CATHELICIDINS AND SURFACTANT PROTEINS IN CHRONIC RHINOSINUSITIS: A CLINICAL AND EXPERIMENTAL STUDY

ENG HOOI OOI

Department of Surgery, Faculty of Health Sciences, The Queen Elizabeth Hospital,

University of Adelaide, South Australia, Australia

A thesis submitted in fulfilments of the requirement for the Degree of Doctor of Philosophy

on 7th June 2007

TABLE OF CONTENTS

Acknowledgement

I wish to acknowledge the Garnett Passe and Rodney Williams Memorial Foundation for providing me with the scholarship that has allowed me to perform the research for this thesis. I also wish to acknowledge the supervision of Professor PJ Wormald whose enthusiasm and encouragement led me to develop an interest in research and to undertake this project as part of my Otolaryngology Head and Neck Surgery training. I am grateful to Dr Lor Wai Tan my co-supervisor who spent countless hours teaching me the laboratory skills required to independently perform the experiments in this thesis. Dr Tan's attention to detail, enthusiasm for science, and her support is gratefully acknowledged. I also wish to acknowledge the support of Professor Maddern, Dr Cowled, Nicholas Hatziridos, Alkis Psaltis and other laboratory colleagues. Finally this thesis would not have been possible without the support, love, and sacrifice of my wife Li Lian and my children Sean and Marcus who are a constant inspiration.

Eng Hooi Ooi

Abstract

Objectives: To study the expression of cathelicidin antimicrobial peptides (CAMP) and surfactant protein D (SP-D) in patients with chronic rhinosinusitis (CRS) and eosinophilic mucus chronic rhinosinusitis (EMCRS) and by a nasal explant *in vitro* model cultured with fungal allergens.

Methods: Nasal biopsies from 59 CRS and EMCRS patients, stratified into Allergic fungal sinusitis (AFS), Nonallergic fungal eosinophilic sinusitis (NAFES), and Nonallergic nonfungal eosinophilic sinusitis (NANFES) were studied by quantitative real-time (q)RT-PCR, Western blot, immunostaining and ELISA. Nasal tissue from CRS and EMCRS patients were cultured with increasing concentrations of fungal allergens in a nasal explant *in vitro* model for 24 hours and CAMP and SP-D mRNA and protein levels in response to the fungi were determined by qRT-PCR and ELISA.

Results: The expression of CAMP mRNA was significantly increased in EMCRS patients compared to CRS patients ($p=0.0004$). By immunohistochemistry, expression of CAMP was localised to nasal epithelial, submucosal glands and inflammatory subepithelial cells. Western blotting demonstrated the presence of CAMP in the study patients. Culturing nasal explants with fungal allergens demonstrated significant upregulation of CAMP mRNA expression in CRS, but not EMCRS patients, by *Aspergillus* (mean 4-fold increase) and *Alternaria* (mean 6-fold increase) extracts with a significant dose-response effect ($p<0.001$). CAMP protein levels in the nasal tissue from CRS patients increased in response to *Alternaria* ($p<0.05$). In contrast, with EMCRS patients the expression of CAMP peptide in nasal tissue increased with *Aspergillus* ($p<0.001$) but decreased with *Alternaria*. Staining for SP-D was detected in the submucosal glands from the nasal biopsies in all patient groups except for AFS. By ELISA, SP-D was undetectable in AFS and decreased in NAFES, NANFES, and CRS compared to controls. CRS patients cultured with *Aspergillus* and *Alternaria* allergens in the *in vitro* nasal explant model induced significant upregulation

of SP-D mRNA ($p<0.0001$). In contrast, NANFES nasal tissue explants cultured with *Aspergillus* allergens induced downregulation of SP-D and only a modest upregulation of SP-D mRNA to *Alternaria* allergens.

Conclusion: This study demonstrates expression of cathelicidin antimicrobial peptides and surfactant proteins in nasal mucosa supporting its potential role in innate defences against inhaled pathogens. There is significant upregulation of CAMP mRNA in the EMCRS group implying an increased inflammatory state. *In vitro,* CAMP is significantly upregulated at the mRNA and protein level in CRS tissue explants to *Aspergillus* and *Alternaria* allergens. However, EMCRS tissue cultured with *Alternaria in vitro* does not demonstrate increased CAMP at the mRNA or protein level. The expression of SP-D in nasal tissue is reported for the first time. SP-D expression in the CRS, but not the EMCRS group, is upregulated *in vitro* by *Aspergillus* and *Alternaria*. The EMCRS group compared to CRS group demonstrate abnormal CAMP and SP-D expression to common fungal allergens. These important findings in understanding the pathogenesis of chronic rhinosinusitis are discussed in this thesis and may provide potential novel therapies for chronic rhinosinusitis in the future.

Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any university of other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis being made available in the University Library.

The author acknowledges that copyright of published works contained within this thesis (as listed below in the statement of authorship) resides with the copyright holder/s of those works.

Chapter 1

Literature review

Chronic rhinosinusitis

Chronic rhinosinusitis (CRS) is a highly prevalent disease estimated to affect approximately 16% of the adult population annually and cost approximately $5.8 billion in direct health care expenditure in the United States [1-3]. It causes significant physical symptoms, adversely affecting quality of life (QOL) resulting in a poorer general health status [4,5].

Clinical features of chronic rhinosinusitis

Patients with CRS usually suffer - symptoms of facial pain/pressure, nasal obstruction, nasal discharge that may be purulent, postnasal drip, hyposmia/anosmia, fever, headache, halitosis, fatigue, cough, or otalgia [6]. On examination with nasal endoscopy, there is often mucosa hyperemia, oedema, crusting, purulence, or nasal polyps [7]. Imaging of the paranasal sinuses in CRS often demonstrates mucosal thickening, bone changes, and air-fluid levels [8]. Chronic rhinosinusitis is defined as a group of disorders characterized by inflammation of the mucosa of the nose and paranasal sinuses of at least 12 consecutive weeks duration according to the Sinus and Allergy Health Partnership Task Force as outlined in Table 1 [9].

Table 1

Symptoms of nasal obstruction/blockage, nasal discharge/purulence/discoloured postnasal drainage, Hyposmia/anosmia, Facial pain/pressure, Headache	Continuous symptoms for > 12 weeks duration
Nasal endoscopy identifying (i) discoloured nasal discharge, nasal polyps, polypoid swelling; (ii) oedema or erythema of the middle meatus or ethmoid bulla; (iii) generalised or localised erythema, oedema, or granulation tissue.	At least one sign identified on nasal endoscopy in the decongested state
CT scan demonstrating isolated or diffuse mucosal thickening, bone changes, air-fluid level.	CT scans performed in all patients in this study prior to surgery

The presence of nasal polyps in CRS is usually associated with more severe symptoms, higher CT scan scores, and a significantly greater need for revision surgery [10]. The prevalence of nasal polyps has been estimated to be 4% from large epidemiology studies [11]. The incidence of nasal polyps is higher in the poorer prognostic group of patients with allergic fungal sinusitis (AFS), Samter's triad, asthma, cystic fibrosis, Churg-Strauss syndrome, Kartagener's syndrome, and Young syndrome [11]. The estimated recurrence rate of nasal polyps after surgery is 40% but the rate is even higher in the poor prognostic group [11].

Treatment for chronic rhinosinusitis

There are very few published prospective randomised controlled clinical trials comparing the different medical treatments and surgery [12-14]. It is generally agreed that medical

treatment consisting of topical or systemic corticosteroids, antibiotics, nasal douching, and allergen avoidance is effective in the management of CRS [12,14,15]. However, in those patients with CRS refractory to medical treatment, functional endoscopic sinus surgery (ESS) is accepted as the gold standard for surgical treatment [16]. ESS is excellent in relieving many of the symptoms of CRS and improving the patient's quality of life [17]. Surgery for chronic rhinosinusitis results in significant improvement by 95% in mean chronic sinusitis survey symptom scores [4]. Direct and indirect costs of treating a cohort of 100 patients with CRS were estimated per patient and compared to the cost of surgery with the use of economic modelling. Significant direct and indirect costs may be achieved with surgery for CRS more than comparable to the cost of medical treatment for CRS [18]. Long-term follow-up studies (average 7.8 years) of CRS patients after ESS demonstrate good outcomes if the original disease can be eradicated allowing the sinus cavity to be normalized [19]. The revision rate is estimated at 4% to 18% of patients [16,19,20]. Revision surgery can still be successful in providing significant symptomatic relief, however the patients often require intensive medical management with corticosteroids but need fewer courses of antibiotics post-operatively [21].

A history of previous sinus surgery, pansinusitis on computed tomography (CT) sinus scans, and smoking has been shown to correlate with poor outcomes after surgery and a frequent need for revision surgery [22,23]. In patients with aspirin sensitivity or AFS the prognosis is poorer with these patients frequently requiring multiple operations [24,25].

Factors associated with chronic rhinosinusitis

The causes of CRS are numerous and various factors have been associated with either its pathogenesis or exacerbating its course. The factors include allergy and disorders of immunity, biofilms, superantigens, colonizing fungi that induce and sustain eosinophilic inflammation, and aspirin sensitivity [26]. The classification of CRS is confusing as various systems have been used classifying on the basis of clinical features as CRS is still a clinical

diagnosis [26-28]. A recent classification system for CRS has been proposed subclassifying patients on the basis of (1) presence or absence of nasal polyps; (2) presence or absence of eosinophilia; and (3) presence or absence of fungal hyphae in sinus mucus [26]. Precise classification of a patient in terms of inflammatory features for this thesis was possible through the meticulous collection of sinus tissue and mucus and its thorough evaluation by histology and microbiology using a similar sub-classification system developed in our department [28].

Allergic Fungal Sinusitis (AFS)

Allergic fungal sinusitis (AFS) is a distinct clinical subset of CRS that is particularly aggressive and associated with the presence of fungi. Historically, Millar et al first described 5 patients with chronic sinusitis and Aspergillus infection of the maxillary sinus with immediate cutaneous reactivity to *Aspergillus fumigatus* antigen, diagnosing these patients as "allergic aspergillosis of the paranasal sinuses" [29,30]. Allergic aspergillus sinusitis was described by Katzenstein in 1983 and thought to be the sinonasal equivalent of allergic bronchopulmonary aspergillosis (ABPA) because of similar histological features [31]. Material that was indistinguishable from mucoid impaction of bronchi was found in the paranasal sinuses [31]. This material contained clumps of necrotic eosinophils and other cellular debris arranged in multilayered rows, within a background of amorphous mucin, Charcot-Leyden crystals, and fungal hyphae within the allergic mucin. Other cases of patients with recurrent nasal polyps, radiographic evidence of pansinusitis, asthma, and mucinous material containing eosinophils, fungal hyphae, and Charcot-Leyden crystals were subsequently described in the literature [32]. Numerous cases of AFS have since been reported in the literature as recognition of this clinical entity has increased [33-38].

The criteria for diagnosing AFS were formalized based on clinical characteristics shared by a cohort of 15 patients as described by Bent and Kuhn [39]. The five diagnostic criteria were: positive evidence of atopy, nasal polyposis, and characteristic CT findings of areas of high

attenuation, presence of eosinophilic mucus, and positive evidence of fungus. Positive skin tests (not just to fungi) or an allergic history is currently considered sufficient evidence of atopy or type I hypersensitivity [40].

The clinical presentation of AFS is with symptoms of chronic rhinosinusitis, predominantly nasal obstruction from extensive nasal polyposis, and a history of multiple operations [41]. Most patients will have a history of producing dark green rubbery nasal casts or plugs [42]. Occasionally, the presentation of AFS may be dramatic with acute visual loss, facial deformity and intracranial extension [43-45]. Nasal polyps and allergic mucin are often found on endoscopy. Typical CT scan findings demonstrate bony expansion, remodelling, or thinning of involved sinus walls, areas of high attenuation and heterogeneous areas of signal intensity within the sinuses [41]. MRI features highly specific for AFS include a hypointense central T1 signal, central T2 signal void, and the presence of increased peripheral T1/T2 enhancement [41]. Fungi associated with AFS are ubiquitous and predominantly of the dematiaceous family which include *Bipolaris, Curvularia, Exserohilum,* and *Alternaria* species while *Aspergillus* species account for the non-dematiaceous group [42,46 43].

The prevalence of AFS as defined by the Bent and Kuhn criteria is reported to vary from 4% to 10% in the literature [33,47,48]. Cases of AFS have been reported worldwide with an interesting geographical variation in incidence [48]. It appears that AFS is more common in warm humid or dry -desert areas. In the USA, most cases have been reported from central Texas, Alabama, Georgia, Oklahoma, Florida, Arizona, and southern California [48]. Some investigators have speculated that the high recurrence rates for AFS in the southwestern United States is due to the intermittently high *Bipolaris* species mold spore counts leading to significant reinoculation of the mold in the paranasal sinuses [42].

Allergic mucin which is also known as eosinophilic mucus or eosinophilic mucin (EM), or fungal mucin has a gross appearance of being thick, tenacious, highly viscous, peanut-butter like and green to light tan in colour [28,41]. Sheets of eosinophils within an amorphous

matrix of mucin and Charcot-Leyden crystals are present [41,46]. Specific fungal stains such as Gomori's methanine silver (GMS) and Periodic-acid-schiff (PAS) stains are required to identify the fungal elements. Frequently, but not always, noninvasive fungal hyphae are found within the mucin. The use of prednisolone preoperatively may inhibit the identification of fungal hyphae and eosinophils in the sinus mucin [49]. The presence of EM is not pathognomonic for AFS alone. In fact, numerous cases of suspected AFS with the typical presence of EM but no fungi identified have been reported in the literature [36]. Some patients presenting similar to AFS without fungal-allergy have been classified as having AFS-like syndrome [33]. The classification of AFS was originally based on empirically selected criteria [39].

Several alternative classification schemes for patients similar to AFS but without either fungal allergy or fungi identified have been proposed. Ferguson initially proposed that patients sharing histological similarities to AFS except for the presence of fungal hyphae could be classified as eosinophilic mucin rhinosinusitis (EMRS) [50]. This distinction was based on a review of cases described in the literature and Ferguson's personal series of cases. EMRS was defined as the histological presence of eosinophilic mucin but no fungus present by histology or culture. EMRS patients were statistically more likely to be asthmatic, aspirin sensitive, older, and to have an IgG1 deficiency [50]. Although IgE levels were increased in EMRS and AFS patients, the IgE levels in the AFS group was statistically higher compared with the EMRS group. Ferguson proposed that even though EMRS and AFS shared some features, EMRS was postulated to be a systemic dysregulation of normal immunological controls whereas AFS is an allergic response to fungi [50].

A retrospective review of 25 surgical cases with allergic mucin identified was recently published [51]. Its characteristic histological features confirmed the presence of allergic mucin. All specimens were evaluated with GMS and PAS stains to identify fungal organisms. Fungal organisms were identified in 10 cases (40%) and compared to the other

15 cases without fungi identified. Both groups had pansinusitis and nasal polyps. Clinical presentation, radiographic findings, atopy and laterality were not significantly different between the two groups. The finding of allergic mucin without fungus is interesting. It demonstrates that the presence of allergic mucin is not unique to AFS but perhaps a result of other inflammatory processes. The fungus may be perpetuating the process or be an entrapped bystander. The investigators proposed that AFS is better described as "eosinophilic mucinous rhinosinusitis" as the patients all possess in common the presence of EM.

A prospective study conducted by our department investigated 349 consecutive chronic rhinosinusitis patients undergoing endoscopic sinus surgery and identified 134 patients (38%) with characteristic thick eosinophilic mucus (EM) suspicious of fungal sinusitis [47]. The EM was subjected to fungal culture and histology and patients were defined, as fungal positive if either fungal stains or culture were positive. These CRS patients with EM were defined as eosinophilic mucus chronic rhinosinusitis (EMCRS) and further subdivided into various subgroups depending on the presence of fungus and allergy results into allergic fungal sinusitis (AFS 8.6%), AFS-like (1.7%), Nonallergic fungal eosinophilic sinusitis (NAFES 15.2%), and Nonallergic nonfungal eosinophilic sinusitis (NANFES 69%) [47]. This classification system based on the common feature of EM being present in all these patients is used throughout this thesis.

Pathogenesis of AFS

IgE vs Non-IgE mediated inflammation

AFS by definition is extra-mucosal and noninvasive. Allergic fungal sinusitis was initially thought to be an allergic or IgE mediated hypersensitivity to fungi rather than an infectious process [52]. This theory was based on the observation that AFS patients often tested positive to fungal antigens using RAST or skin prick testing [53]. In a study by Manning et al, patients with *Bipolaris* culture positive AFS after surgery were selected for testing by skin prick,

inhibition-RAST, and -ELISA for *Bipolaris* specific-IgG and –IgE and compared to a control group of healthy volunteers [53]. All eight AFS patients had positive skin test reactions to *Bipolaris* antigen and all showed positive RAST and ELISA inhibition for *Bipolaris*-specific IgE and IgG respectively whereas only one of the control patients tested positive. The total serum IgE is generally elevated (>199 IU/ml) in approximately two-thirds of AFS cases [54]. Total serum IgE has been proposed to be useful for follow-up as it tends correlate with AFS recurrence [54]. Immunologic evaluation confirms normal immune function in all cases of AFS [42].

There continues to be significant debate concerning the role of IgE in AFS. Stewart et al used a nine-mold RAST panel to evaluate fungal-specific IgG and IgE levels showed that AFS and AFS-like patients have a higher total and fungal specific serum IgE compared to non-AFS CRS polypoid patients [55]. Fungal specific IgG levels of class III and greater were also significantly greater for AFS and AFS-like patients compared to non-AFS polypoid CRS [55]. However, Pant et al investigating fungal-specific humoral responses used an ELISA to measure *A. fumigatus* and *A. alternata* specific serum IgG, IgE, IgM, and IgA levels in CRS and EMCRS patients [56]. The EMCRS patients including AFS patients were compared to CRS, allergic rhinitis with fungal allergy (ARFA), and healthy volunteers and no significant difference in fungal-specific IgE levels were detected between the different subgroups [56]. Furthermore there were no differences in total serum IgE and fungal-specific-IgE levels between EMCRS and fungal-allergic disease controls (ARFA). Only IgG3 levels were elevated significantly in the EMCRS subgroup compared to healthy volunteers, ARFA and CRS [56]. Pant et al concluded that fungal allergy or serum fungal-specific IgE alone was unlikely to be involved in the pathogenesis of AFS al [56]. The difference in IgE findings between Pant et al [56] and Stewart [55] could be explained by different methods used to measure fungal specific IgE levels and the comparison disease control group.

There is also evidence that nasal mucosa can produce local IgE which may contribute to localized IgE mediated sinus inflammation [57,58]. Collins et al liquefied the eosinophilic

mucus from patients undergoing surgery for suspected AFS and tested it for fungal-specific IgE using the Pharmacia UniCAP system [28]. The investigators were able to demonstrate the presence of fungal-specific IgE in the mucus and AFS patients were significantly more likely to have fungal-specific IgE in their eosinophilic mucus. Furthermore, 19% of patients with negative serum fungal-specific IgE and positive fungal cultures had a positive mucus fungal-specific IgE suggesting they could be reclassified as AFS [28].

Controversies of the role of fungi in the pathogenesis of CRS and AFS

Ponikau et al controversially hypothesized that most cases of chronic rhinosinusitis are due to fungal colonization acting as an important inflammatory stimulus for eosinophils in the allergic mucin [59]. The investigators cultured fungi from the lavage fluid of 96% of chronic rhinosinusitis patients with an average of 2.7 fungi per patient. The high culture rate was due to subjecting the collected lavage fluid to dithiothreotol to break apart the disulfide bonds, thus liquefying the mucus. This allows a much higher positive fungal culture rate than generally reported in the literature [47]. Their positive detection rate in identifying fungal elements (hyphae, conidia, and spores) from intraoperative allergic mucin samples subjected to histology was 81%. Allergic mucin was identified histologically in 96% of cases, a rate higher than other reported series [47]. The same investigators also detected no significant differences in total IgE and fungal-specific IgE between this CRS group and the control group. Interestingly, the normal control group cultured 100% fungi from the nasal lavage fluid. Therefore fungi can be found in both diseased and healthy controls implying that the presence of fungi in the nose and sinuses does not equate pathogenicity. Ponikau proposed an alternative classification for AFS to eosinophilic fungal rhinosinusitis (EFRS) and suggested that most cases of CRS can be reclassified as EFRS.

An independent study from Europe using Ponikau's techniques also identified a high proportion of CRS patients with fungi [60]. The study group consisted of 104 CRS patients with mucus obtained by flushing, 106 CRS patients with mucus obtained at ESS, and 23

healthy volunteers with samples obtained by flushing [60]. Altogether 619 strains of fungi were cultured from 233 samples with 91.3% fungal growth in the samples from flushing in CRS patients, 91.3% positive growth in the healthy control group, and 84% positive in the samples from CRS patients obtained at surgery [60]. The most common fungi isolated were *Aspergillus, Penicillium, Cladosporium, Candida, Alternaria,* and *Aureobasidium*. Positive identification of fungal elements by microscopy was 70%. A further follow-up study confirmed similar findings [61]. Lackner et al showed that fungi could be detected in nasal secretions of neonates implying that fungi are ubiquitous and inhaled from the environment soon after birth [62,63].

There are numerous other studies reporting a lower rate of fungal detection. The differences may be due to a more meticulous technique for specimen collection and different detection methods. Fungal culture of the EM were shown to be positive in 65% of non-invasive fungal sinusitis patients in a previous study conducted in our department [28,64]. This is in agreement with the reported 67% positive culture rate in fungal sinusitis cases [38]. A recent study using Ponikau's method of nasal lavage and specimen processing resulted in a lower positive fungal growth rate of 49% although it was higher than using middle meatus swabs alone [65]. PCR is a very sensitive molecular biology technique for amplifying small amounts of DNA. Nasal swabs and DNA samples were collected with a nasal cytology brush from CRS and control patients [66]. PCR was significantly more sensitive detecting fungal DNA from specimens collected with a nasal cytology brush compared to culture from nasal swabs [66]. The study detected fungal DNA in 42% of controls and 40% of CRS patients implying that fungi alone are insufficient to explain most cases of CRS. The detection rate of *Aspergillus* spp. by PCR and culture from nasal lavage fluid subjected to dithiothreitol was lower and only positive in four of 33 (12%) CRS patients with nasal polyposis [67]. Recently, Gosepath et al using PCR detected fungal DNA from polypoid tissue taken from the middle meatus and paranasal sinuses in all 27 CRS patients and 66.7% of the nasal mucosa from healthy sinus mucosa [68]. The investigators used a panfungal primer and an

Alternaria-specific primer for the PCR. All healthy sinus mucosa controls were negative whereas 100% of CRS patients tested positive for the *Alternaria*-specific primer. In summary the detection rate for fungi in sinus mucus is variable depending on techniques used and the role of fungi in CRS remains open to further investigation.

