kern et al., 2008)

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, bactericidal permeability protein, and cathepsin G), various proteases, and cationic antimicrobial peptides (cathelicidins and defensins) (Delves and Roitt, 2000). 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 cytokines (*Prussin and Metcalfe, 2003*). Natural killer cells (NKC_s) can target immunoglobulin G (IgG) coated cells and kill them by a process called antibody-dependent cellular cytotoxicity (*Delves and Roitt, 2000*).

Secreted Factors in Innate Immune Responses:

Complement is a system of more than 30 proteins in plasma and on cell surfaces in which the main physiological activities 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 (*Walport, 2001a; Walport, 2001b*). 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 (*Lane et al., 2006a*).

Cytokines are low molecular weight proteins that regulate differentiation, cell growth, inflammation, immunity, proliferation, and function of immune cells. Example of these cytokines are interleukin (IL), chemokines, interferons, tumor necrosis factor alpha (TNF α). Interleukin

(IL-1 β , IL6, and IL8) have been implicated as important factors in innate immune response (*Janeway and Medzhitov, 2002*). Infectious inflammation is associated with increased neutrophil influx and cytokine levels of IL-1 β and IL6 in sinus tissue secreted by epithelial cells in response to bacteria or viruses. Chemokines are a superfamily of small protein that acting as activation and chemattractants for leukocyte (*Bachert et al., 1998*).

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 (*Ganz, 2004*).

Human defensins (HD_S) are members of the antimicrobial peptides family, which can be divided further into two classes, α -and β -defensins based on structural characteristics. The defensins are small (29-40 aminoacids) cationic peptide. The α -defensins are found in neutrophils (human neutrophil peptides {HNP_S} 1-4) (*Gudmundsson and Agerberth, 1999*), nasal epithelial cells (*Frye et al., 2000*), and paneth cells of small intestine (HD-5 and -6) (*Cunliffe and Mahida, 2004*), Human β -defensins (HBD-1 to 4) are found at epithelial surfaces such as the lung, skin, and gut (*Bals and Hiemstra, 2004; van Wetering et al., 2005*).

The defensins are both constitutive and inducible, possessing broad-spectrum antimicrobial activity, and have

a role in innate immunity and wound healing (*van Wetering et al., 2005*). Using reverse transcription polymerase chain reaction (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 HNP6 expression was not detected in normal controls (*Carothers et al., 2001; Lee et al., 2002*). However, in one study western blot detected HBD₁ and HBD₂ peptide in nasal lavage fluid, suggesting that HBD_S were secreted from nasal mucosa (*Carothers et al., 2001*).

Cathelicidine are synthesized as prepropeptides characterized by a highly conserved signal peptide (29-30 amino acid), an N-terminal prosequence termed cathelin (100 amino acids), and a highly heterogenous C-terminal domain (10-40 amino acids) (*Zanetti et al., 1995*).

Most cathelicidins undergo extracellular proteolytic cleavage that releases the C-terminal peptide containing the antimicrobial activity. Cathelicidine have been named by using acronyms (e.g., cathelicidin antimicrobial peptide {CAMP}) or one-letter symbols of key amino acid residues present in 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, was initially identified in specific granules of human neutrophils. The free C-terminal peptide of hCAP18 is called LL-37 (37 amino acids; the two N-terminal amino acids are leucines) (*Sorensen et al., 1997*).