Putative inflammatory mediators in AFS

The nasal mucosa is thought to be damaged over time by the release of inflammatory mediators such as tumor necrosis factor-α, interleukin (IL)-4, IL-5, IL-10, IL-13, major basic protein (MBP), eosinophil cationic protein (ECP), eosinophil peroxidase, and eosinophil derived neurotoxin (EDN) [40]. Immunohistochemistry staining of sinus mucosa from AFS patients demonstrated strong reactivity for the eosinophil mediators MBP, EDN, and neutrophil elastase [53]. In another study eosinophil MBP, neutrophil elastase, and mast cell tryptase were localized to cells in the respiratory mucosa adjacent to the allergic mucin [69]. Diffuse extracellular staining for neutrophil elastase was also detected in the mucin for the first time [69]. This important study provides evidence of the involvement of neutrophils and its degranulation products in the pathogenesis of AFS [69]. The results from indirect immunofluorescence suggest that activation of both eosinophils and neutrophils releasing their toxic products contributes to tissue inflammation, destruction, and formation of allergic mucin [69]. Further evidence that eosinophils cluster and release MBP at toxic levels within the mucus compared to normal controls was provided in a recent study of CRS patients with mucus [70].

Treatment of AFS

Treatment of AFS requires both medical and surgical treatment [40]. Surgery is required to remove all fungal mucin in the sinuses, reestablish aeration of the sinuses, and obtain specimens for histological diagnosis. Postoperatively 6 months of systemic corticosteroid therapy has been recommended to reduce the chances of disease recurrences [49]. Decreasing the prednisolone dose too soon results in earlier relapse of disease. Endoscopy is useful to

detect disease recurrences often before clinical symptoms appear [7,24,40]. An endoscopic mucosal staging system by Kupferberg has been used to allow monitoring of the disease progression and prognosis [24]. The longest time to recurrence of disease without prednisolone was found to be 34 months in a series of AFS patients [49]. A recurrence of disease in patients is presumably due to reinoculation of their paranasal sinuses with inhaled fungi in their environment [49]. Eventually the disease appears to become quiescent or "burned out" once control of the disease is achieved by medical and surgical treatments. Immunotherapy was described as a possible treatment for AFS after surgery [71]. The immunotherapy protocol involved testing AFS patients for hypersensitivity by skin prick tests against 11 fungal antigens. The antigens that produced a positive response were incorporated into a treatment mixture and administered on a weekly basis to the highest tolerated dose that did not cause a systemic reaction or localized hypersensitive reaction. After 6 months of this therapy, other non-fungal antigens, which the patients had tested positive to, were added into the treatment mixture. The immunotherapy regimen did not appear to worsen the AFS patient's symptoms and may have reduced formation of nasal crusts and mucin and reduced the need for systemic and topical steroids [72]. However criticism of the study included its short-term follow-up, lack of a placebo and a control group. No recurrences of disease were reported in 8 AFS patients who had received immunotherapy for at least 3 years and then discontinued treatment [73]. However, long-term studies of a 4- to 10-year follow up of AFS patients receiving immunotherapy post operatively compared to another group of AFS patients who did not receive immunotherapy failed to detect any significant differences between the two groups [74]. It appeared that use of immunotherapy in AFS patients did not have a significant impact on the long-term control of the disease; also implying that IgE mediated hypersensitivity reaction to fungi was not the key process in AFS.

Summary of AFS and EMCRS

EMCRS is a subgroup of CRS characterized by the presence of EM within the paranasal sinuses. AFS is the most aggressive form of EMCRS characterized by fungi within the EM and recurrent nasal polyposis. The role of fungal allergy has been questioned by recent studies on the humoral responses to fungal allergens. The recent findings that fungi can be cultured from nasal secretions of most CRS patients and healthy controls strongly suggest that fungi are ubiquitous in the paranasal sinuses. Therefore fungi might play an alternate role in the pathogenesis of CRS and AFS whereby patients mount abnormal local innate immune responses to colonizing fungi. These responses are hypothesized to be abnormal leading to inflammation, tissue injury, or failure to clear the fungi. Further research is required to understand the innate immune responses of paranasal sinus mucosa to the presence of fungal allergens.

Biofilms

Biofilms are sessile communities of bacteria embedded in a self-produced exopolysaccharide matrix and irreversibly attached to a mucosal surface [75]. 99% of bacteria exist in biofilms whereas 1% of bacteria exist in a free-floating planktonic form. Bacteria in biofilms have an altered phenotype, lower metabolic requirements, and altered gene transcription, and are extremely difficult to culture [76]. Culture is possible only when bacteria at the edge of the biofilm break off to become a free-floating planktonic form. *Pseudomonas aeruginosa* and *Staphylococcus aureus* are bacteria well known to form biofilms [76]. Fungi, for example *Candida albicans* and *Cryptococcus neofromans* form biofilms as well [77,78]. Biofilms are extremely resistant to antibiotics and provoke both cellular and humoral responses in the host but the inflammatory response is ineffective once the biofilm is established. Biofilms associated with chronic diseases such as OME in a chinchilla model, discharging tympanostomy tubes and infected tracheostomy tubes have been demonstrated with electron scanning microscopy [75,79]. The presence of biofilms has

recently been demonstrated on frontal recess stents in patients with chronic frontal rhinosinusitis [80]. Biofilms were identified using scanning electron microscopy and transmission electron microscopy associated with intraoperative sinus tissue biopsies from 80% of CRS patients and none detected in the healthy controls [81].

Superantigens

Superantigens are microbial toxins that have the ability to bind to MHC class II molecules on antigen-presenting cells (APCs) and T cells and cross-link the MHC Class II molecule on APCs with the variable β-region of the T-cell receptor [82]. The ability of superantigens to bypass normal internal processing by the APCs and classical antigen specificity causes activation of up to 30% of the body's lymphocytes in contrast to the normal activation of <0.01% in classical antigen-specific activation of adaptive immune T-cell responses [82]. Activation of T-cells by superantigens does not require the classical co-stimulation molecules expressed by APCs or T-helper cells. *S. aureus* is well known to produce many toxins that act as superantigens [83]. The presence of specific IgE to staphylococcal enterotoxins A and B (SEA and SEB) was demonstrated in nasal polyp homogenates supporting a possible role for bacterial superantigens [84]. A small study observed that seven of 13 patients with nasal polyposis cultured *S. aureus* from nasal mucus while the other six patients had coagulase negative *Staphylococcus*. Two patients had *Staphylococcus* enterotoxin A (SEA), two patients had *Staphylococcus* enterotoxin B (SEB), and three patients had toxic shock syndrome toxin 1 (TSST-1) [85]. In this same study the investigators identified clonal expansion of the variable β-region of the T-cell receptor that corresponds to the detected staphylococcal enterotoxin [85]. More recently, staphylococcal enterotoxins were detected in 50% of nasal polyp homogenates and 30% of mucus samples from CRS patients with and without nasal polyps whereas none were detected in the control group [86]. Although these studies provide evidence of the presence of staphylococcal enterotoxins in CRS patients potentially acting as superantigens, at least half of the patients do not have

staphylococcal enterotoxins detected suggesting other aetiologies must be causing CRS pathogenicity.

Aspirin sensitivity

Samter's triad is a well-known clinical syndrome of asthma, aspirin sensitivity, and nasal polyposis [87]. In this group of patients, arachidonic acid metabolism is shunted into the pro inflammatory leukotriene pathway producing excessive leukotrienes. It is thought that the pro inflammatory state predisposes to the formation of nasal polyps. There is some recent evidence that genetic predisposition plays a role in nasal polyp formation [88]. Genotyping was performed on patients with polyps and compared to normal controls. Patients carrying the HLA-DR7-DQA1*0201, and -DQB1*0202 haplotype were found to have a two to three times higher odds ratios for developing the disease, compared with controls. Patients with ASA triad carried the above-mentioned DR7 allele with the linked alleles significantly more often [88].

Immune disorders

A retrospective review of 79 patients with refractory sinusitis, (excluding AFS, cystic fibrosis and primary ciliary dyskinesia patients), that evaluated immunoglobulin levels and T lymphocyte function, detected several abnormalities of T-cell function and decreased levels of the different immunoglobulin subclasses [89]. 54.8% of the patients showed abnormal proliferation in response to recall antigens tetanus and mumps, 11.3% had decreased response to alloantigen, and 26.3% demonstrated decreased response to T-cell mitogens. Low IgG in 14 of 78 patients (17.9%), low IgA in 13 of 78 (16.7%), and low IgM in 4 of 78 (5.1%) and selective IgA deficiency in 6.2% were also detected.

Innate immunity in the human sinonasal tract

Introduction of innate immunity

The sinonasal tract plays an important role in innate immunity, as it is the first point of contact with airborne pathogens. In particular, exposure to inhaled allergenic fungal spores and pollen occurs under normal everyday circumstances both indoors and outdoors [90]. Pathogens are cleared from the sinonasal tract by various mechanisms otherwise there would be a very high incidence of rhinosinusitis. The innate immune system is the first line of defence protecting against infection and damage caused by airborne pathogens. The innate immune system has other roles, which include signalling and activating the acquired immune system to the presence of pathogens. This review will focus on the innate immune system and the potential role of innate immunity in the form of cathelicidin and surfactant proteins in chronic rhinosinusitis.

The sinonasal tract and innate immunity

The sinonasal tract is lined by respiratory epithelium covered by a superficial layer of mucus and a deeper serous periciliary layer [91]. The secreted mucus traps inhaled particles allowing it to be transported by mucociliary action to the posterior pharynx where it can be swallowed. Chronic rhinosinusitis results in impaired mucociliary clearance as measured by saccharin clearance time [92]. Electron microscopy of biopsies of the middle turbinate in CRS demonstrate loss of cilia and differentiated epithelium, structural defects in the cilia, basal cell hyperplasia and squamous metaplasia [93]. The changes in ciliary function are secondary to the CRS disease process rather than the cause of CRS as the abnormalities are reversed with treatment [92,93]. Mucociliary clearance improves with endoscopic sinus surgery as demonstrated by shorter saccharin clearance times and regeneration of cilia but it takes at least 3 months for significant recovery to be observed [94-96]. Antral lavage and nasal douching with hypertonic saline also improve mucociliary clearance [15,92].

The respiratory epithelium is actively involved in inflammation and host defence in multiple ways: a mechanical barrier, through mucociliary clearance allowing the physical removal of inhaled pathogens [97], recognition of microbial exposure by pattern recognition receptors expressed on epithelial cells [98], secretion of pro- and anti-inflammatory mediators [91], and a variety of antimicrobial peptides [99].

The inflammatory response in chronic rhinosinusitis

Infection with a pathogen triggers the inflammatory response that involves cells and molecules of the immune system [100]. Cytokines are low molecular weight proteins that act, generally in a paracrine fashion, to regulate differentiation, cell growth, inflammation, immunity, proliferation, and function of the immune cells [101]. Chemokines are a superfamily of small proteins that act mainly as activators and chemoattractants for leucocytes. T and B-lymphocytes, macrophages, fibroblasts, endothelial, epithelial, and bone marrow stromal cells produce various cytokines and chemokines during the inflammatory response [101]. Bachert studied the cytokine profile in twenty-three patients with nasal polyps compared to inferior turbinate tissue from eighteen control patients [102]. The cytokine concentrations of Interleukins (IL)-1β, -3, -4, -5, -6, -8, -10, Tumor necrosis factor (TNF)-α, granulocyte-macrophage colony-stimulating factor (GM-CSF), regulated on activation normal T expressed and secreted (RANTES) and GRO-α were determined by ELISA. The key finding was significantly elevated IL-5 concentrations (median 11.45 pg/ml) in nasal polyp homogenates compared to the controls (only one of 18 controls) whereas the concentrations of IL-6, IL-8, and IL-10 were not significantly different [102]. The histological features of acute and chronic rhinosinusitis from animal studies and biopsies from CRS patients will be reviewed to highlight the important findings. A mouse model of acute viral rhinosinusitis was developed using intranasal reovirus inoculation [103]. The infected mice displayed subepithelial oedema and sinus opacification with an influx of B cells at post infection day 4 and influx of T cells at post infection day 7 with undetectable

levels of B and T cells at day 21. Staining for reovirus in the specimens was undetectable by day 10-post infection. These results suggest that the innate immune system is able to clear the virus but release of local factors after infection even in the absence of significant virus promotes the influx of T and B cells in an adaptive immune response.

Histological findings on surgical biopsies of sinus mucosa showed desquamation of superficial columnar cells, marked thickening of the basement membrane, odema of the submucosa, metaplasia into stratified squamous epithelium, and prominent infiltration by eosinophils and some neutrophils [104]. Hamilos et al have reported that biopsies from middle turbinates of normal control subjects contain minimal numbers of inflammatory cells whereas biopsies from chronic hyperplastic sinusitis (CHS) patients contain intense inflammatory infiltrates of mainly eosinophils and some neutrophils [105]. Tissue eosinophilia is a prominent feature in both allergic and nonallergic patients [106]. Important histological features of CRS with nasal polyps are predominance of eosinophils, IL-5 producing T lymphocytes, expression of RANTES, and eotaxin and VCAM-1 adhesion molecule in epithelial cells [107]. Infectious inflammation is associated with increased neutrophil influx, IL-1β, IL-6 levels in sinus tissue by epithelial cells in response to bacteria or viruses [101]. Biopsies of sinus mucosa from 22 CRS patients undergoing ESS were subjected to histological and immunohistochemical analysis [108]. All CRS patients demonstrated epithelial shedding and basement membrane thickening whereas healthy controls maintained an intact epithelial barrier. Staining for major basic protein was used to measure eosinophilic inflammation with no differences detected between allergic and non-allergic patients but was not detected in healthy controls. The histological features of CRS demonstrate similar features of inflammation and tissue remodeling with those seen in asthma.

The immune system

The immune system is broadly divided into two different types of responses against pathogens. Innate responses consist of all the immune defenses that lack immunologic memory [100]. Their characteristic feature is the responses occur to the same extent, however often the antigen is encountered. These types of responses evolved earlier than the acquired (adaptive) responses. Whereas, the acquired response has memory and the response is greater on repeated exposure to the same pathogen [100]. The important features of innate and acquired immunity are summarized in Table 2.

Table 2

Innate	Acquired (or adaptive)
Responses evolved early	Responses acquired with exposure to
Present from birth	pathogens
Immediate response	Slower response (3 to 5 days)
Germ-line encoded receptors	T and B-cell receptors specific to the
(Nonspecific, hundreds of receptors only)	antigen (10^{14}-10^{18} receptors)
Not dependent of prior exposure	Has memory
Immune response the same regardless of	More effective immune response on
exposure to antigen	subsequent encounter with the antigen

Acquired responses include the proliferation of antigen-specific B and T lymphocytes that occurs when an antigen binds to the surface receptors of these cells [100]. Specialized cells, called antigen-presenting cells, display the antigen to lymphocytes and activate these cells by expression of co-stimulatory molecules such as B7, CD40, CD80, CD83, CD86 in association with MHC class II [109]. B cells are stimulated to secrete immunoglobulins, the antigen-specific antibodies, responsible for eliminating microorganisms. T cells help the B

cells to produce antibodies and can also eradicate intracellular pathogens by cytoxicity and activating macrophages.

The major histocompatibility complex (MHC) is encoded on the short arm of chromosome 6 and can be divided into two classes. MHC class I genes encode for human leucocyte antigens (HLA) A, B, and C that are present on all nucleated cells. MHC class II genes encode for human leucocyte antigen-D (and subclasses –DP, -DQ, and -DR) that are only found on certain lymphocytes, macrophages, epidermal cells and sperm. T-cells recognize antigens presented in association with the appropriate MHC whereas B-cells can recognize the antigen alone. The function of the HLA is to act as receptors for foreign antigens and present them to MHC class I ($CD8^+$ T-cells) and MHC class II ($CD4^+$ T-cells) restricted cells.

Innate and acquired responses work together to eliminate pathogens. All these cells develop in the fetal liver and bone marrow and are then released into circulation. B cells reach maturity within the bone marrow, but T cells must complete their development within the thymus. Acquired responses are generated in the lymph nodes, spleen, and mucosa-associated lymphoid tissue. The T-cell and B-cell receptors are generated randomly during their development, so that each lymphocyte has a single receptor specific to an antigen. An extremely large and diverse range of receptors (10^{14}-10^{18}) is generated randomly to increase the probability that an individual lymphocyte will recognize and bind to an antigen triggering activation and proliferation of clones of lymphocytes bearing that receptor. Lymphocytes expressing receptors specific for the pathogen are subsequently selected for clonal expansion by encountering antigens for which they happen to be specific. Clonal selection is important for the development of the acquired immune system. Clonal expansion of lymphocytes takes three to five days for sufficient numbers of clones to be generated and to differentiate into effector cells. However, these T- and B-cell receptors are not encoded in the germ line, so they cannot be passed on to the next generation even if they provide an evolutionary survival benefit. These T- and B-cell receptors may contain

binding sites against ubiquitous environmental allergens and self-antigens. The activation of the acquired response can be harmful to the host if the antigens are self or environmental antigens, since immune responses to these antigens can lead to autoimmune diseases and allergies. In contrast, innate immunity is activated immediately after infection and serves to rapidly eliminate or control replication of the infecting pathogen [110].

Innate immune recognition

A key difference between the innate and acquired immune response is the mechanisms of recognizing antigens. Innate immune recognition is mediated by germ-line encoded receptors, which means that the specificity of each receptor is already genetically predetermined. There are approximately 10^{14} and 10^{18} different somatically generated B and T cell receptors. However, there are only a few hundred innate immunity receptors [110]. The innate immune response focuses on recognizing a few highly conserved structures present on microorganisms, rather than every possible antigen. These structures are referred to as pathogen-associated molecular patterns (PAMPs). Examples of PAMPs include lipopolysaccharide (LPS), lipoteichoic acid (LTA), peptidoglycan, mannans, bacterial DNA, and double stranded RNA [110]. PAMPs have several features important in understanding the innate immune system. (i) PAMPs are produced by microbial pathogens but not by the host cells. This allows the innate immune system to differentiate self from non-self molecules. (ii) PAMPs represent conserved molecular patterns essential for the survival or pathogenicity of the microbial pathogen. (iii) PAMPs are usually shared by entire classes of pathogens. For example, gram-negative bacteria all have LPS, and gram-positive bacteria all have LTA. This property of PAMPs allows a limited number of germ-line encoded pattern recognition receptors to recognize a vast variety of pathogens.

Pattern-recognition receptors

The receptors of the innate immune system are expressed on many effector cells of the innate immune response such as macrophages, dendritic cells, B cells, and respiratory

epithelial cells [111]. Functionally, pattern-recognition receptors can be divided into three classes: secreted, endocytic, and signalling [110]. Secreted pattern-recognition receptors function as opsonins for phagocytosis or activation of the lectin pathways of complement. The best-characterized receptor of this class is the mannan-binding lectin and surfactant proteins A and D belonging to the family called collectins because structurally they consist of a collagenous domain linked to the calcium-dependent lectin domain [112]. Endocytic pattern recognition receptors occur on the surface of phagocytes. These receptors mediate the engulfment of pathogens by phagocytes and dendritic cells where it can be processed and presented to lymphocytes. An example is the macrophage mannose receptor [113]. Signalling receptors trigger signalling pathways that result in the induction of transcription of a variety of immune response genes, such as inflammatory cytokines and the antimicrobial peptides [100].

The recently described Toll-like receptors (TLRs) are signalling transmembrane receptors that contain an extracellular leucine rich repeat domain and a cytoplasmic signalling domain [98]. The first receptor of the toll family was discovered in the fly *Drosophilia* Toll gene. TLRs are homologues of *Drosophila* toll and key players in mediating innate immunity and the activation of adaptive immunity. Studies of gene knock out mice have identified the roles for some of the TLRs. TLR4 has been identified as the key receptor for recognising LPS in gram-negative bacteria [98]. TLR2 is involved in recognising LTA and peptidoglycan from gram-positive bacteria [114]. Infections with *C. albicans* and *A. fumigatus* have involved TLR2 and TLR4 in the activation of innate immune responses [115]. Expression of mRNA for all TLRs has been detected by RT-PCR in human sinonasal tissue from patients with chronic rhinosinusitis [116].

Linking innate immune responses and the acquired immune response

The acquired immune system responds to a pathogen only after it has been recognised by the pattern-recognition receptors on antigen-presenting cells. The peptides are processed

and presented in a complex with MHC class II molecules to T-cell receptors. Furthermore, the APC needs to express both the antigen and co-stimulatory molecules (e.g. CD80, CD86) so that the T cell can be activated. Recognition of an antigen in the absence of the co-stimulatory molecule will cause apoptosis of the T-cell [110]. The expression of these co-stimulatory molecules is controlled by the innate immune system. Self-antigens are not recognised by the pattern-recognition receptors and therefore do not induce the expression of co-stimulatory molecules. This ensures that usually only pathogen-specific T-cells are activated. Recent studies have demonstrated that the innate immune system plays an important role in signalling and activating the acquired immune system [110].

Cellular components of innate responses

The polymorphonuclear neutrophil (PMN) and the macrophage are the major phagocytes in the body, whereas the eosinophil and basophil are only weakly phagocytic [100]. Phagocytosis involves several steps: attracting the phagocyte to the bacteria by chemotactic factors, binding of the organism to receptors on phagocytes, and engulfing the organism. Opsonisation or coating of the organism with antibodies, complement, and collectins enhances the binding to phagocytes [112]. Phagocytes also play an important role removing the body's own apoptotic cells.

Killing of the engulfed microorganism occurs by both oxygen-dependent and –independent mechanisms. The respiratory burst refers to the increased oxidative metabolism following activation of phagocytes to generate reactive oxygen species (superoxide anion, hydroxyl radicals, hypochlorous acid, and nitric oxide). The neutrophil contains preformed bactericidal peptides (lysozyme, lactoferrin, bactericidal permeability protein, cathepsin G), various proteases and cationic antimicrobial peptides (cathelicidins and defensins) within the larger primary azurophil and the smaller specific granules [100].

Basophils and mast cells possess high-affinity receptors for IgE (FcεR) and become activated by the binding of allergens to IgE, thus cross-linking the FcεR. This triggers the

release of preformed inflammatory mediators such as histamine, prostaglandin, and leukotrienes [117]. Eosinophils are granulocytes typically seen in the blood and tissue in diseases such as asthma, helminth infection, and allergy. Eosinophils contain both primary and specific granules. Upon activation, it releases granule-stored cationic peptides Major basic protein (MBP), Eosinophil-derived neurotoxin (EDN), leukotrienes, and various cytokines [117].

Natural killer cells (NKC) destroy infected and malignant cells by two methods [100,118]. They possess Fc receptors that bind IgG (FcγR). NKC's can target IgG-coated cells and kill them by a process called antibody-dependent cellular cytotoxicity. 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, which recognize MHC class I molecules, the killer-activating receptors signal the NKC's to attack and kill the cell. The NKC inserts the pore-forming molecule perforin into the cell membrane of the target cell and releases cytotoxic granzymes into the cell [100].

The dendritic cell (DC) is a key cellular component of innate immunity. All DC's are derived from CD34+ pluripotent bone marrow progenitor cells and are characterized by different phenotypes and functions during their maturation [109]. Immature dendritic cells (iDC's) have a high capacity to endocytose and process antigens but only a weak capacity to activate T cells. However, upon maturation they lose ability to take up and process antigens but there are upregulation of co-stimulatory molecules and a translocation of MHC class II to the surface. Mature dendritic cells (mDC) become activated as antigen-presenting cells when pattern recognition receptors on their surface recognize distinctive pathogen-associated molecular patterns on the surface of organisms and are one of the most potent inducers of T-cells [109]. DC's can also present exogenous antigens together with MHC class I in certain conditions by a process called *cross priming* allowing activation of CD8+ cytotoxic T cells [109].

Nasal epithelial cells can also serve as antigen presenting cells and amplify the inflammatory response after the immediate innate response at the local tissue level [119]. Primary nasal epithelial cells from the middle meatus were grown at the air-liquid interface and expression of mRNA for HLA-B, HLA-DR, HLAB7 to 1 (CD80), and HLB7 to 2 (CD86), B7-H2, B7-H3 and B7-DC1 genes associated with antigen presenting functions was examined by real-time RT-PCR [119]. Expression of HLA-B and HLA-DR increased significantly with cellular maturation of the nasal epithelial cells suggesting the potential for epithelial cells to interact with T cells.

Soluble factors in innate responses

Complement is a system of more than 30 proteins in plasma and on cell surfaces [120]. The main physiologic activities of complement [120,121] 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, enhancement of immunologic memory); and (iii) disposing of immune complexes and products of inflammatory injury. Complement can be activated by antibody binding (classical pathway), by cell surface carbohydrates and proteins (alternative pathway), and by binding of mannose groups on the bacterial cell surface (mannose binding lectin pathway). For example C3b is a major opsonin, C3a, C4a, C5a cause mast cells to degranulate, C5a is a powerful chemoattractant, C5b, C6, C7, C8, C9 form the membrane attack complex which causes cell lysis. Mannose-binding lectin and surfactant proteins A and D are members of the family of collectins that bind to arrays of terminal mannose groups on a variety of bacteria [112,120].

Acute phase proteins are a group of plasma molecules whose concentration either decreases or increases by at least twenty five percent during inflammatory disorders [122]. They enhance resistance to infection; promote the repair of damaged tissue and other systemic effects. Human acute phase proteins include the coagulation and fibrinolytic system, antiproteases, transport proteins (ceruloplasmin), C-reactive protein, albumin, transferrin etc [122].

IL-1β, IL-6, and IL-8 have been implicated as important factors in the innate immune response [123]. The cytokine response has also been used to characterize the type of T helper cells (Th) involved [124]. A Th_1 cytokine response includes IL-2, -12, and IFN-γ, which activate macrophages and cytotoxic T cells. A Th_2 cytokine response is characterized by IL-4, IL-5, IL-6, IL-10 secretion to activate B cells to become antibody secreting plasma cells. Recently, a TH_0 cytokine response consisting of mixed Th_1 and Th_2 responses has been characterized in house dust mite (HDM) and egg ovalbumin allergen sensitivity [125].

Disorders of Innate immunity

Two general types of disorders affecting the innate immune system could lead to immunological abnormalities [110]. Firstly, mutations that inactivate the receptors or effector mechanisms such as chronic granulomatous disease, an inherited disorder of phagocytic cells that result in their inability to undergo the respiratory burst necessary to kill bacteria and fungi. This leads to recurrent life threatening infections. The second type of disorder affecting molecules involved in innate immune recognition would contribute to a wide variety of chronic inflammatory conditions such as asthma [126], allergy, arthritis [127], and possibly chronic rhinosinusitis.

Host-defence functions of human nasal antimicrobial polypeptides

The two major families of cationic antimicrobial peptides involved in innate immunity at mucosal surfaces are cathelicidins and defensins [99]. Lactoferrin, lysozyme, secretory leukoprotease inhibitor (SLPI) are other antimicrobial peptides that have been identified in nasal secretions and sinus mucosa [128-130]. This review will focus on cathelicidins and surfactant proteins based on evidence from studies on the respiratory tract.

The two types of innate immune responses are constitutive and inducible. The constitutive mechanisms include the barrier functions of the mucosal epithelium. Mucosal epithelial cells, mucous and serous cells of the submucosal glands, constitutively produce antimicrobial peptides (e.g. HBD-1 and enteric α-defensins) and an antibacterial enzyme,

lysozyme, into the airway surface fluid. However, most of the innate immune response is inducible and activated by infecting pathogens.

Nasal secretions analysed by minimally manipulated suction or nasal lavage

Analysing the constituents of nasal secretions from healthy volunteers demonstrates presence of numerous antimicrobial factors. Some of the constituents of human nasal secretions such as lactoferrin, lysozyme and SLPI are derived from the serous cells of the submucosal glands [129,131]. Lysozyme and lactoferrin are bactericidal peptides contained within neutrophil granules and epithelial cells with *in vitro* antibacterial activity against *Vibrio cholerae, Salmonella typhimurium, and Escherichia coli* [128]. Lysozyme is a 14-kDa enzyme directed against the β 1 → 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. 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 can also be microbicidal and block biofilm formation by *P. aeruginosa* [132]. SLPI is a 12-kDa nonglycosylated protein with modest antimicrobial and antifungal activity in vitro [133,134].

Human defensins

Human defensins (HD) are members of the family of antimicrobial peptides, which can be further divided 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 disulphide bonds. α-defensins are found in neutrophils (human neutrophil peptides (HNP) 1-4) [135], Paneth cells of the small intestine (HD-5, -6) [136] and nasal epithelial cells [137]. Human β-defensins (HBD 1-4) is found at epithelial surfaces such as the lung, skin and gut [138,139]. The defensins are both constitutive and inducible, possessing broad-spectrum antimicrobial activity, and have roles in innate immunity and wound

healing [138]. Using RT-PCR and immunohistochemical staining, α-defensins 1, 2, and 3, β-defensins1 and 2 expression was upregulated in maxillary sinus epithelium and nasal polyps whereas HBD-2, HNP-5, -6 expression was not detected in normal controls [140,141]. However, in one study Western blot detected HBD-1 and HBD-2 peptide in nasal lavage fluid suggesting that HBDs were secreted from paranasal sinuses [141].

Human cathelicidins

Introduction

Cathelicidins are members of a large family of cationic antimicrobial peptides that was first identified in mammalian myeloid cells [142]. Cathelicidins are synthesized as pre-propeptides characterized by a highly conserved signal peptide (29-30 amino acids), an N-terminal pro-sequence termed cathelin (~100 amino acids), and a highly heterogenous C-terminal domain (~10-40 amino acids). 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. 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, PR-39). Cathelicidins have been identified in mouse [143] (CRAMP), horse [144] (eCATH1-3), guinea pig [145] (CAP11), sheep [146] (SMAP-29, -34), rat [147] (rCRAMP), rabbit [147] (CAP-18), and pig [148] (PR-39).

The only known human cathelicidin hCAP18 (human cationic antimicrobial peptide-18kDa) or CAMP was initially identified by three groups who described its cDNA, gene, and peptide sequences [149-151]. Human cathelicidin hCAP18 was first independently cloned from a bone marrow cDNA library using oligonucleotide probes designed from the rabbit CAP18 [149] and porcine PR-39 [152]. The cDNA encodes a protein composed of a 30 amino acid signal peptide, a 103 amino acid N-terminal domain, and a 37 amino acid C-terminal domain. The free C-terminal peptide of hCAP18 is called LL-37 (37 amino acids, the two N-terminal amino acids are leucines). Initially hCAP18 was identified synthesized at the

myelocyte and metamyelocyte stage and stored in specific granules of human neutrophils [153]. Further studies using Northern blot and *in situ* hybridisation confirmed hCAP18 mRNA was expressed abundantly in bone marrow cells, is neutrophil lineage-specific and predominantly in myelocytes (>95%), than mature neutrophils [154]. Western blot analysis indicated that the hCAP18 protein was more abundant in mature neutrophils than bone marrow cells. These observations suggest that hCAP18 gene transcription occurs early on at the myelocyte stage of neutrophil maturation in the bone marrow that results in the synthesis and storage of hCAP18 in mature neutrophil granules. The gene for hCAP18 called FALL39 has been mapped to chromosome 3 [150]. It is a compact gene of 1963 bp with four exons. Exons 1-3 code for the signal sequence and the cathelin sequence whereas exon 4 contains the information for the mature antibacterial peptide. The abbreviations CAMP, hCAP18, or LL-37 are used interchangeably in the literature by various authors to refer to human cathelicidin antimicrobial peptides [155,156].

Expression of hCAP18 has also been identified by RT-PCR, *in* situ hybridization, and immunohistochemistry in NKC, γδT cells, B cells, monocytes/macrophages [157], EG2-expressing eosinophils, Langerhans cells, CD1a-expressing dendritic cells [158], and mast cells [159]. hCAP18 is also expressed in airway epithelial cells and the serous and mucous cells of the submucosal glands [160], gastric epithelial cells [161], keratinocytes [162], squamous epithelia of the mouth, tongue, oesophagus, vagina, cervix [163], saliva [164], salivary glands [165], seminal plasma [166,167], epididymis, spermatozoa [167], testis [152], breast milk [168], colon [169] and recently nasal mucosa [170].

Initial studies showed that hCAP18 is cleaved by proteinase 3 from azurophil granules present in neutrophils to generate LL-37 after exocytosis [171]. However in more recent studies, hCAP18 in seminal plasma derived from epididymal cells is cleaved by gastricsin from the prostate and seminal vesicles. Significant processing by gastricsin requires activation by the low pH in the vagina. By studying secreted sweat, it was shown that hCAP18 was cleaved in a serine-protease manner to generate multiple smaller peptides

distinct from LL-37 [172]. These peptides displayed enhanced antimicrobial activity against *S. aureus* and *C. albicans*. However, these processed peptides lost the LL-37 ability to induce IL-8 production by keratinocytes, suggesting a loss of immunomodulatory activities while enhancing its antibacterial activity with subsequent processing.

Role of the cathelin-like domain

A recombinant human cathelin-like protein and the full-length hCAP18 were expressed in *E. coli*. The cathelin-like domain, but not the full-length hCAP18, was shown to inhibit protease activity and kill *E. coli* and methicillin resistant *S. aureus* at concentrations of 16 and 32 μM respectively [173]. Interestingly, the full-length hCAP18 was inactive against Gram positive and Gram-negative bacteria even at higher concentrations (>64uM). It suggests that the cathelin-like domain of hCAP18 may also contribute to innate host defenses and inhibit cysteine-proteinase mediated tissue damage. Hypothetically the inhibition of proteases released during an inflammatory response may be an adaptive function of the cathelin-like domain.

Antimicrobial activity of cathelicidin hCAP18 or LL-37

The net positive charge of LL-37 facilitates its binding to negatively charged bacterial surfaces of bacteria [174]. It is thought to kill cells by disrupting the cell membrane by one of two mechanisms: (i) transmembrane pore formation via a "barrel-stave" mechanism; and (ii) membrane destruction via a "carpet-like" mechanism. Current evidence supports a detergent-like effect via a carpet-like mechanism [175,176]. The α-helical conformation of LL-37 and its antibacterial activity is pH, electrolyte, and concentration dependent [177]. In contrast to human β-defensins which is inactive at high salt concentrations seen in cystic fibrosis patients, LL-37 generally retains its antibacterial activity *in vitro* against *P. aeruginosa, S. typhimurium, E. coli, Listeria monocytogenes, Staphylococcus epidermidis,* and *S. aureus* at moderate to high salt concentrations [177-179]. Methicillin-resistant *S. aureus*

(MRSA) and *C. albicans* were resistant to LL-37 at 100 mM NaCl but susceptible at low salt conditions [178]. LL-37 is also bactericidal *in vitro* against several *Helicobacter pylori* strains [180]. LL-37 is synergistic with lysozyme, lactoferrin [160], defensins [181], and SLPI [182]. Indirect evidence that endogenous expression of LL-37 protects from infections comes from studies with mice null for the CRAMP cathelicidin gene who were more susceptible to develop larger areas of skin infection by group A *Streptococcus* compared to wild-type mice [183]. Using transgenic mice induced to over-express the cathelicidin PR-39 the same investigators then showed increased resistance to group A *Streptococcus* skin infections [148]. Murine mast cells deficient in cathelicidin had a 50% reduction in their ability to kill group A *Streptococcus* even with normal amounts of defensins, supporting the observation that cathelicidins are important in microbial killing [159]. LL-37, HBD-1 and HBD-2 have also been detected in tracheal aspirates of premature and mature newborns and upregulated in response to infection [184]. These studies demonstrate that LL-37 is involved in innate immunity at epithelial surfaces during the perinatal period.

Cathelicidins immunomological functions

Chemotaxis

LL-37 is chemotactic for neutrophils and CD4+ T helper cells *in vitro* in a dose-dependent manner, maximal at 0.1 μmol/L for neutrophils and 5 μmol/L for CD4+ cells [157]. By a checkerboard analysis, 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 known to be expressed on these cells [185]. The optimal chemotactic concentration of LL-37 was 10 μM (approximately 50 μg/ml). This relatively high concentration greater than that required for antibacterial activity is likely to occur only at sites of inflammation and is slightly less than the concentrations known to cause eukaryotic cell cytotoxicity (13-25 μM) [177]. In this manner, LL-37 may play a role in infected tissue as a direct microbial agent and a positive amplification of immune responses.

Modulation of gene expression in immune cells

LL-37 is able to induce degranulation, histamine release and prostaglandin D_2 production in mast cells which is a recognised innate immune response [186]. Using gene-array studies, LL-37 at 50 - 100 μg/ml directly upregulated 29 genes and down-regulated 20 genes in the Murine macrophage cell line RAW 264.7 [187]. The genes upregulated were cytokines, chemokines and chemokine receptors whereas the genes downregulated were DNA repair proteins and some cytokines [187]. LL-37 is a multipotential molecule able to modify dendritic cell (DC) differentiation [188]. Functionally the LL-37 derived iDC displayed a significantly greater uptake of antigen in association with complement coated IgM sheep red blood cell. This is thought to increase the amount of antigen presentation, which will enhance T-cell stimulation.

Wound healing and angiogenesis

Antimicrobial peptides secreted into wound fluid were originally found to induce mesenchymal cells to express cell surface heparan sulfate proteoglycans important for wound healing [189]. In one study, LL-37 expression was not detected initially in normal skin but upregulation of LL-37 expression was observed following wounding in both human and mice skin [190]. LL-37 was detected within the keratinocytes at the wound edge and within granulocytes migrating to the injury. Other studies have confirmed this *in vivo* observation and shown that hCAP18 both at the RNA and protein level (by *in situ* hybridisation and immunohistochemistry) increase immediately and peak at 48 hours at the migrating epithelium during re-epithelialization [191]. However, in chronic non-healing venous ulcers, hCAP18 immunoreactivity was absent in the keratinocytes. Then using an *ex vivo* wound healing model, a polyclonal anti-LL-37 antibody inhibited reepithelialization in a concentration-dependent manner. The *ex vivo* wounds treated with anti-LL-37 lacked immunoreactivity for the epithelial proliferation marker Ki67 suggesting that LL-37 plays a role in epithelial cell proliferation and wound healing [191]. Administering a neutrophil

elastase inhibitor significantly decreased the bacterial clearance from a surgically created wound in an *in vitro* porcine skin wound model suggesting a failure of cleavage of hCAP18 to LL-37 [192]. LL-37 also appears to play a role in wound repair and the inflammatory response by promoting neovascularisation [193]. Application of exogenous LL-37 results in angiogenesis in both a chorioallantoic membrane assay and a rabbit hind-limb model of ischaemia [193]. LL-37 appears to promote angiogenesis by directly stimulating the FPRL1 receptor on human umbilical vein endothelial cells to proliferate and sprout blood vessels [193].

Binding to Lipopolysaccharide (LPS)

LL-37 at concentrations of 1 – 50 μg/ml blocked LPS-induced or LTA-induced TNF-α production by macrophages and protected mice from death by endotoxic shock compared to untreated controls [187]. LL-37 also reduced the ability of LPS to stimulate A459 lung epithelial cells to secrete IL-8 and monocyte chemoattractant protein 1 (MCP-1)[187]. The ability of recombinant LL-37 to inhibit LPS-induced endotoxic shock has subsequently been verified by other investigators [194].

Putative receptors and signaling pathways involved in the effects of LL-37 on cells

The role of FPRL1 in LL-37 mediated chemotaxis has already been discussed [185]. LL-37 was shown to induce a dose- and time-dependent (at 33.3 μg/ml) increase in IL-8 release in both the mucoepidermoid lung carcinoma cell line NCH-H292 and primary cultures of bronchial epithelial cells [195]. The LL-37 induced IL-8 release was mediated via the epidermal growth factor receptor (EGFR) and activation of the mitogen-activated protein kinase (MAPK) extracellular signal-regulated kinase ERK1/2 in airway epithelial cells. MAPK/ERK inhibitors, epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors, blocking anti-EGFR, and anti-EGFR-ligand antibodies, and by metalloproteinase

inhibitors [195]. These results suggest that LL-37 activates epithelial cells to produce IL-8 via the metalloproteinase-mediated cleavage of EGFR.

Further studies in peripheral blood monocytes (PBMC), B and T lymphocytes using RT-PCR and ELISA demonstrated that LL-37 (at 25 – 50 μg/ml) induced activation of PBMC to produce IL-8 via MAPK, ERK1/2 and p38 transcription factors [196]. The presence of GM-CSF allowed activation of ERK1/2 and p38 by LL-37 at lower concentrations (5 – 10 μg/ml) i.e. enhanced, whereas inhibitors of ERK1/2 or p38 inhibited IL-8 upregulation. It is possible that exposure of LL-37 to epithelial cells activates a metalloproteinase, subsequently cleaving membrane-anchored EGFR ligands that activate EGFR resulting in ERK1/2 activation and gene transcription.

Elsner et al provided indirect evidence that LL-37 interacts with the $P2X_7$ purinergic receptor (a nucleotide gated channel) predominantly expressed on monocytes, macrophages, and dendritic cells [197]. LL-37 (10 – 20 μM) induces LPS-primed but not fresh monocytes to release IL-1β release via the $P2X_7$ receptor [197]. LL-37 also induced a transient release of ATP, causing membrane permeability, and activating caspase-1. Pre-treatment with inhibitors of $P2X_7$ receptor inhibited the LL-37 effects.

Regulation of cathelicidin gene expression

The hCAP18 gene is upregulated in skin in response to wounding, infection [190] and inflammatory disorders such as psoriasis [198,199]. It is also upregulated by *H. pylori* infection in gastric epithelial cells [161,180], colonocyte cell differentiation [169,180], upregulated by human synovial membranes in the inflammatory conditions rheumatoid arthritis, osteoarthritis and pyogenic arthritis [200] and pulmonary inflammation in the lung such as pneumonia [184] or sarcoidosis [201].

The cytokine IL-6 may be involved in the regulation of hCAP18 because there is a potential binding site for IL-6 on the promoter region of the hCAP18 gene [150]. Using immunohistochemical methods, hCAP18 and IL-6 were shown to colocalize in squamous

epithelium [163]. However the evidence for IL-6 regulation is conflicting. In one study, IL-6 increased expression and secretion of the LL-37 peptide from peripheral blood mononuclear cells (PMBC) but not NKC or T-cells but this effect was lost after 24 hours [157]. The same study showed enhanced secretion of the mature LL-37 peptide by IFN-γ but down-regulation of LL-37 expression in PBMC and down-regulation was faster with IL-6 [157]. In another study, challenging the human colon epithelial cell line HCA-7 with several cytokines IL-1α, TNF-α, IFN-γ, IL-6, LPS, or PMA for 6 hours did not alter regulation of hCAP18/LL-37 mRNA levels [180]. The short duration of stimulation may be a possible explanation for the observed lack of change. Production of insulin-like growth factor IGF-1 induced expression of hCAP18/LL-37 in an *in vitro* primary human keratinocyte cell culture model [202]. There was an increase in the LL-37 peptide in the cells and the secreted conditioned medium.

Inactivation of cathelicidins

Relatively little is known about the inactivation of cathelicidins but presumably proteases in the environment inhibit its activity. Serum inactivates the antibacterial and cytotoxic properties of LL-37 without affecting the other functions [177]. Apolipoprotein A-I binds to and inhibits the cytotoxic properties of LL-37 in plasma [203]. The binding of cathelicidins to lipoproteins has been suggested to serve as a reservoir of LL-37 in plasma [204]. Increase in the phosphocholine content of the cell wall of *Haemophilus influenzae* decreased the organism's susceptibility to LL-37 [205]. *Streptococcus pyogenes* has been reported to secrete a cysteine proteinase SpeB that is retained at its surface thus protecting it by degrading and inactivating LL-37 [206]. *S. aureus* also secretes a metalloproteinase aureolysin that can cleave and inactivate LL-37 [207].

Studies of cathelicidins in otorhinolaryngology associated diseases

There have been few studies on the expression of cathelicidins in the field of otorhinolaryngology. In one study twenty patients with cholesteatoma, a chronic ear condition, were studied prospectively using semi quantitative RT-PCR and immunohistochemistry compared to normal skin [208]. Cathelicidin mRNA was located in all layers of the cholesteatoma and upregulated compared to normal skin of the ear canal. There have been two studies to date investigating cathelicidins in the paranasal sinuses. One study examined inferior turbinate tissue from 15 chronic rhinitis patients comparing them to six inferior turbinate tissues obtained from healthy controls using immunohistochemistry and RT-PCR [170]. The investigators showed that LL-37 peptide was localized in the surface epithelia, in the serous and mucous cells of the submucosal glands and in stromal inflammatory cells of the rhinitis patients (11/15) and control patients (2/6). RT-PCR showed expression of LL-37 mRNA in all rhinitis patients and only in 3 of six control patients. A more recent study examined nasal polyps from 12 patients compared to 7 inferior turbinate tissues from healthy controls using RT-PCR and immunohistochemistry [209]. Expression of LL-37 mRNA was increased in nasal polyps compared to normal nasal mucosa as shown by semi quantitative RT-PCR analysis suggesting chronic sinonasal inflammation upregulates LL-37 expression.

Surfactant Proteins

Introduction

A major function of pulmonary surfactant is to reduce surface liquid tension at the air-liquid interface of the alveoli, thereby reducing alveolar collapse at the end of expiration. Pulmonary surfactant is a mixture of lipids (90%) and proteins (10%). The surfactant lipids are mainly phospholipids. There are four surfactant proteins: SP-A, SP-B, SP-C, and SP-D. The hydrophobic and lipophilic SP-B and SP-C are important for the surface tension lowering properties of surfactant and deficiency of SP-B is incompatible with life [210].

However, the hydrophilic SP-A and SP-D is not essential for respiration and pulmonary function. Artificial surfactant (e.g. Exosurf Neonatal®) and natural surfactant extracts (Survanta®and Curosurf®) do not contain SP-A and SP-D, which is lost during the process of extraction from animal lungs.

Surfactant protein A and D are members of the family of collagenous carbohydrate binding proteins, involved in innate immunity, known as collectins because they have collagenous and lectin binding domains [112]. Collectins are part of the family that includes mannose-binding lectin (MBL) and conglutinin. The human SP-A and SP-D genes have been localised to the region of 10q22.2-23.1 near the MBL gene [112]. There is increasing evidence that the collectins are involved in innate immunity against various bacteria, fungi, and viruses [112].

Protein Structure

Type II lung pneumocytes, clara cells and non-ciliated bronchiolar epithelial cells synthesize 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 recognises and binds carbohydrates on allergen 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 [211]. The collagen-like region forms triple helices whereas the neck region forms stable trimeric α-helical coiled-coils [212].

Surfactant Protein A

SP-A is the most abundant surfactant protein. In humans, there are at least two SP-A genes expressed (SP-A1 and SP-A2) [213]. The functional significance of this is unclear at present. Several post-translational modifications, results in monomeric protein, which assemble via disulfide bonding and formation of triple helices to form a bouquet-like complex with up to six trimers. Evidence is accumulating that the main function of SP-A is a role in innate

immunity [214]. Mice lacking a functional SP-A gene did not demonstrate any significant difference in lung function or surfactant lipid metabolism [214]. Surfactant from an SP-A deficient mouse lacks tubular myelin, is more easily inhibited by plasma proteins and forms less dense lipid aggregates [215]. However, SP-A deficient mice are more susceptible to bacterial [216,217], and virus [218,219] infections. The clearance of influenza A virus is decreased and pulmonary inflammation is increased in an SP-A deficient mouse model compared with wild type controls [219]. Treatment with exogenous SP-A enhanced viral clearance and decreased lung inflammation. The levels of proinflammatory cytokines TNF-α and IL-6 and neutrophil infiltration into pulmonary tissues were significantly increased 24 hours after infection with respiratory syncytial virus in a SP-A deficient mouse model [218]. There was a decreased production of superoxide radicals and hydrogen peroxide by alveolar macrophages in the absence of SP-A. Treatment with recombinant SP-A increased clearance of the respiratory syncytial virus. This study demonstrates a role of SP-A in the pulmonary clearance of viruses *in vivo* [218]. It also suggests that SP-A may decrease the release of cytokines in response to a viral infection.

Surfactant protein D

SP-D was first characterised in the secreted conditioned medium of freshly isolated rat alveolar type II cells and was originally designated CP-4 [220]. Human SP-D shares 92% homology with the mouse SP-D protein and gene [221]. A significant amount of SP-D (50% - 90%) is not associated with pulmonary surfactant and accumulates in the air spaces as a soluble protein whereas greater than 90% of SP-A is bound to lipids [222]. The monomeric subunit of SP-D is 43 kDa and it forms a trimer. Four identical trimers (each 160 kDa) extend pair wise from a central hub with the lectin domains at the tip of the collagenous arms that forms a cruciate dodecamer [223]. The dodecamers can also assemble to form higher order structures consisting of two to eight SP-D dodecamers [223]. The human SP-D gene is 11 kb and located on chromosome 10 at 10q22.3-23.1 comprising eight exons [224]. Exon 2

encodes the signal peptide, the N-terminal domain, and the first seven Gly-X-Y repeats. Exons 3-6 encode the rest of the collagen-like region. The neck region is encoded by exon 7, and the entire lectin domain is encoded by exon 8 [224]. The SP-D gene locus is only 80 – 100 kb from the SP-A gene [225]. By Western blot under reducing conditions, the SP-D polypeptide has a molecular mass detected at 43 kDa [223].

Synthesis of SP-D

The biosynthesis of wild-type rat SP-D transfected in Chinese hamster ovary (CHO-K1) cells has been studied [226]. SP-D assembly begins with folding of the C-terminal CRD taking approximately 10 minutes and trimerisation occurring within 30 minutes [226]. The initial formation of the trimeric coiled-coil neck domain and subsequent folding of the collagen domain within the rough endoplasmic reticulum precedes formation of trimers with disulfide cross linking of monomers [226]. Oligomerization of trimers is an intracellular process and the major intracellular form of rat SP-D accumulates in the rough endoplasmic reticulum [226]. The trimers then associate to form stable cross-linked dodecamers that is transported to the Golgi apparatus for final modifications prior to secretion [226]. Interference with collagen helix formation in CHO-K1 cells expressing selected mutant recombinant rat SP-D compared to wild-type mice demonstrated that the secretion of SP-D is dependent on the formation of dodecamers stabilized by efficient disulfide cross-linking of the amino-terminal domain [226]. Interfering with collagen-helix formation prevented the secretion of SP-D. Mutant rat SP-D transfected in CHO-K1 cells and secreted exclusively as trimers bound to the hemagglutinin of influenza A virus but failed to enhance viral aggregation, opsonisation, enhanced respiratory burst) with influenza A virus [227]. Oligomerization of the trimeric subunits of SP-D was deduced to be important for its interaction and immunomodulatory functions with influenza A virus [227]. Transgenic mice expressing trimeric SP-D alone were crossed with SP-D null mice to generate heterozygous mice [228]. Heterozygous mice were bred to then generate wild type or SP-D null mice. The mice

expressing the trimeric SP-D form failed to correct the emphysema and foamy macrophages also seen in SP-D null mice. Therefore it was deduced that disulfide cross-linked SP-D dodecamers are required *in vivo* for the prevention of emphysema and foamy macrophages observed in SP-D null mice [228].

Formation of the coiled-coils neck region takes place usually within milliseconds and isolated SP-D neck regions have been shown to form stable trimers [229]. Isolated lectin domains alone do not trimerize. Formation of intrachain disulfide bonds within the CRD domain takes approximately 10 minutes and the chains then associate to form inter-disulfide bonded trimers [226]. Once the surfactant protein is secreted its quaternary structure does not change [212]. A recombinant polypeptide composed of the α-helical neck region and the CRD has been generated demonstrating the same carbohydrate biding specificities as native SP-D for LPS [230].

By Western blot under reducing conditions, the SP-D polypeptide has a molecular mass detected at 43 kDa [223]. A variant form of SP-D at 50 kDa was detected by several monoclonal and polyclonal antibodies to SP-D in addition to the 43-kDa form by screening BALF of alveolar proteinosis patients with Western blot analysis [231]. The major differences between the 43-kDa and 50-kDa forms were due to post-translational glycosylation at the amino-terminal end [231]. However, its exact physiological function and significance remain unknown. The structure of collectins has previously been reviewed extensively elsewhere [212].

Microbial targets: SP-D as a pattern recognition receptor

The collectins bind to carbohydrate moieties on the surface of bacteria and fungi through its carbohydrate recognition domain in a calcium dependent manner. Individual collectins demonstrate different sugar binding specificity. SP-D preferentially binds to Maltose>L-fucose>mannose>glucose>>glucosamine [212]. Whereas SP-A binds to ManNAc (N-acetyl-D-mannosamine)>L-fucose, maltose>glucose>mannose but not galactose [212]. The

clustering of three CRDs in close proximity ensure binding with hight affinity to dense sugar arrays on the surface of microbes and also providing a large enough docking surface for effector molecules [211]. SP-D binds to both Gram-positive and Gram-negative bacteria including *H. pylori* [232], *Mycobacterium tuberculosis* bacilli [233], Acapsular *Klebsiella pneumoniae* [234], *P. aeruginosa* [235], *E. coli* [236], *Streptococcus pneumoniae*, and *S. aureus* [237] and *Chlamydia trachomatis* [238]. SP-D recognises and aggregates several viruses implicated in nasal and respiratory diseases including influenza A virus [239] and respiratory syncytial virus [240]. Collectins also recognise LPS on the outer membrane of gram-negative bacteria and the mannan-like high-mannose structures on the surface of fungi [236,241]. SP-D binds several fungal species including *A. fumigatus* [241], acapsular *C. neoformans* [242], *C. albicans* [243], and *Pneumocystis carinii* [244]. It has been suggested that collectins represent a unique class of pattern recognition receptors similar to TLRs.

SP-D immunological functions

Phagocytosis

Collectins promote phagocytosis of various microorganisms, either directly after biding to microbes, or indirectly though upregulation of phagocytosis mediated by other phagocytic receptors. Human, rat and recombinant SP-D enhanced the respiratory burst by neutrophils in response to influenza A virus [239]. A putative opsonin or phagocytic receptor for SP-D was identified as glycoprotein-340 (gp-340), a member of the scavenger-receptor superfamily, from the BALF of alveolar proteinosis patients [245]. The binding occurs as a protein-protein interaction via the CRD [245]. Strong staining of gp-340 associated with SP-D was located on and within alveolar macrophages by immunohistochemical methods [245,246]. RT-PCR analysis demonstrated expression of gp-340 in lung, trachea, stomach, small intestine, and salivary gland [246].

Chemotaxis

SP-D is also reported to be a potent chemoattractant for both blood neutrophils and monocytes [247]. The maximal response at 5 ng/ml SP-D was comparable to the migration induced by the positive control *N*-formyl-methionyl-leucyl-phenylalanine (fMLP) [247]. This effect was abolished by maltose suggesting that the CRD of SP-D is important in the stimulation of chemotaxis. Further studies using recombinant SP-D consisting of the neck and CRD demonstrated responses chemotactic for neutrophils similar to fMLP [248]. Preincubating neutrophils with SP-D or using maltose abolished the chemotaxis but did not affect the response to fMLP [248].

Modulation of T cell responses by SP-A and SP-D

SP-D has recently been demonstrated to play a role in dendritic functions. Recombinant rat SP-D binds to mouse bone marrow-derived immature dendritic cells in a dose-, carbohydrate-, and calcium-dependent manner whereas it's binding to mature DCs is reduced [249]. In another study, incubating immature bone marrow-derived dendritic cells with SP-A for 24 hours inhibited basal- and LPS-mediated expression of MCH class II and CD86 in a dose-dependent manner to negatively regulate DC differentiation [250]. These important findings suggest that SP-A and SP-D may also enhance and modulate an adaptive immune response against infections.

Native human SP-D and recombinant SP-D composed of the neck and CRD inhibited PHA or Der p allergen stimulated lymphocyte proliferation in a dose response manner [251]. Similar findings for SP-A and SP-D inhibiting CD3(+)/CD4(+) lymphocyte proliferation induced by PMA and ionomycin in both an IL-2-independent - [252] and IL-2 dependent manner [253] support a dual immune role for SP-D in combating infection and dampening the inflammatory response.

Apoptosis

SP-D may play a role in immune homeostasis by recognizing and removing apoptotic cells thereby limiting inflammation. SP-D binds genomic DNA from mouse alveolar

macrophages and preferentially aggregates macrophages with damaged or nicked-DNA [254]. SP-D null mice have a 5- to 10-fold more apoptotic and necrotic alveolar macrophages than wild type mice [255,256]. Administration of recombinant human SP-D reduced the number of apoptotic and necrotic alveolar macrophages in SP-D null mice [255,256].

Interaction of SP-D with allergens

SP-A has been shown to bind to a variety of pollen grains (Lombardy pollar, Kentucky blue grass, cultivated rye and short ragweed) via its CRDs and enhance their aggregation [257]. SP-A, SP-D, and recombinant SP-D also bind *A. fumigatus* conidia [241,258].

Purified native SP-A and SP-D, isolated from human bronchoalveolar lavage fluid, and recombinant SP-D were found to bind to whole mite extracts (*Dermatophagoides pteronyssinus*) and the purified allergen Der p I, in a carbohydrate-specific and calcium-dependent manner [259]. SP-A and SP-D binding prevented allergen-specific IgE binding to the mite extracts [259]. Furthermore, native SP-A, SP-D and recombinant SP-D inhibited phytohemagglutinin (PHA) or Der p allergen stimulated lymphocyte proliferation, and inhibited allergen-induced histamine release from whole blood of asthmatic children [259].

Human SP-A and SP-D bound to 3-week culture filtrate allergens and purified glycosylated allergens gp45 and gp55 of *A. fumigatus* in a carbohydrate-specific and calcium-dependent manner [260]. Both SP-A and SP-D did not bind to the deglycosylated allergens suggesting the interaction is mediated via the CRD. Both SP-A and SP-D were able to inhibit binding of allergen-specific IgE from Aspergillosis patients to these allergens and also inhibit the *A. fumigatus* allergen-induced histamine release from sensitized basophils of allergic bronchopulmonary aspergillosis patient's [260]. 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 allergic inflammation.

SP-D gene regulation

The mechanism of SP-D gene regulation is unknown but several regulatory elements known to be involved in acute phase [261] and glucocorticoid [262] responses have been found in the 5' flanking region of the SP-D gene [261,262]. These include potential cis-regulatory elements including glucocorticoid response elements, NF-IL-6, interferon response elements, an AP-1 sequence, E-box sequences, PEA3 motifs [262], thyroid transcription factor 1, and the forkhead/winged helix transcription factors HNF-3 (FOXA) [261]. Dexamethasone was able to induce increases in plasmid constructs containing these sequences on transfection of human adenocarcinoma H441 cells [262]. Dexamethasone was administered *in utero* at days 16-18 to timed-pregnant rats and fetal lungs were harvested at days 19, 20, and 21. Dexamethasone increased SP-D mRNA and protein levels in the harvested fetal rat lung to detectable levels by immunoblot, Northern blot and immunohistochemistry at days 19- 20 whereas the equivalent control rats had barely detectable SP-D mRNA [263]. Then the same investigators using a fetal lung explant culture system incubated in the presence or absence of increasing doses of dexamethasone demonstrated dose and time-dependent increases in SP-D protein and mRNA levels [263]. Immunohistochemistry of the fetal lung from the dexamethasone treated rats showed strong staining of the airway epithelial cells compared to weak staining in the control 19-day lungs [263].

Infection at mucosal surfaces has been demonstrated to affect SP-D expression. SP-D mRNA and protein levels have also been demonstrated to be increased using semiquantitative RT-PCR and immunohistochemistry respectively in biopsy specimens from patients undergoing endoscopy as investigation for non-ulcer dyspepsia [232]. A study of BALF from RSV-infected children found decreased SP-A and SP-D protein levels compared to the uninfected control group [264]. An animal study suggested that exhaustion of SP-D protein levels and a lack of upregulation of mRNA levels contribute to chronic infection [265].

Pulmonary and extra-pulmonary distribution of SP-D

An important study examined the distribution of SP-D from archival human fetal, newborn and adult tissues by immunohistochemistry using mouse anti-SP-D antibody that cross-reacts with human SP-D [266]. SP-D was detected in adult lung with diffuse intracellular staining in type II epithelial cells, serous cells of tracheobronchial glands and extracellular staining along the surfaces of airspaces. SP-D was also detected in a variety of non-pulmonary tissues from fetuses, children and adults. SP-D was detected in epithelial cells and in luminal material of the ducts of the lacrimal glands, salivary glands, intercalated ducts of the pancreas, hepatocytes and intra- and extrahepatic bile ducts, esophageal glands, breast, sebaceous and eccrine glands sweat glands of the skin, Von Ebner's glands of the tongue, parietal cells of the stomach, proximal and distal renal tubules, podocytes of the glomerulus, seminal vesicles, endocervical glands, adrenal cortex, myocardial cells from the right atrium, and in follicular stellate cells of the anterior pituitary gland [266]. SP-D was detected in fetal lung from 10 weeks gestation, with staining increasing in the distal airways and decreasing in the proximal airways with advancing gestation [266]. Adult tissues without detectable SP-D staining included tongue muscle, skeletal muscle, esophageal muscle, large intestine, synovial membrane, and testes [266]. SP-D was detected by ELISA in human amniotic fluid and saliva, but not in seminal fluid, urine, breast milk, or tears [266].

Porcine Eustachian tube was examined by in situ hybridization, electron microscopy, and immunoelectron microscopy with SP-D mRNA detected in basal epithelial cells [267,268]. Staining for SP-D protein revealed staining in the mucosal folds of the porcine Eustachian tube, on the surface of the apical cells, and as amorphous material lying on the epithelial cells indicating that SP-D was secreted rather than associated with intracellular organelles as with SP-A [268].

A panel of mRNAs from human tissue was screened for SP-D mRNA by RT-PCR [269]. Expression of SP-D mRNA was strongest in the lung, but was also detected in the kidney, trachea, brain, testis, pancreas, salivary gland, heart, prostate, small intestine, and placenta.

Low levels of expression were detected in the uterus, stomach, mammary glands, spleen, adrenal gland, and liver [269].

The extra-pulmonary distribution of SP-A has been investigated using RT-PCR and immunohistochemical screening of a panel mRNA's and human tissues [270]. SP-A1 and SP-A2 transcripts were amplified in the lung and thymus whereas SP-A2 expression was only detected in the lung and prostate. Strong SP-A immunoreactivity was seen in alveolar type-II cells, Clara cells, and on and within alveolar macrophages, but no extra pulmonary SP-A immunoreactivity was detected. In contrast to lung surfactant protein D (SP-D), which is generally expressed on mucosal surfaces, SP-A seems to be mainly expressed in the respiratory tract.

Surfactant protein-D gene targeted mice

Two groups working independently generated homozygous and heterozygous SP-D deficient mice in 1998 by disrupting embryonic stem cells using homologous recombination [261,271]. Mice totally deficient in SP-D were healthy to 7 months but had a progressive accumulation of surfactant lipids, SP-A, and SP-B protein levels but no change in mRNA levels, in the alveolar space using Western blot analysis on the BALF and Northern blot analysis on lung homogenates [272]. However, Korfhagen et al detected reduced SP-A mRNA and protein levels and no changes in SP-B or SP-C protein or mRNA levels in the SP-D deficient mice using similar assays to Botas et al [271,272]. The SP-D deficient mice also had type II cell hyperplasia with massive enlargement of intracellular lamellar bodies and an accumulation of foamy alveolar macrophage [271,272]. These changes in surfactant homeostasis were not associated with detectable changes in surfactant surface activity, postnatal respiratory function, or survival [272]. With longer observation, the SP-D deficient mice progressively developed chronic lung inflammation, emphysema, and fibrosis [273]. These changes were reversible, except for emphysema, by replacing SP-D *in vivo* suggesting that SP-D is required for maintenance of normal lung architecture [274]. The

fibrosis developed in association with increased MMP-2 and MMP-9 activity detected in conditioned media from isolated alveolar macrophages but not in the BALF compared to wild type mice [273]. The SP-D mRNA levels in the whole lung by Northern blot analysis were similar between SP-D deficient and wild type mice [273]. The pro-inflammatory cytokines TNF-α, IL-1β, IL-6 and MIP-2 in BALF from SP-D deficient mice were not detected [273]. However, alveolar macrophages isolated from SP-D deficient mice produced 10-fold more H_2O_2 [273]. These findings support another unsuspected role for SP-D in modulating alveolar macrophage activation, oxidant production, and MMP activity leading to progressive fibrosis and remodeling at a local level given there were no changes in MMP2 and MMP9 mRNA levels as assessed in the whole lung.

A construct with the human SP-C promoter and a rat SP-D cDNA clone was developed to produce transgenic mice that over-expressed rat SP-D in respiratory epithelial cells of wild type mice (rSP-D, SP-D (+/+)) and bred with SP-D null mice [275]. The distal respiratory epithelial cell-specific expression of rSP-D in the mouse had no effect on lung morphology, surfactant phospholipid content, metabolism, and SP-A, -B, -C mRNA levels *in vivo* [275]. However, the local expression of rSP-D in the SP-D null mice fully corrected the increased surfactant phospholipid content and alveolar macrophage morphology seen in SP-D null mice [275].

Inactivation of SP-D

Neutrophil serine proteinases elastase, proteinase 3, and cathepsin G have recently been shown to degrade human and rat SP-D *in vitro* in a temperature (decreased at 4°C and restored at 37°C) and calcium dependent manner [276]. Native SP-D was unable to bind to maltose-coated surfaces or agglutinate *P. aeruginosa* any more after incubating with neutrophil elastase [277]. Further evidence *in vivo* that SP-D can be degraded was provided in a study of BALF from cystic fibrosis patients [278]. Those patients with high levels of neutrophil elastase in their BALF and also degraded fragments of SP-D detected on

immunoblotting [278]. Human multimeric SP-D can be degraded *in vitro* by neutrophil elastase, cathepsin G, and proteinase 3 resulting in fragments of 32-41 kDa detected by immunoblotting [278]. Degradation of SP-D occurred after 10 minutes of incubation with neutrophil elastase and within 8 hours with the other proteases. Inactivation or inhibition of the proteases completely prevented the degradation of SP-D [278]. The serine proteinases released by infiltrating neutrophils may serve as a feedback mechanism to limit the actions of SP-D at sites of inflammation.

Studies of SP-A, SP-D in otorhinolaryngology associated disease

SP-A mRNA was detected in the epithelial cells of submucosal glands in rabbit maxillary sinus tissues by Northern blot analysis and RT-PCR [279]. In comparison to rabbit lung, SP-A transcripts were less in the middle ear and sinus tissues [279]. In the middle ear SP-A mRNA was present primarily in the surface epithelium. RT-PCR detected the presence of SP-B and SP-C mRNA in middle ear and sinus tissues [279]. Bands for SP-A protein were detected at 29 kDa, 35 kDa and 70 kDa in middle ear and sinus tissues by immunoblot analysis indicating different posttranslational modification from the lungs. The investigators did not screen for the presence of SP-D, but expression of SP-A in sinus tissue suggests that SP-D may also be present in the paranasal sinuses. More recently SP-A transcript was detected in nasal biopsies from CRS patients without nasal polyps by RT-PCR and increased compared to normal controls [280]. Immunohistochemical staining demonstrated SP-A present in the epithelial cells and submucosal glands of sinus mucosa in both control and CRS patients [280].

Conclusion

Currently there is a limited understanding of immune responses in CRS pathogenesis especially against fungi. EMCRS is a subgroup of CRS characterized by thick EM in the sinuses and AFS is the most aggressive form of EMCRS. From evidence in the literature, I postulate that innate immunity at the nasal tissue level involving the expression of

cathelicidins (CAMP) and surfactant protein-D (SP-D) is present in CRS and EMCRS patients. The findings from my research were published in high impact peer-reviewed journals in the field of rhinology. I investigated expression of CAMP and SP-D in nasal biopsies from CRS and EMCRS patients by both quantitative and descriptive molecular analyses. The *in vitro* experiments consisted of challenging nasal tissue explants from CRS and EMCRS patients with common fungal allergens and analyzing the expression of CAMP and SP-D by quantitative real-time RT-PCR and ELISA. This thesis is submitted as a thesis by publication with three published peer-reviewed scientific papers forming chapters 2, 3 and 4 and a concluding chapter to discuss these findings.

Chapter 2

Human cathelicidin antimicrobial peptide is upregulated in the eosinophilic mucus subgroup of chronic rhinosinusitis patients [American Journal of Rhinology In press]

Eng Hooi Ooi [a], Peter-John Wormald [a], A. Simon Carney [a], Craig Lloyd James [b], Lor Wai Tan [a].

[a]The Department of Surgery - Otorhinolaryngology Head and Neck Surgery University of Adelaide and Flinders University; and [b]Adelaide Pathology Partners, Adelaide, South Australia, Australia.

Declaration of sources of funding:

This study was supported by grants from the Garnett Passe and Rodney Williams Memorial Foundation, Victoria, Australia.

Ooi, E.H., Wormald, P-J., Carney, A.S., James, C.L. and Tan, L.W. (2007) Human cathelicidin antimicrobial peptide is upregulated in the eosinophilic mucus subgroup of chronic rhinosinusitis patients.
American Journal of Rhinology, v.21 (4) pp. 395-401 July/August 2007

Chapter 3

Fungal allergens induce cathelicidin LL-37 expression in chronic rhinosinusitis patients in a nasal explant model [American Journal of Rhinology In press]

Eng Hooi Ooi [a], Peter-John Wormald [a], A. Simon Carney [a], Craig Lloyd James [b], Lor Wai Tan [a].

[a]The Department of Surgery - Otorhinolaryngology Head and Neck Surgery University of Adelaide and Flinders University; and [b]Adelaide Pathology Partners, Adelaide, South Australia, Australia.

Declaration of sources of funding:

This study was supported by grants from the Garnett Passe and Rodney Williams Memorial Foundation, Victoria, Australia.

Ooi, E.H., Wormald, P-J., Carney, A.S., James, C.L. and Tan, L.W. (2007) Fungal allergens induce cathelicidin LL-37 expression in chronic rhinosinusitis patients in a nasal explant model.
American Journal of Rhinology, v.21 (3) pp. 367-372 May/June 2007

Chapter 4

Surfactant protein D expression in chronic rhinosinusitis patients and immune responses in vitro to Aspergillus and Alternaria in a nasal explant model [The Laryngoscope, Jan 2007; 117(1): 51-7]

Eng Hooi Ooi [a], Peter-John Wormald [a], A. Simon Carney [a], Craig Lloyd James [b], Lor Wai Tan [a].

[a]The Department of Surgery - Otorhinolaryngology Head and Neck Surgery University of Adelaide and Flinders University; and [b]Adelaide Pathology Partners, Adelaide, South Australia, Australia.

Declaration of sources of funding:

This study was supported by grants from the Garnett Passe and Rodney Williams Memorial Foundation, Victoria, Australia.

Ooi, E.H., Wormald, P-J., Carney, A.S., James, C.L. and Tan, L.W. (2007) Surfactant protein D expression in chronic rhinosinusitis patients and immune responses in vitro to Aspergillus and Alternaria in a nasal explant model.
The Laryngoscope, v.117 (1) pp. 51-57 January 2007

Chapter 5

Discussion

This thesis consisted of a number of different studies investigating the innate immune system in the paranasal sinuses in a cohort of CRS and EMCRS patients compared to normal controls.

Clinical characteristics of the study group

The CT Lund-Mackay scores for the AFS and NANFES group were significantly higher than the CRS group while the AFS patients tended to be younger. Our overall fungal detection rate was 33.8 % (20/59) in the cohort of patients with chronic rhinosinusitis and 60 % (20/33) in the subgroup EMCRS which is agreement with our previous published studies [28,47,64].

Cathelicidins are upregulated in chronic rhinosinusitis

CAMP is widely expressed at epithelial surfaces throughout the body [161-163,167,169]. Previous studies have demonstrated expression of CAMP in airway epithelial cells and the serous and mucous cells of the submucosal therefore playing a role in innate immunity [160,209]. CAMP acts as an antimicrobial peptide and multipotent immune modulator amplifying innate and adaptive immune responses by signalling and recruiting T cells, promoting neoangiogenesis and dendritic cell differentiation to increase expression of costimulatory molecules important for T cell activation [160,188,193].

Our findings as detailed in chapter 2 demonstrated CAMP immunostaining was localized to mainly inflammatory cells in the subepithelial layer, nasal epithelial cells and cells of the submucosal glands in agreement with other published studies [170,209]. This suggests that CAMP is constitutively expressed in the nasal mucosa and submucosal glands which are sites of constant challenge from viruses, bacteria, and fungi. Using quantitative real-time

RT-PCR, CAMP mRNA was detected and upregulated in all patients with chronic rhinosinusitis and significantly in the EMCRS subgroup compared to the CRS and control groups. The findings from chapter 2 suggest that CAMP gene is expressed but also inducible with inflammation. Certainly CAMP expression in salivary glands is constitutive and inducible [164,165]. Immunostaining for CAMP showed protein was localized to ductal epithelial cells and inflammatory cells in glands with chronic sialadenitis. RT-PCR demonstrated increased expression of CAMP mRNA in the chronic sialadenitis compared to normal salivary glands [165].

The ELISA results (in chapter 2) from nasal tissue homogenates did not demonstrate any significantly increased CAMP protein levels between the different groups. Several reasons for this observed discrepancy between CAMP mRNA and protein levels potentially exist in this study: There may be increased production of CAMP but this increase could be accompanied by direct binding of CAMP to fungi within the EM or CAMP was secreted into the paranasal sinuses rather than the tissue. CAMP has been identified in airway surface fluid from human lung and from a bronchial xenograft model suggesting that it is secreted in normal host defense in the lung [160]. Alternatively the discrepancy between protein and mRNA results may be due to post-translational modifications of CAMP in the EMCRS group but this is less likely as I was unable to detect this on Western blot analysis. The findings demonstrate the presence of cathelicidins in patients with chronic rhinosinusitis thus provding evidence of an active innate immune system in nasal mucosa. Cathelicidins have multiple inflammatory actions, e.g. chemotaxis, angiogenesis, augmenting dendritic cell maturation, and enhancing expression of inflammatory genes. Expression of CAMP mRNA was increased significantly in the EMCRS subgroup which may represent a more severe sinus disease group [281]. Suppression of cathelicidin expression may in fact inhibit inflammation. Relatively little is known in the literature regarding the effects of glucocorticoids on human cathelicidin expression. It has been reported that glucocorticoids decrease the secretion of antimicrobial peptides from the skin of a frog

through effects on NF-κB transcription [282]. Glucocorticoids have a profound inhibitory effect on the production of inflammatory cytokines and chemokines and induce apoptosis of inflammatory cells from *in vivo* and *in vitro* studies when delivered topically or systemically [283-285]. An exacerbation of the inflammatory disease in CRS is usually triggered by infections of the mucosal surface by viruses, bacteria, and fungi. Short courses of systemic corticosteroids produce significant improvements in the symptoms and size of nasal polyps [286]. Modulation of the innate immune system and indirectly the adaptive immune response may be a novel form of therapy in the future. Studying cathelicidins and their expression in nasal mucosa is therefore important in understanding the pathogenesis and improving treatment of chronic rhinosinusitis.

Common fungal allergens induce cathelicidin expression in a nasal explant model

The experimental *in vitro* study (chapter 3) was undertaken to determine if CAMP was being produced and possibly secreted in response to common fungal allergens. The nasal tissue explant model rather than primary epithelial cell culture allows us the possibility to investigate whole nasal tissue immune responses to fungal allergens.

CAMP is significantly upregulated *in vitro* at the mRNA and protein level in CRS patient's nasal tissue in response to the fungal allergens *Aspergillus* and *Alternaria* within the first 24 hours and with an increasing does-response effect. The *in vitro* experiments also demonstrate that CAMP is secreted by nasal tissue into the culture supernatant suggesting that cathelicidins are probably secreted into the sinuses *in vivo*. The results suggest that CAMP from the nasal tissue homogenates is constitutive and production of CAMP is increased in the nasal explants cultured with fungal allergens *in vitro*. *Alternaria* but not *Aspergillus* increased tissue CAMP protein levels compared to untreated tissue controls in the CRS group. However, *Alternaria* did not induce increased CAMP at the mRNA or protein level in EMCRS patients. CAMP mRNA is significantly upregulated due to the

more severe sinus disease and therefore it is not surprising that the additional challenge with fungal allergens is not associated with further increases in mRNA levels in the EMCRS group. Challenging nasal tissue from EMCRS patients with the highest dose of *Aspergillus* used in this study produced an increase in tissue CAMP levels. PBMCs from CRS patients produced more IL-5, IL-13, and IFN-γ *in vitro* when cultured with *Alternaria* allergens compared to *Aspergillus* [287]. The possible reasons for different tissue responses to different fungi have already been discussed in chapter 3 and are considered to reflect a more severe pathogenicity of *Alternaria* in airway inflammation. Increases in the airborne concentrations of *Alternaria* spores are shown to be associated with increased symptoms of airway responsiveness in children with asthma [288].

A trend was observed in this study for CAMP levels in the culture supernatant to decrease in response to increasing fungal allergens in both CRS and EMCRS groups. Deficiency in the secretion of LL-37 into airway surface fluid has been implicated in chronic bacterial infections in cystic fibrosis [289]. Therefore a similar model may exist where fungal allergens play a role affecting the secretion of LL-37 and this immune response may be a pathogenic marker in chronic rhinosinusitis.

Other *in vitro* studies of cathelicidins have found that it is not secreted in the absence of stimulation [162]. The cathelicidin hCAP18 or LL-37 peptide was not detected in the conditioned media of HaCaT keratinocytes cultured to confluence [162]. A model using sinus tissue cultured at the air-liquid interface *in vitro* to promote epithelial differentiation demonstrated that culturing with lipopolysaccharide (LPS) and lipoteichoic acid (LTA) increased goblet cell mucus production and a decrease in ciliated cells [290]. This is similar to the changes involved in rhinosinusitis. The same investigators examined cathelicidin expression in sinus epithelium from normal transsphenoidal pituitary surgery patients cultured at the air-liquid interface *in vitro* with LPS and LTA [291]. In that study the cells cultured for 14 days with 100 ng/ml LPS or 10 μg/ml LTA demonstrated a significantly increased expression of cathelicidins as assessed by immunohistochemistry. LPS and LTA

also stimulated increased mucus and IL-8 production being a common innate immune response in the upper airways. It is concluded from those studies that cathelicidin expression is inducible in sinus epithelium by bacterial antigens.

Transient down-regulation of LL-37 in the gut from *Shigella* infection may also be due to interference with the transcription of LL-37 [292]. Here the investigators reported that infection with *Shigella* spp downregulated LL-37 mRNA expression and protein levels from colon biopsies taken between day 1 and day 30 as assessed by RT-PCR and immunohistochemical analysis respectively. However, by days 60 when the patients were clinically recovered there was LL-37 mRNA and immunostaining detected by RT-PCR and immunohistochemistry [292]. This observation was confirmed *in vitro* by incubating the epithelial cell line HT-29 and monocyte cell line U937 with *Shigella* and demonstrating a down-regulation in LL-37 mRNA by RT-PCR in those cell lines infected with *Shigella* compared to uninfected controls [292]. This down-regulation of immune defences is thought to be mediated by *Shigella* plasmid DNA might promote bacterial adherence and invasion into host epithelium [292].

Cathelicidins are effective against *C. albicans* in a hypertonic environment [293]. Nasal douching has been proposed to be an effective method of irrigating the maxillary sinuses and frontal recesses after surgery and improve mucociliary clearance [294,295]. Nasal douching with hypertonic solutions potentially has a dual effect of mechanically removing fungal debris as well as improving the local environment for cathelicidins to be effective.

Currently there are phase II clinical trials using the cathelicidin protegrin analogue IB-367 in the treatment of oral mucositis in immunocompromised patients showing promising results [296,297].

There is also the potential of gene transfer therapy in cystic fibrosis, which may be applicable for selected cases of chronic rhinosinusitis [289]. An adenovirus-mediated gene transfer of hCAP18/LL-37 into a cystic fibrosis human bronchial xenograft model to cause overexpression of LL-37 into the airway fluid reversed the cystic-fibrosis specific defects in

bacterial killing [298]. Further evidence from animal studies involving transfer of the hCAP18/L-37 gene into the mouse results in a decreased bacterial load and inflammatory response after intra-tracheal challenge with *P. aeruginosa* and *E. coli* [298]. It is possible that recombinant LL-37 may be useful as an antimicrobial agent in the treatment of sinonasal infections.

Surfactant protein D expression in chronic rhinosinusitis and to common fungal allergens in a nasal explant model

Ours is the first published report of SP-D in nasal tissue. By immunohistochemistry we found SP-D to be highly expressed in the duct epithelial cells and lumen of the submucosal glands in the CRS and NANFES patients. Our data are consistent with the wide distribution of SP-D at mucosal surfaces exposed to pathogens. However, we were unable to detect staining for SP-D in the AFS patients indicating possible reduction in SP-D synthesis or intracellular reserves. Staining was strongest in the controls, CRS and NANFES groups, which is in general agreement with the ELISA results. The SP-D protein levels were below the detection limit of the assay in the AFS patients and majority of NAFES patients in support of our immunhistochemistry results and we observed a trend for decreased SP-D levels in CRS and NANFES patients compared to controls. SP-D levels have been found reduced in various diseases such as cystic fibrosis, RSV infection, and chronic pneumonia [264,265,299,300].

Surfactant proteins A and D belong to the family of collectins, which are pattern recognition molecules involved in innate immunity. Binding of collectins to microbes through the lectin domains leads to activation of immunological processes such as complement activation, phagocytosis, chemotaxis, and modulating lymphocyte proliferation and differentiation [211]. SP-D may act as an anti-inflammatory regulator by binding to signal inhibitory regulatory protein α (SIRPα) through its C-lectin domain inhibiting production of inflammatory mediators and blocking P38 MAP kinase activation

[301]. SP-D appears upregulated by an acute inflammatory stimulus analogous to acute phase proteins but with the potential to maintain normal homeostasis at sites of tissue inflammation by downregulation [301]. This may be a protective response in CRS patients; however in contrast, EMCS patients do not demonstrate a similar response.

SP-D mRNA has been detected by RT-PCR in a wide range of tissues, including the salivary gland, lacrimal gland, ovary, uterus, esophagus, stomach, testes, thyroid, and heart [269,302]. SP-D protein expression was more restricted in distribution compared to mRNA, and detected in lung, trachea, uterus, ovary and lacrimal gland. More recently, SP-D mRNA and protein was detected in human prostate by RT-PCR, in situ hybridization, Western blot and immunohistochemistry. Using a polyclonal anti-SP-D antibody that cross-reacts with human SP-D, staining was detected in the epithelial cells lining the prostate glands and the lumen of the glands indicating that glandular epithelium secretes SP-D protein [238]. However, SP-D staining was not uniformly present in all prostate glands with some glands staining intensely and others staining weakly [238].

A possible explanation for the absence of significant SP-D gene upregulation is due to depletion of SP-D protein reserves during the progression from acute to chronic rhinosinusitis that may result in failure to clear the pathogen from the sinuses and increase the chances of re-infection or re-colonization post-operatively. Grubor et al using an ovine model of chronic pneumonia, demonstrated decreased SP-D expression as measured by real-time RT-PCR and immunohistochemistry (Chapter 4) [303]. Clinically decreased protein levels with inflammation has been reported in a study of BALF from RSV-infected children that found decreased SP-A and SP-D protein levels compared to the uninfected control group [264].

SP-D mRNA and protein responses to fungal allergens in a nasal explant model have not previously been described. Challenging the nasal tissue in our explant *in vitro* model with *Aspergillus* and *Alternaria* extracts induced a significant dose response increase in SP-D mRNA expression in CRS patients. However, in NANFES patients only the highest dose of

Alternaria used *in vitro* induced upregulation of SP-D. AFS and NAFES patients were not studied because they did not demonstrate significant SP-D protein levels in the initial clinical study. This study also demonstrated that SP-D is secreted into culture supernatant *in vitro* as detected by ELISA therefore indirectly providing evidence that SP-D may be secreted *in vivo* in the paranasal sinuses. The levels of SP-D protein were lower in the NANFES patients challenged with fungal allergens relative to the untreated control tissue indicating possibly that SP-D protein reserves become exhausted with ongoing stimulation. The relative deficiency in SP-D levels of CF patients compared to normal controls or those with acute lung infection is implicated as a cause in the increased frequency of bacterial colonization in CF children [299]. Deficiency of SP-A and SP-D in cystic fibrosis patients may explain their increased frequency of bacterial colonization and infections [299]. SP-A and SP-D concentrations were measured in BALF from children with cystic fibrosis (CF), acute lung infection, and controls (investigation of structural abnormalities without infection). The levels of SP-D were significantly decreased in CF patients (median 0.1 μg/ml) compared to the infection (12.17 μg/ml) and control (641 μg/ml) group. In fact, more than half of the CF children were below the detectable limits of the SP-D assay [299]. A similar model potentially exists with chronic rhinosinusitis where defects in SP-D production or secretion may contribute to ongoing inflammation from bacterial or fungal stimuli. A recent review highlighted the potential of recombinant SP-D as therapy in neonatal chronic lung disease, cystic fibrosis, and emphysema [304].

Intranasal treatment with SP-A (3 μg/mouse), SP-D (1 μg/mouse) and recombinant SP-D (1 μg/mouse) in a mice model of allergic bronchopulmonary aspergillosis (ABPA) decreased blood eosinophilia, pulmonary infiltration, and levels of allergen-specific IgG and IgE [258]. 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 [258]. Other investigators using 1-week culture filtrate rich in the non-glycosylated allergen Asp f1 have demonstrated similar findings with decreased allergen-

specific IgG and IgE and decreased levels of IL-4 and increased IL-12 and IFN-γ [305]. *In vivo*, wild type and SP-D null mice were sensitized and challenged with the allergen ovalbumin in a mice model of allergic inflammation and BALF was performed [306]. The SP-D null mice had higher levels of eosinophils and IL-13 but lower levels of IFN-γ in the BALF compared to wild type mice. *In vitro*, splenocytes from wild type and SP-D null mice were isolated and stimulated with the mitogen concanavalin A (ConA) and also lipid A (LpA) a known ligand for Toll-like receptor 4. TLR4 gene expression in the lungs was decreased in SP-D mice challenged with ovalbumin allergen compared with wild type mice. TLR4 has been identified as important in recognition of fungi and activating the innate immune response [114]. Lack of SP-D in an allergen challenge appears to be associated with a greater Th2 like response suggesting that SP-D may dampen the allergic responses through promoting IFN-γ secretion (a Th1 response). The effects of SP-D may potentially be beneficial in chronic rhinosinusitis patients to dampen down a Th2 cytokine inflammatory response.

SP-D expression is increased by common fungal allergens in the nasal explant model. Other factors affecting SP-D expression include dexamethasone, IL-4, keratinocyte growth factor (KGF), foetal lung development, and infections [307]. Dexamethasone increases SP-D mRNA and protein 2-fold in a fetal lung explant culture system [308]. Dexamethasone treatment in neonates with respiratory distress syndrome increases SP-A and SP-D in tracheal aspirates (mean SP-D from 100 ng/ml to 254 ng/ml) from days 3 to 14 after birth compared to controls [309].

A model is proposed where CAMP is produced by epithelial and inflammatory cells in the subepithelial layer and secreted into the sinuses in response to the presence of fungal allergens. The function of CAMP is to disrupt the cell membrane of the bacteria or fungus. CAMPs also are chemotactic for neutrophils, T helper cells, and promotion of mast cell degranulation. CAMP mRNA is upregulated as an inflammatory response in CRS and EMCRS patients. However, the EMCRS patients are unable to increase CAMP in response

to the increasing inflammatory state. Therefore fungi or bacteria are able to colonize, possibly causing micro-invasions, and further tissue injury therefore leading to a chronic inflammatory state. SP-D is produced predominantly by the submucosal glands and secreted into the sinuses. SP-D's main role is binding and agglutination of pathogens, enhancing phagocytosis, rapid clearance of bacterial endotoxin, moderating the inflammatory response to infection and allergens, and clearance of apoptotic cells. SP-D thus acts to both clear and limit the inflammatory response to the bacterial and fungal pathogens. Defects in its production and cellular reserves, or lack of production of SP-D in AFS patients, is proposed to lead to a chronic inflammatory state from bacterial and/or fungal colonization in chronic rhinosinusitis and especially the EMCRS group.

Conclusion

The findings of the novel innate defense proteins CAMP and SP-D expressed in human nasal tissue provides further evidence that innate immune responses potentially play a significant role in chronic rhinosinusitis. EMCRS patients demonstrate increased CAMP expression, perhaps as a marker or inducer of severe mucosal inflammation. Fungal allergens induce mucosal innate immune responses in CRS and EMCRS patients and their responses are characterised in this study. It is hoped that the findings from this study will lead to further research and development of novel therapies in the understanding and treatment of chronic rhinosinusitis.

References

1. Anand VK. Epidemiology and economic impact of rhinosinusitis. *Ann Otol Rhinol Laryngol Suppl* 2004;193:3-5.
2. Gross CW, Schlosser RJ. Prevalence and economic impact of rhinosinusitis. *Curr Opin Otolaryngol Head Neck Surg* 2001;9:8-10.
3. Van Cauwenberge P, Watelet JB. Epidemiology of chronic rhinosinusitis. *Thorax* 2000;55 Suppl 2:S20-21.
4. Metson RB, Gliklich RE. Clinical outcomes in patients with chronic sinusitis. *Laryngoscope* 2000;110:24-28.
5. Gliklich RE, Metson R. Techniques for outcomes research in chronic sinusitis. *Laryngoscope* 1995;105:387-390.
6. Lanza DC, Kennedy DW. Adult rhinosinusitis defined. *Otolaryngol Head Neck Surg* 1997;117:S1-7.
7. Kuhn FA. Role of endoscopy in the management of chronic rhinosinusitis. *Ann Otol Rhinol Laryngol Suppl* 2004;193:15-18.
8. Zinreich SJ. Imaging for staging of rhinosinusitis. *Ann Otol Rhinol Laryngol Suppl* 2004;193:19-23.
9. Benninger MS, Ferguson BJ, Hadley JAet al. Adult chronic rhinosinusitis: definitions, diagnosis, epidemiology, and pathophysiology. *Otolaryngol Head Neck Surg* 2003;129:S1-32.
10. Deal RT, Kountakis SE. Significance of nasal polyps in chronic rhinosinusitis: symptoms and surgical outcomes. *Laryngoscope* 2004;114:1932-1935.
11. Settipane GA. Epidemiology of nasal polyps. *Allergy Asthma Proc* 1996;17:231-236.

12. Ragab SM, Lund VJ, Scadding G. Evaluation of the medical and surgical treatment of chronic rhinosinusitis: a prospective, randomised, controlled trial. *Laryngoscope* 2004;114:923-930.

13. Dijkstra MD, Ebbens FA, Poublon RM, Fokkens WJ. Fluticasone propionate aqueous nasal spray does not influence the recurrence rate of chronic rhinosinusitis and nasal polyps 1 year after functional endoscopic sinus surgery. *Clin Exp Allergy* 2004;34:1395-1400.

14. Lildholdt T, Rundcrantz H, Bende M, Larsen K. Glucocorticoid treatment for nasal polyps. The use of topical budesonide powder, intramuscular betamethasone, and surgical treatment. *Arch Otolaryngol Head Neck Surg* 1997;123:595-600.

15. Homer JJ, Dowley AC, Condon L, El-Jassar P, Sood S. The effect of hypertonicity on nasal mucociliary clearance. *Clin Otolaryngol Allied Sci* 2000;25:558-560.

16. Kennedy DW. Prognostic factors, outcomes and staging in ethmoid sinus surgery. *Laryngoscope* 1992;102:1-18.

17. Damm M, Quante G, Jungehuelsing M, Stennert E. Impact of functional endoscopic sinus surgery on symptoms and quality of life in chronic rhinosinusitis. *Laryngoscope* 2002;112:310-315.

18. Gliklich RE, Metson R. Economic implications of chronic sinusitis. *Otolaryngol Head Neck Surg* 1998;118:344-349.

19. Senior BA, Kennedy DW, Tanabodee J, Kroger H, Hassab M, Lanza D. Long-term results of functional endoscopic sinus surgery. *Laryngoscope* 1998;108:151-157.

20. Sobol SE, Wright ED, Frenkiel S. One-year outcome analysis of functional endoscopic sinus surgery for chronic sinusitis. *J Otolaryngol* 1998;27:252-257.

21. Bhattacharyya N. Clinical outcomes after revision endoscopic sinus surgery. *Arch Otolaryngol Head Neck Surg* 2004;130:975-978.

22. Marks SC, Shamsa F. Evaluation of prognostic factors in endoscopic sinus surgery. *Am J Rhinol* 1997;11:187-191.

23. Briggs RD, Wright ST, Cordes S, Calhoun KH. Smoking in chronic rhinosinusitis: a predictor of poor long-term outcome after endoscopic sinus surgery. *Laryngoscope* 2004;114:126-128.

24. Kupferberg SB, Bent JP, 3rd, Kuhn FA. Prognosis for allergic fungal sinusitis. *Otolaryngol Head Neck Surg* 1997;117:35-41.

25. Amar YG, Frenkiel S, Sobol SE. Outcome analysis of endoscopic sinus surgery for chronic sinusitis in patients having Samter's triad. *J Otolaryngol* 2000;29:7-12.

26. Meltzer EO, Hamilos DL, Hadley JAet al. Rhinosinusitis: establishing definitions for clinical research and patient care. *J Allergy Clin Immunol* 2004;114:155-212.

27. Kountakis SE, Arango P, Bradley D, Wade ZK, Borish L. Molecular and Cellular Staging for the Severity of Chronic Rhinosinusitis. *Laryngoscope* 2004;114:1895-1905.

28. Collins M, Nair S, Smith W, Kette F, Gillis D, Wormald PJ. Role of local immunoglobulin E production in the pathophysiology of noninvasive fungal sinusitis. *Laryngoscope* 2004;114:1242-1246.

29. Lamb D, Miller J, Johnston A. Allergic aspergillosis of the maxillary sinuses. *Thorax* 1981;36:710.

30. Lamb D, Miller J, Johnston A. Allergic aspergillosis of the para-nasal sinuses. *Journal of Pathology* 1982;137:56.

31. Katzenstein AL, Sale SR, Greenberger PA. Allergic Aspergillus sinusitis: a newly recognized form of sinusitis. *J Allergy Clin Immunol* 1983;72:89-93.

32. Waxman JE, Spector JG, Sale SR, Katzenstein AL. Allergic Aspergillus sinusitis: concepts in diagnosis and treatment of a new clinical entity. *Laryngoscope* 1987;97:261-266.

33. Cody DT, 2nd, Neel HB, 3rd, Ferreiro JA, Roberts GD. Allergic fungal sinusitis: the Mayo Clinic experience. *Laryngoscope* 1994;104:1074-1079.

34. Thakar A, Sarkar C, Dhiwakar M, Bahadur S, Dahiya S. Allergic fungal sinusitis: expanding the clinicopathologic spectrum. *Otolaryngol Head Neck Surg* 2004;130:209-216.

35. deShazo RD, Swain RE. Diagnostic criteria for allergic fungal sinusitis. *J Allergy Clin Immunol* 1995;96:24-35.

36. Allphin AL, Strauss M, Abdul-Karim FW. Allergic fungal sinusitis: problems in diagnosis and treatment. *Laryngoscope* 1991;101:815-820.

37. Torres C, Ro JY, el-Naggar AK, Sim SJ, Weber RS, Ayala AG. Allergic fungal sinusitis: a clinicopathologic study of 16 cases. *Hum Pathol* 1996;27:793-799.

38. Granville L, Chirala M, Cernoch P, Ostrowski M, Truong LD. Fungal sinusitis: histologic spectrum and correlation with culture. *Hum Pathol* 2004;35:474-481.

39. Bent JP, 3rd, Kuhn FA. Diagnosis of allergic fungal sinusitis. *Otolaryngol Head Neck Surg* 1994;111:580-588.

40. Kuhn FA, Swain Jr. R. Allergic Fungal Sinusitis: diagnosis and treatment. *Curr Opin Otolaryngol Head Neck Surg* 2003;11:1-5.

41. Marple BF. Allergic fungal rhinosinusitis: current theories and management strategies. *Laryngoscope* 2001;111:1006-1019.

42. Schubert MS, Goetz DW. Evaluation and treatment of allergic fungal sinusitis. I. Demographics and diagnosis. *J Allergy Clin Immunol* 1998;102:387-394.

43. Manning SC, Schaefer SD, Close LG, Vuitch F. Culture-positive allergic fungal sinusitis. *Arch Otolaryngol Head Neck Surg* 1991;117:174-178.

44. Manning SC, Vuitch F, Weinberg AG, Brown OE. Allergic aspergillosis: a newly recognized form of sinusitis in the pediatric population. *Laryngoscope* 1989;99:681-685.

45. Fryen A, Mayser P, Glanz H, Fussle R, Breithaupt H, de Hoog GS. Allergic fungal sinusitis caused by Bipolaris (Drechslera) hawaiiensis. *Eur Arch Otorhinolaryngol* 1999;256:330-334.

46. Corey JP, Delsupehe KG, Ferguson BJ. Allergic fungal sinusitis: allergic, infectious, or both? *Otolaryngol Head Neck Surg* 1995;113:110-119.

47. Collins MM, Nair SB, Wormald PJ. Prevalence of noninvasive fungal sinusitis in South Australia. *Am J Rhinol* 2003;17:127-132.

48. Ferguson BJ, Barnes L, Bernstein JMet al. Geographic variation in allergic fungal rhinosinusitis. *Otolaryngol Clin North Am* 2000;33:441-449.

49. Kuhn FA, Javer AR. Allergic fungal sinusitis: a four-year follow-up. *Am J Rhinol* 2000;14:149-156.

50. Ferguson BJ. Eosinophilic mucin rhinosinusitis: a distinct clinicopathological entity. *Laryngoscope* 2000;110:799-813.

51. Lara JF, Gomez JD. Allergic mucin with and without fungus: a comparative clinicopathologic analysis. *Arch Pathol Lab Med* 2001;125:1442-1447.

52. Manning SC, Mabry RL, Schaefer SD, Close LG. Evidence of IgE-mediated hypersensitivity in allergic fungal sinusitis. *Laryngoscope* 1993;103:717-721.

53. Manning SC, Holman M. Further evidence for allergic pathophysiology in allergic fungal sinusitis. *Laryngoscope* 1998;108:1485-1496.

54. Schubert MS, Goetz DW. Evaluation and treatment of allergic fungal sinusitis. II. Treatment and follow-up. *J Allergy Clin Immunol* 1998;102:395-402.

55. Stewart AE, Hunsaker DH. Fungus-specific IgG and IgE in allergic fungal rhinosinusitis. *Otolaryngol Head Neck Surg* 2002;127:324-332.

56. Pant H, Kette FE, Smith WB, Wormald PJ, Macardle PJ. Fungal-specific humoral response in eosinophilic mucus chronic rhinosinusitis. *Laryngoscope* 2005;115:601-606.

57. Fokkens WJ, Vinke JG, KleinJan A. Local IgE production in the nasal mucosa: a review. *Am J Rhinol* 2000;14:299-303.

58. Durham SR, Smurthwaite L, Gould HJ. Local IgE production. *Am J Rhinol* 2000;14:305-307.

59. Ponikau JU, Sherris DA, Kern EBet al. The diagnosis and incidence of allergic fungal sinusitis. *Mayo Clin Proc* 1999;74:877-884.

60. Buzina W, Braun H, Freudenschuss K, Lackner A, Habermann W, Stammberger H. Fungal biodiversity--as found in nasal mucus. *Med Mycol* 2003;41:149-161.

61. Braun H, Buzina W, Freudenschuss K, Beham A, Stammberger H. 'Eosinophilic fungal rhinosinusitis': a common disorder in Europe? *Laryngoscope* 2003;113:264-269.

62. Lackner A, Freudenschuss K, Buzina Wet al. [From when on can fungi be identified in nasal mucus of humans?]. *Laryngorhinootologie* 2004;83:117-121.

63. Lackner A, Stammberger H, Buzina Wet al. Fungi: a normal content of human nasal mucus. *Am J Rhinol* 2005;19:125-129.

64. Collins MM, Nair SB, Der-Haroutian Vet al. Effect of using multiple culture media for the diagnosis of noninvasive fungal sinusitis. *Am J Rhinol* 2005;19:41-45.

65. Jiang RS, Su MC, Lin JF. Nasal mycology of chronic rhinosinusitis. *Am J Rhinol* 2005;19:131-133.

66. Catten MD, Murr AH, Goldstein JA, Mhatre AN, Lalwani AK. Detection of fungi in the nasal mucosa using polymerase chain reaction. *Laryngoscope* 2001;111:399-403.

67. Weschta M, Rimek D, Formanek M, Polzehl D, Riechelmann H. Local production of Aspergillus fumigatus specific immunoglobulin E in nasal polyps. *Laryngoscope* 2003;113:1798-1802.

68. Gosepath J, Brieger J, Vlachtsis K, Mann WJ. Fungal DNA is present in tissue specimens of patients with chronic rhinosinusitis. *Am J Rhinol* 2004;18:9-13.

69. Khan DA, Cody DT, 2nd, George TJ, Gleich GJ, Leiferman KM. Allergic fungal sinusitis: an immunohistologic analysis. *J Allergy Clin Immunol* 2000;106:1096-1101.

70. Ponikau JU, Sherris DA, Kephart GMet al. Striking deposition of toxic eosinophil major basic protein in mucus: implications for chronic rhinosinusitis. *J Allergy Clin Immunol* 2005;116:362-369.

71. Mabry RL, Manning SC, Mabry CS. Immunotherapy in the treatment of allergic fungal sinusitis. *Otolaryngol Head Neck Surg* 1997;116:31-35.

72. Folker RJ, Marple BF, Mabry RL, Mabry CS. Treatment of allergic fungal sinusitis: a comparison trial of postoperative immunotherapy with specific fungal antigens. *Laryngoscope* 1998;108:1623-1627.

73. Mabry RL, Marple BF, Mabry CS. Outcomes after discontinuing immunotherapy for allergic fungal sinusitis. *Otolaryngol Head Neck Surg* 2000;122:104-106.

74. Marple B, Newcomer M, Schwade N, Mabry R. Natural history of allergic fungal rhinosinusitis: a 4- to 10-year follow-up. *Otolaryngol Head Neck Surg* 2002;127:361-366.

75. Manning SC. Basics of biofilm in clinical otolaryngology. *Ear Nose Throat J* 2003;82:18-20.

76. Shirtliff ME, Mader JT, Camper AK. Molecular interactions in biofilms. *Chem Biol* 2002;9:859-871.

77. Ramage G, Saville SP, Thomas DP, Lopez-Ribot JL. Candida biofilms: an update. *Eukaryot Cell* 2005;4:633-638.

78. Martinez LR, Casadevall A. Specific antibody can prevent fungal biofilm formation and this effect correlates with protective efficacy. *Infect Immun* 2005;73:6350-6362.

79. Ehrlich GD, Veeh R, Wang Xet al. Mucosal biofilm formation on middle-ear mucosa in the chinchilla model of otitis media. *Jama* 2002;287:1710-1715.

80. Perloff JR, Palmer JN. Evidence of bacterial biofilms on frontal recess stents in patients with chronic rhinosinusitis. *Am J Rhinol* 2004;18:377-380.

81. Sanclement JA, Webster P, Thomas J, Ramadan HH. Bacterial biofilms in surgical specimens of patients with chronic rhinosinusitis. *Laryngoscope* 2005;115:578-582.

82. Schubert MS. A superantigen hypothesis for the pathogenesis of chronic hypertrophic rhinosinusitis, allergic fungal sinusitis, and related disorders. *Ann Allergy Asthma Immunol* 2001;87:181-188.

83. Choi YW, Kotzin B, Herron L, Callahan J, Marrack P, Kappler J. Interaction of Staphylococcus aureus toxin "superantigens" with human T cells. *Proc Natl Acad Sci U S A* 1989;86:8941-8945.

84. Bachert C, Gevaert P, Holtappels G, Johansson SG, van Cauwenberge P. Total and specific IgE in nasal polyps is related to local eosinophilic inflammation. *J Allergy Clin Immunol* 2001;107:607-614.

85. Bernstein JM, Ballow M, Schlievert PM, Rich G, Allen C, Dryja D. A superantigen hypothesis for the pathogenesis of chronic hyperplastic sinusitis with massive nasal polyposis. *Am J Rhinol* 2003;17:321-326.

86. Seiberling KA, Conley DB, Tripathi Aet al. Superantigens and chronic rhinosinusitis: detection of staphylococcal exotoxins in nasal polyps. *Laryngoscope* 2005;115:1580-1585.

87. Cook P. The role of aspirin sensitivity and nasal polyposis. *Curr Opin Otolaryngol Head Neck Surg* 2002;10:213-216.

88. Molnar-Gabor E, Endreffy E, Rozsasi A. HLA-DRB1, -DQA1, and -DQB1 genotypes in patients with nasal polyposis. *Laryngoscope* 2000;110:422-425.

89. Chee L, Graham SM, Carothers DG, Ballas ZK. Immune dysfunction in refractory sinusitis in a tertiary care setting. *Laryngoscope* 2001;111:233-235.

90. Mitakakis TZ, Tovey ER, Xuan W, Marks GB. Personal exposure to allergenic pollen and mould spores in inland New South Wales, Australia. *Clin Exp Allergy* 2000;30:1733-1739.

91. Kaliner MA. Human nasal host defense and sinusitis. *J Allergy Clin Immunol* 1992;90:424-430.

92. Sakakura Y, Majima Y, Saida S, Ukai K, Miyoshi Y. Reversibility of reduced mucociliary clearance in chronic sinusitis. *Clin Otolaryngol* 1985;10:79-83.

93. Al-Rawi MM, Edelstein DR, Erlandson RA. Changes in nasal epithelium in patients with severe chronic sinusitis: a clinicopathologic and electron microscopic study. *Laryngoscope* 1998;108:1816-1823.

94. Inanli S, Tutkun A, Batman C, Okar I, Uneri C, Sehitoglu MA. The effect of endoscopic sinus surgery on mucociliary activity and healing of maxillary sinus mucosa. *Rhinology* 2000;38:120-123.

95. Asai K, Haruna S, Otori N, Yanagi K, Fukami M, Moriyama H. Saccharin test of maxillary sinus mucociliary function after endoscopic sinus surgery. *Laryngoscope* 2000;110:117-122.

96. Elwany S, Hisham M, Gamaee R. The effect of endoscopic sinus surgery on mucociliary clearance in patients with chronic sinusitis. *Eur Arch Otorhinolaryngol* 1998;255:511-514.

97. Liote H, Zahm JM, Pierrot D, Puchelle E. Role of mucus and cilia in nasal mucociliary clearance in healthy subjects. *Am Rev Respir Dis* 1989;140:132-136.

98. Medzhitov R, Janeway C, Jr. Innate immune recognition: mechanisms and pathways. *Immunol Rev* 2000;173:89-97.

99. Ganz T. Antimicrobial polypeptides. *J Leukoc Biol* 2004;75:34-38.

100. Delves PJ, Roitt IM. The immune system. First of two parts. *N Engl J Med* 2000;343:37-49.

101. Bachert C, Wagenmann M, Rudack Cet al. The role of cytokines in infectious sinusitis and nasal polyposis. *Allergy* 1998;53:2-13.

102. Bachert C, Wagenmann M, Hauser U, Rudack C. IL-5 synthesis is upregulated in human nasal polyp tissue. *J Allergy Clin Immunol* 1997;99:837-842.

103. Ramadan HH, Meek RB, Dawson GS, Spirou GA, Cuff CF, Berrebi AS. Histologic and immunologic observations of viral-induced rhinosinusitis in the mouse. *Am J Rhinol* 2002;16:61-67.

104. Harlin SL, Ansel DG, Lane SR, Myers J, Kephart GM, Gleich GJ. A clinical and pathologic study of chronic sinusitis: the role of the eosinophil. *J Allergy Clin Immunol* 1988;81:867-875.

105. Hamilos DL, Leung DY, Wood Ret al. Chronic hyperplastic sinusitis: association of tissue eosinophilia with mRNA expression of granulocyte-macrophage colony-stimulating factor and interleukin-3. *J Allergy Clin Immunol* 1993;92:39-48.

106. Hamilos DL, Leung DY, Huston DP, Kamil A, Wood R, Hamid Q. GM-CSF, IL-5 and RANTES immunoreactivity and mRNA expression in chronic hyperplastic sinusitis with nasal polyposis (NP). *Clin Exp Allergy* 1998;28:1145-1152.

107. Hamilos DL. Chronic sinusitis. *J Allergy Clin Immunol* 2000;106:213-227.

108. Ponikau JU, Sherris DA, Kephart GMet al. Features of airway remodeling and eosinophilic inflammation in chronic rhinosinusitis: is the histopathology similar to asthma? *J Allergy Clin Immunol* 2003;112:877-882.

109. von Bubnoff D, Geiger E, Bieber T. Antigen-presenting cells in allergy. *J Allergy Clin Immunol* 2001;108:329-339.

110. Medzhitov R, Janeway C, Jr. Innate immunity. *N Engl J Med* 2000;343:338-344.

111. Diamond G, Legarda D, Ryan LK. The innate immune response of the respiratory epithelium. *Immunol Rev* 2000;173:27-38.

112. Crouch E, Hartshorn K, Ofek I. Collectins and pulmonary innate immunity. *Immunol Rev* 2000;173:52-65.

113. Fraser IP, Koziel H, Ezekowitz RA. The serum mannose-binding protein and the macrophage mannose receptor are pattern recognition molecules that link innate and adaptive immunity. *Semin Immunol* 1998;10:363-372.

114. Polito AJ, Proud D. Epithelia cells as regulators of airway inflammation. *J Allergy Clin Immunol* 1998;102:714-718.

115. Roeder A, Kirschning CJ, Rupec RA, Schaller M, Korting HC. Toll-like receptors and innate antifungal responses. *Trends Microbiol* 2004;12:44-49.

116. Vandermeer J, Sha Q, Lane AP, Schleimer RP. Innate immunity of the sinonasal cavity: expression of messenger RNA for complement cascade components and toll-like receptors. *Arch Otolaryngol Head Neck Surg* 2004;130:1374-1380.

117. Prussin C, Metcalfe DD. 4. IgE, mast cells, basophils, and eosinophils. *J Allergy Clin Immunol* 2003;111:S486-494.

118. Biron CA, Nguyen KB, Pien GC, Cousens LP, Salazar-Mather TP. Natural killer cells in antiviral defense: function and regulation by innate cytokines. *Annu Rev Immunol* 1999;17:189-220.

119. Lane AP, Saatian B, Yu XY, Spannhake EW. mRNA for genes associated with antigen presentation are expressed by human middle meatal epithelial cells in culture. *Laryngoscope* 2004;114:1827-1832.

120. Walport MJ. Complement. First of two parts. *N Engl J Med* 2001;344:1058-1066.

121. Walport MJ. Complement. Second of two parts. *N Engl J Med* 2001;344:1140-1144.

122. Gabay C, Kushner I. Acute-phase proteins and other systemic responses to inflammation. *N Engl J Med* 1999;340:448-454.

123. Janeway CA, Jr., Medzhitov R. Innate immune recognition. *Annu Rev Immunol* 2002;20:197-216.

124. Delves PJ, Roitt IM. The immune system. Second of two parts. *N Engl J Med* 2000;343:108-117.

125. Ng TW, Holt PG, Prescott SL. Cellular immune responses to ovalbumin and house dust mite in egg-allergic children. *Allergy* 2002;57:207-214.

126. Sha Q, Truong-Tran AQ, Plitt JR, Beck LA, Schleimer RP. Activation of airway epithelial cells by toll-like receptor agonists. *Am J Respir Cell Mol Biol* 2004;31:358-364.

127. Kilpatrick DC. Mannan-binding lectin and its role in innate immunity. *Transfus Med* 2002;12:335-352.

128. Ellison RT, 3rd, Giehl TJ. Killing of gram-negative bacteria by lactoferrin and lysozyme. *J Clin Invest* 1991;88:1080-1091.

129. Raphael GD, Jeney EV, Baraniuk JN, Kim I, Meredith SD, Kaliner MA. Pathophysiology of rhinitis. Lactoferrin and lysozyme in nasal secretions. *J Clin Invest* 1989;84:1528-1535.

130. Fukami M, Stierna P, Veress B, Carlsoo B. Lysozyme and lactoferrin in human maxillary sinus mucosa during chronic sinusitis. An immunohistochemical study. *Eur Arch Otorhinolaryngol* 1993;250:133-139.

131. Lee CH, Igarashi Y, Hohman RJ, Kaulbach H, White MV, Kaliner MA. Distribution of secretory leukoprotease inhibitor in the human nasal airway. *Am Rev Respir Dis* 1993;147:710-716.

132. Singh PK, Parsek MR, Greenberg EP, Welsh MJ. A component of innate immunity prevents bacterial biofilm development. *Nature* 2002;417:552-555.

133. Tomee JF, Hiemstra PS, Heinzel-Wieland R, Kauffman HF. Antileukoprotease: an endogenous protein in the innate mucosal defense against fungi. *J Infect Dis* 1997;176:740-747.

134. Tomee JF, Koeter GH, Hiemstra PS, Kauffman HF. Secretory leukoprotease inhibitor: a native antimicrobial protein presenting a new therapeutic option? *Thorax* 1998;53:114-116.

135. Gudmundsson GH, Agerberth B. Neutrophil antibacterial peptides, multifunctional effector molecules in the mammalian immune system. *J Immunol Methods* 1999;232:45-54.

136. Cunliffe RN, Mahida YR. Expression and regulation of antimicrobial peptides in the gastrointestinal tract. *J Leukoc Biol* 2004;75:49-58.

137. Frye M, Bargon J, Dauletbaev N, Weber A, Wagner TO, Gropp R. Expression of human alpha-defensin 5 (HD5) mRNA in nasal and bronchial epithelial cells. *J Clin Pathol* 2000;53:770-773.

138. van Wetering S, Tjabringa GS, Hiemstra PS. Interactions between neutrophil-derived antimicrobial peptides and airway epithelial cells. *J Leukoc Biol* 2004.

139. Bals R, Hiemstra PS. Innate immunity in the lung: how epithelial cells fight against respiratory pathogens. *Eur Respir J* 2004;23:327-333.

140. Lee SH, Kim JE, Lim HH, Lee HM, Choi JO. Antimicrobial defensin peptides of the human nasal mucosa. *Ann Otol Rhinol Laryngol* 2002;111:135-141.

141. Carothers DG, Graham SM, Jia HP, Ackermann MR, Tack BF, McCray PB, Jr. Production of beta-defensin antimicrobial peptides by maxillary sinus mucosa. *Am J Rhinol* 2001;15:175-179.

142. Zanetti M, Gennaro R, Romeo D. Cathelicidins: a novel protein family with a common proregion and a variable C-terminal antimicrobial domain. *FEBS Lett* 1995;374:1-5.

143. Gallo RL, Kim KJ, Bernfield Met al. Identification of CRAMP, a cathelin-related antimicrobial peptide expressed in the embryonic and adult mouse. *J Biol Chem* 1997;272:13088-13093.

144. Skerlavaj B, Scocchi M, Gennaro R, Risso A, Zanetti M. Structural and functional analysis of horse cathelicidin peptides. *Antimicrob Agents Chemother* 2001;45:715-722.

145. Nagaoka I, Tsutsumi-Ishii Y, Yomogida S, Yamashita T. Isolation of cDNA encoding guinea pig neutrophil cationic antibacterial polypeptide of 11 kDa (CAP11) and evaluation of CAP11 mRNA expression during neutrophil maturation. *J Biol Chem* 1997;272:22742-22750.

146. Brogden KA, Kalfa VC, Ackermann MR, Palmquist DE, McCray PB, Jr., Tack BF. The ovine cathelicidin SMAP29 kills ovine respiratory pathogens in vitro and in an ovine model of pulmonary infection. *Antimicrob Agents Chemother* 2001;45:331-334.

147. Travis SM, Anderson NN, Forsyth WRet al. Bactericidal activity of mammalian cathelicidin-derived peptides. *Infect Immun* 2000;68:2748-2755.

148. Lee PH, Ohtake T, Zaiou Met al. Expression of an additional cathelicidin antimicrobial peptide protects against bacterial skin infection. *Proc Natl Acad Sci U S A* 2005;102:3750-3755.

149. Larrick JW, Hirata M, Balint RF, Lee J, Zhong J, Wright SC. Human CAP18: a novel antimicrobial lipopolysaccharide-binding protein. *Infect Immun* 1995;63:1291-1297.

150. Gudmundsson GH, Agerberth B, Odeberg J, Bergman T, Olsson B, Salcedo R. The human gene FALL39 and processing of the cathelin precursor to the antibacterial peptide LL-37 in granulocytes. *Eur J Biochem* 1996;238:325-332.

151. Cowland JB, Johnsen AH, Borregaard N. hCAP-18, a cathelin/pro-bactenecin-like protein of human neutrophil specific granules. *FEBS Lett* 1995;368:173-176.

152. Agerberth B, Gunne H, Odeberg J, Kogner P, Boman HG, Gudmundsson GH. FALL-39, a putative human peptide antibiotic, is cysteine-free and expressed in bone marrow and testis. *Proc Natl Acad Sci U S A* 1995;92:195-199.

153. Sorensen O, Arnljots K, Cowland JB, Bainton DF, Borregaard N. The human antibacterial cathelicidin, hCAP-18, is synthesized in myelocytes and metamyelocytes and localized to specific granules in neutrophils. *Blood* 1997;90:2796-2803.

154. Nagaoka I, Hirata M, Sugimoto Ket al. Evaluation of the expression of human CAP18 gene during neutrophil maturation in the bone marrow. *J Leukoc Biol* 1998;64:845-852.

155. Zanetti M. Cathelicidins, multifunctional peptides of the innate immunity. *J Leukoc Biol* 2004;75:39-48.

156. Bals R, Wilson JM. Cathelicidins--a family of multifunctional antimicrobial peptides. *Cell Mol Life Sci* 2003;60:711-720.

157. Agerberth B, Charo J, Werr Jet al. The human antimicrobial and chemotactic peptides LL-37 and alpha-defensins are expressed by specific lymphocyte and monocyte populations. *Blood* 2000;96:3086-3093.

158. Marchini G, Lindow S, Brismar Het al. The newborn infant is protected by an innate antimicrobial barrier: peptide antibiotics are present in the skin and vernix caseosa. *Br J Dermatol* 2002;147:1127-1134.

159. Di Nardo A, Vitiello A, Gallo RL. Cutting Edge: Mast Cell Antimicrobial Activity Is Mediated by Expression of Cathelicidin Antimicrobial Peptide. *J Immunol* 2003;170:2274-2278.

160. Bals R, Wang X, Zasloff M, Wilson JM. The peptide antibiotic LL-37/hCAP-18 is expressed in epithelia of the human lung where it has broad antimicrobial activity at the airway surface. *Proc Natl Acad Sci U S A* 1998;95:9541-9546.

161. Hase K, Murakami M, Iimura Met al. Expression of LL-37 by human gastric epithelial cells as a potential host defense mechanism against Helicobacter pylori. *Gastroenterology* 2003;125:1613-1625.

162. Braff MH, Nardo AD, Gallo RL. Keratinocytes store the antimicrobial Peptide cathelicidin in lamellar bodies. *J Invest Dermatol* 2005;124:394-400.

163. Frohm Nilsson M, Sandstedt B, Sorensen O, Weber G, Borregaard N, Stahle-Backdahl M. The human cationic antimicrobial protein (hCAP18), a peptide antibiotic, is widely expressed in human squamous epithelia and colocalizes with interleukin-6. *Infect Immun* 1999;67:2561-2566.

164. Murakami M, Ohtake T, Dorschner RA, Gallo RL. Cathelicidin antimicrobial peptides are expressed in salivary glands and saliva. *J Dent Res* 2002;81:845-850.

165. Woo JS, Jeong JY, Hwang YJ, Chae SW, Hwang SJ, Lee HM. Expression of cathelicidin in human salivary glands. *Arch Otolaryngol Head Neck Surg* 2003;129:211-214.

166. Andersson E, Sorensen OE, Frohm B, Borregaard N, Egesten A, Malm J. Isolation of human cationic antimicrobial protein-18 from seminal plasma and its association with prostasomes. *Hum Reprod* 2002;17:2529-2534.

167. Malm J, Sorensen O, Persson Tet al. The human cationic antimicrobial protein (hCAP-18) is expressed in the epithelium of human epididymis, is present in seminal plasma at high concentrations, and is attached to spermatozoa. *Infect Immun* 2000;68:4297-4302.

168. Murakami M, Dorschner RA, Stern LJ, Lin KH, Gallo RL. Expression and secretion of cathelicidin antimicrobial peptides in murine mammary glands and human milk. *Pediatr Res* 2005;57:10-15.

169. Schauber J, Svanholm C, Termen Set al. Expression of the cathelicidin LL-37 is modulated by short chain fatty acids in colonocytes: relevance of signalling pathways. *Gut* 2003;52:735-741.

170. Kim ST, Cha HE, Kim DYet al. Antimicrobial peptide LL-37 is upregulated in chronic nasal inflammatory disease. *Acta Otolaryngol* 2003;123:81-85.

171. Sorensen OE, Follin P, Johnsen AHet al. Human cathelicidin, hCAP-18, is processed to the antimicrobial peptide LL-37 by extracellular cleavage with proteinase 3. *Blood* 2001;97:3951-3959.

172. Murakami M, Lopez-Garcia B, Braff M, Dorschner RA, Gallo RL. Postsecretory processing generates multiple cathelicidins for enhanced topical antimicrobial defense. *J Immunol* 2004;172:3070-3077.

173. Zaiou M, Nizet V, Gallo RL. Antimicrobial and protease inhibitory functions of the human cathelicidin (hCAP18/LL-37) prosequence. *J Invest Dermatol* 2003;120:810-816.

174. Oren Z, Shai Y. Mode of action of linear amphipathic alpha-helical antimicrobial peptides. *Biopolymers* 1998;47:451-463.

175. Oren Z, Lerman JC, Gudmundsson GH, Agerberth B, Shai Y. Structure and organization of the human antimicrobial peptide LL-37 in phospholipid membranes: relevance to the molecular basis for its non-cell-selective activity. *Biochem J* 1999;341:501-513.

176. Henzler Wildman KA, Lee DK, Ramamoorthy A. Mechanism of lipid bilayer disruption by the human antimicrobial peptide, LL-37. *Biochemistry* 2003;42:6545-6558.

177. Johansson J, Gudmundsson GH, Rottenberg ME, Berndt KD, Agerberth B. Conformation-dependent antibacterial activity of the naturally occurring human peptide LL-37. *J Biol Chem* 1998;273:3718-3724.

178. Turner J, Cho Y, Dinh NN, Waring AJ, Lehrer RI. Activities of LL-37, a cathelin-associated antimicrobial peptide of human neutrophils. *Antimicrob Agents Chemother* 1998;42:2206-2214.

179. Travis SM, Conway BA, Zabner Jet al. Activity of abundant antimicrobials of the human airway. *Am J Respir Cell Mol Biol* 1999;20:872-879.

180. Hase K, Eckmann L, Leopard JD, Varki N, Kagnoff MF. Cell differentiation is a key determinant of cathelicidin LL-37/human cationic antimicrobial protein 18 expression by human colon epithelium. *Infect Immun* 2002;70:953-963.

181. Nagaoka I, Hirota S, Yomogida S, Ohwada A, Hirata M. Synergistic actions of antibacterial neutrophil defensins and cathelicidins. *Inflamm Res* 2000;49:73-79.

182. Singh PK, Tack BF, McCray PB, Jr., Welsh MJ. Synergistic and additive killing by antimicrobial factors found in human airway surface liquid. *Am J Physiol Lung Cell Mol Physiol* 2000;279:L799-805.

183. Nizet V, Ohtake T, Lauth Xet al. Innate antimicrobial peptide protects the skin from invasive bacterial infection. *Nature* 2001;414:454-457.

184. Schaller-Bals S, Schulze A, Bals R. Increased levels of antimicrobial peptides in tracheal aspirates of newborn infants during infection. *Am J Respir Crit Care Med* 2002;165:992-995.

185. De Y, Chen Q, Schmidt APet al. LL-37, the neutrophil granule- and epithelial cell-derived cathelicidin, utilizes formyl peptide receptor-like 1 (FPRL1) as a receptor to chemoattract human peripheral blood neutrophils, monocytes, and T cells. *J Exp Med* 2000;192:1069-1074.

186. Niyonsaba F, Hirata M, Ogawa H, Nagaoka I. Epithelial cell-derived antibacterial peptides human beta-defensins and cathelicidin: multifunctional activities on mast cells. *Curr Drug Targets Inflamm Allergy* 2003;2:224-231.

187. Scott MG, Davidson DJ, Gold MR, Bowdish D, Hancock RE. The human antimicrobial peptide LL-37 is a multifunctional modulator of innate immune responses. *J Immunol* 2002;169:3883-3891.

188. Davidson DJ, Currie AJ, Reid GSet al. The cationic antimicrobial peptide LL-37 modulates dendritic cell differentiation and dendritic cell-induced T cell polarization. *J Immunol* 2004;172:1146-1156.

189. Gallo RL, Ono M, Povsic Tet al. Syndecans, cell surface heparan sulfate proteoglycans, are induced by a proline-rich antimicrobial peptide from wounds. *Proc Natl Acad Sci U S A* 1994;91:11035-11039.

190. Dorschner RA, Pestonjamasp VK, Tamakuwala Set al. Cutaneous injury induces the release of cathelicidin anti-microbial peptides active against group A Streptococcus. *J Invest Dermatol* 2001;117:91-97.

191. Heilborn JD, Nilsson MF, Kratz Get al. The cathelicidin anti-microbial peptide LL-37 is involved in re-epithelialization of human skin wounds and is lacking in chronic ulcer epithelium. *J Invest Dermatol* 2003;120:379-389.

192. Cole AM, Shi J, Ceccarelli A, Kim YH, Park A, Ganz T. Inhibition of neutrophil elastase prevents cathelicidin activation and impairs clearance of bacteria from wounds. *Blood* 2001;97:297-304.

193. Koczulla R, von Degenfeld G, Kupatt Cet al. An angiogenic role for the human peptide antibiotic LL-37/hCAP-18. *J Clin Invest* 2003;111:1665-1672.

194. Nagaoka I, Hirota S, Niyonsaba Fet al. Augmentation of the lipopolysaccharide-neutralizing activities of human cathelicidin CAP18/LL-37-derived antimicrobial peptides by replacement with hydrophobic and cationic amino acid residues. *Clin Diagn Lab Immunol* 2002;9:972-982.

195. Tjabringa GS, Aarbiou J, Ninaber DKet al. The antimicrobial peptide LL-37 activates innate immunity at the airway epithelial surface by transactivation of the epidermal growth factor receptor. *J Immunol* 2003;171:6690-6696.

196. Bowdish DM, Davidson DJ, Speert DP, Hancock RE. The human cationic peptide LL-37 induces activation of the extracellular signal-regulated kinase and p38 kinase pathways in primary human monocytes. *J Immunol* 2004;172:3758-3765.

197. Elssner A, Duncan M, Gavrilin M, Wewers MD. A novel P2X7 receptor activator, the human cathelicidin-derived peptide LL37, induces IL-1 beta processing and release. *J Immunol* 2004;172:4987-4994.

198. Ong PY, Ohtake T, Brandt Cet al. Endogenous antimicrobial peptides and skin infections in atopic dermatitis. *N Engl J Med* 2002;347:1151-1160.

199. Frohm M, Agerberth B, Ahangari Get al. The expression of the gene coding for the antibacterial peptide LL-37 is induced in human keratinocytes during inflammatory disorders. *J Biol Chem* 1997;272:15258-15263.

200. Paulsen F, Pufe T, Conradi Let al. Antimicrobial peptides are expressed and produced in healthy and inflamed human synovial membranes. *J Pathol* 2002;198:369-377.

201. Agerberth B, Grunewald J, Castanos-Velez Eet al. Antibacterial components in bronchoalveolar lavage fluid from healthy individuals and sarcoidosis patients. *Am J Respir Crit Care Med* 1999;160:283-290.

202. Sorensen OE, Cowland JB, Theilgaard-Monch K, Liu L, Ganz T, Borregaard N. Wound healing and expression of antimicrobial peptides/polypeptides in human keratinocytes, a consequence of common growth factors. *J Immunol* 2003;170:5583-5589.

203. Wang Y, Agerberth B, Lothgren A, Almstedt A, Johansson J. Apolipoprotein A-I binds and inhibits the human antibacterial/cytotoxic peptide LL-37. *J Biol Chem* 1998;273:33115-33118.

204. Sorensen O, Bratt T, Johnsen AH, Madsen MT, Borregaard N. The human antibacterial cathelicidin, hCAP-18, is bound to lipoproteins in plasma. *J Biol Chem* 1999;274:22445-22451.

205. Lysenko ES, Gould J, Bals R, Wilson JM, Weiser JN. Bacterial phosphorylcholine decreases susceptibility to the antimicrobial peptide LL-37/hCAP18 expressed in the upper respiratory tract. *Infect Immun* 2000;68:1664-1671.

206. Nyberg P, Rasmussen M, Bjorck L. alpha2-Macroglobulin-proteinase complexes protect Streptococcus pyogenes from killing by the antimicrobial peptide LL-37. *J Biol Chem* 2004;279:52820-52823.

207. Sieprawska-Lupa M, Mydel P, Krawczyk Ket al. Degradation of human antimicrobial peptide LL-37 by Staphylococcus aureus-derived proteinases. *Antimicrob Agents Chemother* 2004;48:4673-4679.

208. Jung HH, Chae SW, Jung SK, Kim ST, Lee HM, Hwang SJ. Expression of a cathelicidin antimicrobial peptide is augmented in cholesteatoma. *Laryngoscope* 2003;113:432-435.

209. Chen PH, Fang SY. The expression of human antimicrobial peptide LL-37 in the human nasal mucosa. *Am J Rhinol* 2004;18:381-385.

210. Pryhuber GS. Regulation and function of pulmonary surfactant protein B. *Mol Genet Metab* 1998;64:217-228.

211. Lu J, Teh C, Kishore U, Reid KB. Collectins and ficolins: sugar pattern recognition molecules of the mammalian innate immune system. *Biochim Biophys Acta* 2002;1572:387-400.

212. Hakansson K, Reid KB. Collectin structure: a review. *Protein Sci* 2000;9:1607-1617.

213. Karinch AM, Floros J. 5' splicing and allelic variants of the human pulmonary surfactant protein A genes. *Am J Respir Cell Mol Biol* 1995;12:77-88.

214. Korfhagen TR, Bruno MD, Ross GFet al. Altered surfactant function and structure in SP-A gene targeted mice. *Proc Natl Acad Sci U S A* 1996;93:9594-9599.

215. Ikegami M, Korfhagen TR, Whitsett JAet al. Characteristics of surfactant from SP-A-deficient mice. *Am J Physiol* 1998;275:L247-254.

216. LeVine AM, Kurak KE, Bruno MD, Stark JM, Whitsett JA, Korfhagen TR. Surfactant protein-A-deficient mice are susceptible to Pseudomonas aeruginosa infection. *Am J Respir Cell Mol Biol* 1998;19:700-708.

217. LeVine AM, Kurak KE, Wright JRet al. Surfactant protein-A binds group B streptococcus enhancing phagocytosis and clearance from lungs of surfactant protein-A-deficient mice. *Am J Respir Cell Mol Biol* 1999;20:279-286.

218. LeVine AM, Gwozdz J, Stark J, Bruno M, Whitsett J, Korfhagen T. Surfactant protein-A enhances respiratory syncytial virus clearance in vivo. *J Clin Invest* 1999;103:1015-1021.

219. LeVine AM, Hartshorn K, Elliott J, Whitsett J, Korfhagen T. Absence of SP-A modulates innate and adaptive defense responses to pulmonary influenza infection. *Am J Physiol Lung Cell Mol Physiol* 2002;282:L563-572.

220. Persson A, Rust K, Chang D, Moxley M, Longmore W, Crouch E. CP4: a pneumocyte-derived collagenous surfactant-associated protein. Evidence for heterogeneity of collagenous surfactant proteins. *Biochemistry* 1988;27:8576-8584.

221. Motwani M, White RA, Guo N, Dowler LL, Tauber AI, Sastry KN. Mouse surfactant protein-D. cDNA cloning, characterization, and gene localization to chromosome 14. *J Immunol* 1995;155:5671-5677.

222. van Rozendaal BA, van Golde LM, Haagsman HP. Localization and functions of SP-A and SP-D at mucosal surfaces. *Pediatr Pathol Mol Med* 2001;20:319-339.

223. Crouch E, Persson A, Chang D, Heuser J. Molecular structure of pulmonary surfactant protein D (SP-D). *J Biol Chem* 1994;269:17311-17319.

224. Crouch E, Rust K, Veile R, Donis-Keller H, Grosso L. Genomic organization of human surfactant protein D (SP-D). SP-D is encoded on chromosome 10q22.2-23.1. *J Biol Chem* 1993;268:2976-2983.

225. Floros J, Hoover RR. Genetics of the hydrophilic surfactant proteins A and D. *Biochim Biophys Acta* 1998;1408:312-322.

226. Brown-Augsburger P, Chang D, Rust K, Crouch EC. Biosynthesis of surfactant protein D. Contributions of conserved NH2-terminal cysteine residues and collagen helix formation to assembly and secretion. *J Biol Chem* 1996;271:18912-18919.

227. Brown-Augsburger P, Hartshorn K, Chang Det al. Site-directed mutagenesis of Cys-15 and Cys-20 of pulmonary surfactant protein D. Expression of a trimeric protein with altered anti-viral properties. *J Biol Chem* 1996;271:13724-13730.

228. Zhang L, Ikegami M, Crouch EC, Korfhagen TR, Whitsett JA. Activity of pulmonary surfactant protein-D (SP-D) in vivo is dependent on oligomeric structure. *J Biol Chem* 2001;276:19214-19219.

229. Hopfer U, Jacobberger JW, Gruenert DC, Eckert RL, Jat PS, Whitsett JA. Immortalization of epithelial cells. *Am J Physiol* 1996;270:C1-11.

230. Wang JY, Kishore U, Reid KB. A recombinant polypeptide, composed of the alpha-helical neck region and the carbohydrate recognition domain of conglutinin, self-associates to give a functionally intact homotrimer. *FEBS Lett* 1995;376:6-10.

231. Mason RJ, Nielsen LD, Kuroki Y, Matsuura E, Freed JH, Shannon JM. A 50-kDa variant form of human surfactant protein D. *Eur Respir J* 1998;12:1147-1155.

232. Murray E, Khamri W, Walker MMet al. Expression of surfactant protein D in the human gastric mucosa and during Helicobacter pylori infection. *Infect Immun* 2002;70:1481-1487.

233. Ferguson JS, Voelker DR, McCormack FX, Schlesinger LS. Surfactant protein D binds to Mycobacterium tuberculosis bacilli and lipoarabinomannan via carbohydrate-lectin interactions resulting in reduced phagocytosis of the bacteria by macrophages. *J Immunol* 1999;163:312-321.

234. Ofek I, Mesika A, Kalina Met al. Surfactant protein D enhances phagocytosis and killing of unencapsulated phase variants of Klebsiella pneumoniae. *Infect Immun* 2001;69:24-33.

235. Restrepo CI, Dong Q, Savov J, Mariencheck WI, Wright JR. Surfactant protein D stimulates phagocytosis of Pseudomonas aeruginosa by alveolar macrophages. *Am J Respir Cell Mol Biol* 1999;21:576-585.

236. Kuan SF, Rust K, Crouch E. Interactions of surfactant protein D with bacterial lipopolysaccharides. Surfactant protein D is an Escherichia coli-binding protein in bronchoalveolar lavage. *J Clin Invest* 1992;90:97-106.

237. Hartshorn KL, Crouch E, White MRet al. Pulmonary surfactant proteins A and D enhance neutrophil uptake of bacteria. *Am J Physiol* 1998;274:L958-969.

238. Oberley RE, Goss KL, Dahmoush L, Ault KA, Crouch EC, Snyder JM. A role for surfactant protein D in innate immunity of the human prostate. *Prostate* 2005.

239. Hartshorn KL, Crouch EC, White MRet al. Evidence for a protective role of pulmonary surfactant protein D (SP-D) against influenza A viruses. *J Clin Invest* 1994;94:311-319.

240. Hickling TP, Bright H, Wing Ket al. A recombinant trimeric surfactant protein D carbohydrate recognition domain inhibits respiratory syncytial virus infection in vitro and in vivo. *Eur J Immunol* 1999;29:3478-3484.

241. Allen MJ, Harbeck R, Smith B, Voelker DR, Mason RJ. Binding of rat and human surfactant proteins A and D to Aspergillus fumigatus conidia. *Infect Immun* 1999;67:4563-4569.

242. Schelenz S, Malhotra R, Sim RB, Holmskov U, Bancroft GJ. Binding of host collectins to the pathogenic yeast Cryptococcus neoformans: human surfactant protein D acts as an agglutinin for acapsular yeast cells. *Infect Immun* 1995;63:3360-3366.

243. van Rozendaal BA, van Spriel AB, van De Winkel JG, Haagsman HP. Role of pulmonary surfactant protein D in innate defense against Candida albicans. *J Infect Dis* 2000;182:917-922.

244. O'Riordan DM, Standing JE, Kwon KY, Chang D, Crouch EC, Limper AH. Surfactant protein D interacts with Pneumocystis carinii and mediates organism adherence to alveolar macrophages. *J Clin Invest* 1995;95:2699-2710.

245. Holmskov U, Lawson P, Teisner Bet al. Isolation and characterization of a new member of the scavenger receptor superfamily, glycoprotein-340 (gp-340), as a lung surfactant protein-D binding molecule. *J Biol Chem* 1997;272:13743-13749.

246. Holmskov U, Mollenhauer J, Madsen Jet al. Cloning of gp-340, a putative opsonin receptor for lung surfactant protein D. *Proc Natl Acad Sci U S A* 1999;96:10794-10799.

247. Crouch EC, Persson A, Griffin GL, Chang D, Senior RM. Interactions of pulmonary surfactant protein D (SP-D) with human blood leukocytes. *Am J Respir Cell Mol Biol* 1995;12:410-415.

248. Cai GZ, Griffin GL, Senior RM, Longmore WJ, Moxley MA. Recombinant SP-D carbohydrate recognition domain is a chemoattractant for human neutrophils. *Am J Physiol* 1999;276:L131-136.

249. Brinker KG, Martin E, Borron Pet al. Surfactant protein D enhances bacterial antigen presentation by bone marrow-derived dendritic cells. *Am J Physiol Lung Cell Mol Physiol* 2001;281:L1453-1463.

250. Brinker KG, Garner H, Wright JR. Surfactant protein A modulates the differentiation of murine bone marrow-derived dendritic cells. *Am J Physiol Lung Cell Mol Physiol* 2003;284:L232-241.

251. Wang JY, Shieh CC, You PF, Lei HY, Reid KB. Inhibitory effect of pulmonary surfactant proteins A and D on allergen-induced lymphocyte proliferation and histamine release in children with asthma. *Am J Respir Crit Care Med* 1998;158:510-518.

252. Borron PJ, Mostaghel EA, Doyle C, Walsh ES, McHeyzer-Williams MG, Wright JR. Pulmonary surfactant proteins A and D directly suppress CD3+/CD4+ cell function: evidence for two shared mechanisms. *J Immunol* 2002;169:5844-5850.

253. Borron PJ, Crouch EC, Lewis JF, Wright JR, Possmayer F, Fraher LJ. Recombinant rat surfactant-associated protein D inhibits human T lymphocyte proliferation and IL-2 production. *J Immunol* 1998;161:4599-4603.

254. Palaniyar N, Clark H, Nadesalingam J, Hawgood S, Reid KB. Surfactant protein D binds genomic DNA and apoptotic cells, and enhances their clearance, in vivo. *Ann N Y Acad Sci* 2003;1010:471-475.

255. Clark H, Palaniyar N, Strong P, Edmondson J, Hawgood S, Reid KB. Surfactant protein D reduces alveolar macrophage apoptosis in vivo. *J Immunol* 2002;169:2892-2899.

256. Clark H, Palaniyar N, Hawgood S, Reid KB. A recombinant fragment of human surfactant protein D reduces alveolar macrophage apoptosis and pro-inflammatory cytokines in mice developing pulmonary emphysema. *Ann N Y Acad Sci* 2003;1010:113-116.

257. Malhotra R, Haurum J, Thiel S, Jensenius JC, Sim RB. Pollen grains bind to lung alveolar type II cells (A549) via lung surfactant protein A (SP-A). *Biosci Rep* 1993;13:79-90.

258. Madan T, Kishore U, Singh Met al. Surfactant proteins A and D protect mice against pulmonary hypersensitivity induced by Aspergillus fumigatus antigens and allergens. *J Clin Invest* 2001;107:467-475.

259. Wang JY, Kishore U, Lim BL, Strong P, Reid KB. Interaction of human lung surfactant proteins A and D with mite (Dermatophagoides pteronyssinus) allergens. *Clin Exp Immunol* 1996;106:367-373.

260. Madan T, Kishore U, Shah Aet al. Lung surfactant proteins A and D can inhibit specific IgE binding to the allergens of Aspergillus fumigatus and block allergen-induced histamine release from human basophils. *Clin Exp Immunol* 1997;110:241-249.

261. He Y, Crouch EC, Rust K, Spaite E, Brody SL. Proximal promoter of the surfactant protein D gene: regulatory roles of AP-1, forkhead box, and GT box binding proteins. *J Biol Chem* 2000;275:31051-31060.

262. Rust K, Bingle L, Mariencheck W, Persson A, Crouch EC. Characterization of the human surfactant protein D promoter: transcriptional regulation of SP-D gene expression by glucocorticoids. *Am J Respir Cell Mol Biol* 1996;14:121-130.

263. Mariencheck W, Crouch E. Modulation of surfactant protein D expression by glucocorticoids in fetal rat lung. *Am J Respir Cell Mol Biol* 1994;10:419-429.

264. Kerr MH, Paton JY. Surfactant protein levels in severe respiratory syncytial virus infection. *Am J Respir Crit Care Med* 1999;159:1115-1118.

265. Grubor B, Gallup JM, Ramirez-Romero Ret al. Surfactant protein D expression in normal and pneumonic ovine lung. *Vet Immunol Immunopathol* 2004;101:235-242.

266. Stahlman MT, Gray ME, Hull WM, Whitsett JA. Immunolocalization of surfactant protein-D (SP-D) in human fetal, newborn, and adult tissues. *J Histochem Cytochem* 2002;50:651-660.

267. Paananen R, Glumoff V, Hallman M. Surfactant protein A and D expression in the porcine Eustachian tube. *FEBS Lett* 1999;452:141-144.

268. Paananen R, Glumoff V, Sormunen R, Voorhout W, Hallman M. Expression and localization of lung surfactant protein B in Eustachian tube epithelium. *Am J Physiol Lung Cell Mol Physiol* 2001;280:L214-220.

269. Madsen J, Kliem A, Tornoe I, Skjodt K, Koch C, Holmskov U. Localization of lung surfactant protein D on mucosal surfaces in human tissues. *J Immunol* 2000;164:5866-5870.

270. Madsen J, Tornoe I, Nielsen O, Koch C, Steinhilber W, Holmskov U. Expression and localization of lung surfactant protein A in human tissues. *Am J Respir Cell Mol Biol* 2003;29:591-597.

271. Korfhagen TR, Sheftelyevich V, Burhans MSet al. Surfactant protein-D regulates surfactant phospholipid homeostasis in vivo. *J Biol Chem* 1998;273:28438-28443.

272. Botas C, Poulain F, Akiyama Jet al. Altered surfactant homeostasis and alveolar type II cell morphology in mice lacking surfactant protein D. *Proc Natl Acad Sci U S A* 1998;95:11869-11874.

273. Wert SE, Yoshida M, LeVine AMet al. Increased metalloproteinase activity, oxidant production, and emphysema in surfactant protein D gene-inactivated mice. *Proc Natl Acad Sci U S A* 2000;97:5972-5977.

274. Zhang L, Ikegami M, Dey CR, Korfhagen TR, Whitsett JA. Reversibility of pulmonary abnormalities by conditional replacement of surfactant protein D (SP-D) in vivo. *J Biol Chem* 2002;277:38709-38713.

275. Fisher JH, Sheftelyevich V, Ho YSet al. Pulmonary-specific expression of SP-D corrects pulmonary lipid accumulation in SP-D gene-targeted mice. *Am J Physiol Lung Cell Mol Physiol* 2000;278:L365-373.

276. Hirche TO, Crouch EC, Espinola Met al. Neutrophil serine proteinases inactivate surfactant protein D by cleaving within a conserved subregion of the carbohydrate recognition domain. *J Biol Chem* 2004;279:27688-27698.

277. Griese M, Wiesener A, Lottspeich F, von Bredow C. Limited proteolysis of surfactant protein D causes a loss of its calcium-dependent lectin functions. *Biochim Biophys Acta* 2003;1638:157-163.

278. von Bredow C, Wiesener A, Griese M. Proteolysis of surfactant protein D by cystic fibrosis relevant proteases. *Lung* 2003;181:79-88.

279. Dutton JM, Goss K, Khubchandani KR, Shah CD, Smith RJ, Snyder JM. Surfactant protein A in rabbit sinus and middle ear mucosa. *Ann Otol Rhinol Laryngol* 1999;108:915-924.

280. Lee HM, Kang HJ, Woo JS, Chae SW, Lee SH, Hwang SJ. Upregulation of surfactant protein A in chronic rhinosinusitis. *Laryngoscope* 2006;116:328-330.

281. Pant H, Kette FE, Smith WB, Macardle PJ, Wormald PJ. Eosinophilic mucus chronic rhinosinusitis: clinical subgroups or a homogeneous pathogenic entity? *Laryngoscope* 2006;116:1241-1247.

282. Simmaco M, Boman A, Mangoni MLet al. Effect of glucocorticoids on the synthesis of antimicrobial peptides in amphibian skin. *FEBS Lett* 1997;416:273-275.

283. Wallwork B, Coman W, Feron F, Mackay-Sim A, Cervin A. Clarithromycin and prednisolone inhibit cytokine production in chronic rhinosinusitis. *Laryngoscope* 2002;112:1827-1830.

284. Wright ED, Frenkiel S, Al-Ghamdi Ket al. Interleukin-4, interleukin-5, and granulocyte-macrophage colony-stimulating factor receptor expression in chronic sinusitis and response to topical steroids. *Otolaryngol Head Neck Surg* 1998;118:490-495.

285. Saunders MW, Wheatley AH, George SJ, Lai T, Birchall MA. Do corticosteroids induce apoptosis in nasal polyp inflammatory cells? In vivo and in vitro studies. *Laryngoscope* 1999;109:785-790.

286. Hissaria P, Smith W, Wormald PJet al. Short course of systemic corticosteroids in sinonasal polyposis: a double-blind, randomized, placebo-controlled trial with evaluation of outcome measures. *J Allergy Clin Immunol* 2006;118:128-133.

287. Shin SH, Ponikau JU, Sherris DAet al. Chronic rhinosinusitis: an enhanced immune response to ubiquitous airborne fungi. *J Allergy Clin Immunol* 2004;114:1369-1375.

288. Downs SH, Mitakakis TZ, Marks GBet al. Clinical importance of Alternaria exposure in children. *Am J Respir Crit Care Med* 2001;164:455-459.

289. Bals R, Weiner DJ, Meegalla RL, Wilson JM. Transfer of a cathelicidin peptide antibiotic gene restores bacterial killing in a cystic fibrosis xenograft model. *J Clin Invest* 1999;103:1113-1117.

290. Nell MJ, Grote JJ. Effects of bacterial toxins on air-exposed cultured human respiratory sinus epithelium. *Ann Otol Rhinol Laryngol* 2003;112:461-468.

291. Nell MJ, Sandra Tjabringa G, Vonk MJ, Hiemstra PS, Grote JJ. Bacterial products increase expression of the human cathelicidin hCAP-18/LL-37 in cultured human sinus epithelial cells. *FEMS Immunol Med Microbiol* 2004;42:225-231.

292. Islam D, Bandholtz L, Nilsson Jet al. Downregulation of bactericidal peptides in enteric infections: a novel immune escape mechanism with bacterial DNA as a potential regulator. *Nat Med* 2001;7:180-185.

293. Lopez-Garcia B, Lee PH, Yamasaki K, Gallo RL. Anti-fungal activity of cathelicidins and their potential role in Candida albicans skin infection. *J Invest Dermatol* 2005;125:108-115.

294. Wormald PJ, Cain T, Oates L, Hawke L, Wong I. A comparative study of three methods of nasal irrigation. *Laryngoscope* 2004;114:2224-2227.

295. Homer JJ, England RJ, Wilde AD, Harwood GR, Stafford ND. The effect of pH of douching solutions on mucociliary clearance. *Clin Otolaryngol Allied Sci* 1999;24:312-315.

296. Chen J, Falla TJ, Liu Het al. Development of protegrins for the treatment and prevention of oral mucositis: structure-activity relationships of synthetic protegrin analogues. *Biopolymers* 2000;55:88-98.

297. Mosca DA, Hurst MA, So W, Viajar BS, Fujii CA, Falla TJ. IB-367, a protegrin peptide with in vitro and in vivo activities against the microflora associated with oral mucositis. *Antimicrob Agents Chemother* 2000;44:1803-1808.

298. Bals R, Weiner DJ, Moscioni AD, Meegalla RL, Wilson JM. Augmentation of innate host defense by expression of a cathelicidin antimicrobial peptide. *Infect Immun* 1999;67:6084-6089.

299. Postle AD, Mander A, Reid KBet al. Deficient hydrophilic lung surfactant proteins A and D with normal surfactant phospholipid molecular species in cystic fibrosis. *Am J Respir Cell Mol Biol* 1999;20:90-98.

300. Leth-Larsen R, Nordenbaek C, Tornoe Iet al. Surfactant protein D (SP-D) serum levels in patients with community-acquired pneumonia. *Clin Immunol* 2003;108:29-37.

301. Gardai SJ, Xiao YQ, Dickinson Met al. By binding SIRPalpha or calreticulin/CD91, lung collectins act as dual function surveillance molecules to suppress or enhance inflammation. *Cell* 2003;115:13-23.

302. Akiyama J, Hoffman A, Brown Cet al. Tissue distribution of surfactant proteins A and D in the mouse. *J Histochem Cytochem* 2002;50:993-996.

303. Grubor B, Gallup JM, Meyerholz DKet al. Enhanced surfactant protein and defensin mRNA levels and reduced viral replication during parainfluenza virus type 3 pneumonia in neonatal lambs. *Clin Diagn Lab Immunol* 2004;11:599-607.

304. Clark H, Reid K. The potential of recombinant surfactant protein D therapy to reduce inflammation in neonatal chronic lung disease, cystic fibrosis, and emphysema. *Arch Dis Child* 2003;88:981-984.

305. Strong P, Reid KB, Clark H. Intranasal delivery of a truncated recombinant human SP-D is effective at down-regulating allergic hypersensitivity in mice sensitized to allergens of Aspergillus fumigatus. *Clin Exp Immunol* 2002;130:19-24.

306. Schaub B, Westlake RM, He Het al. Surfactant protein D deficiency influences allergic immune responses. *Clin Exp Allergy* 2004;34:1819-1826.

307. Pan T, Nielsen LD, Allen MJet al. Serum SP-D is a marker of lung injury in rats. *Am J Physiol Lung Cell Mol Physiol* 2002;282:L824-832.

308. Dulkerian SJ, Gonzales LW, Ning Y, Ballard PL. Regulation of surfactant protein D in human fetal lung. *Am J Respir Cell Mol Biol* 1996;15:781-786.

309. Wang JY, Yeh TF, Lin YC, Miyamura K, Holmskov U, Reid KB. Measurement of pulmonary status and surfactant protein levels during dexamethasone treatment of neonatal respiratory distress syndrome. *Thorax* 1996;51:907-913.

Appendix

Please note: This page is blank in print copies