The Environment and the Host in Chronic Rhinosinusitis

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This thesis is dedicated to those who have sacrificed the most during my scientific endeavors

My amazing family

Julia, Thomas & Will

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Thesis declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any university or 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.

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Dr Sam Boase

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Publications arising from this thesis

The microbiome of chronic rhinosinusitis: culture, molecular diagnostics and biofilm detection

Boase, S., Foreman, F., Tan, L.W., Melton-Kreft, R., Pant, H., Hu, F.Z., Ehrlich, G.D., Wormald, P.J.

BMC Infectious Diseases (in review)

A sheep model to investigate the role of fungal biofilms in sinusitis: fungal and bacterial synergy

Boase, S., Valentine, R., Singhal, D., Tan, L. W., Wormald, P.J.

International Forum of Allergy & Rhinology 2011, 1 (5): 340-347

Bacterial induced cilia damage promotes fungal biofilm formation in a sheep model of sinusitis.

Boase, S., Jervis-Bardy, S., Cleland, C., Pant, H., Tan, L.W., Wormald, P.J.

International Forum of Allergy & Rhinology (in review)

Microorganisms and host immunoglobulin E responses in chronic rhinosinusitis: *Staphylococcus aureus* potentiates inhalant aeroallergen sensitization.

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A model to investigate the role of fungal biofilms in chronic rhinosinusitis

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Fungal biofilm formation in sinusitis: fungal & bacterial interactions in the sheep model of sinusitis.

The Queen Elizabeth Hospital Research Day

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The aetiopathogenesis of CRS

14th Advanced Functional Endoscopic Sinus Surgery Course

Adelaide, November 2011

CRS: microorganisms and the host

The Australian Society of Otolaryngology Head & Neck Surgery Scientific Meeting (SA), Adelaide, November 2011.

Microorganisms and the host in Chronic Rhinosinusitis: Making the link. The Royal Australian College of Surgeons (SA) Annual Scientific Meeting: The RP Jepson Medal, Adelaide, November 2012.

Abbreviations

A. alternata	*Alternaria alternata*
A. flavus	*Aspergillus flavus*
A. fumigatus	*Aspergillus fumigatus*
Aa	*Alternaria alternata*
ABPA	Allergic bronchopulmonary Aspergillosis
AD	Atopic dermatitis
Af	*Aspergillus fumigatus*
AFRS	Allergic fungal rhinosinusitis
AIDS	Acquired immunodeficiency syndrome
APC	Antigen presenting cell
AR	Allergic rhinosinusitis
ARS	Acute rhinosinusitis
ATCC	American Type Culture Collection
BAFF	B cell activating factor
C. albicans	*Candida albicans*
cAMP	3'-5'-cyclic adenosine monophosphate
CAZS	Citric acid zwitterionic surfactant
CD	Cluster of differentiation
CF	Cystic fibrosis
CHIPS	Chemotaxis inhibitory protein of *S. aureus*
CNS	Coagulase-negative *staphylococci*
COPD	Chronic obstructive pulmonary disease

CRS	Chronic rhinosinusitis
CRSsNP	Chronic rhinosinusitis without nasal polyps
CRSwNP	Chronic rhinosinusitis with nasal polyps
CSF	Cerebrospinal fluid
CT	Computed Tomography
CVID	Common variable immunodeficiency
DC	Dendritic cell
DGGE	Denaturing gradient gel electrophoresis
DNA	Deoxyribonucleic acid
ECP	Eosinophilic cationic protein
EM	Eosinophilic mucus
ESI	Electrospray ionisation
ESS	Endoscopic sinus surgery
FISH	Fluorescence *in situ* hybridisation
GM-CSF	Granulocyte macrophage colony stimulating factor
H. influenzae / HI	*Haemophilus influenzae*
H&E	Haematoxylin and Eosin
HLA	Human leukocyte antigen
IgE	Immunoglobulin E
IL	Interleukin
IQR	Interquartile range
IT	Immunotherapy
L-M	Lund-Mackay
MHC	Major histocompatibility complex
MRI	Magnetic resonance imaging

MS	Mass spectroscopy
OR	Odds Ratio
P. aeruginosa	*Pseudomonas aeruginosa*
P. chrysogenum	*Penicillium chrysogenum*
PA	*Pseudomonas aeruginosa*
PAMPs	Pathogen-associated molecular patterns
PCD	Primary ciliary dyskinesia
PNAG	poly-N-acetylglucosamine
RCT	Randomised controlled trial
RNA	Ribonucleic acid
ROS	Reactive oxygen species
rRNA	Ribosomal ribonucleic acid
S. aureus	*Staphylococcus aureus*
S. epidermidis	*Staphylococcus epidermidis*
SA	*Staphylococcus aureus*
SAE	*Staphylococcus aureus* enterotoxin
SAg	Superantigen
SCIN	*Staphylococcal* complement inhibitor
SCV	Small colony variants
SD	Standard deviation
SE	*Staphylococcus epidermidis*
SEA	*Staphylococcus aureus* enterotoxins A
SEB	*Staphylococcus aureus* enterotoxins B
SEC	*Staphylococcus aureus* enterotoxins C
SEM	Scanning electron microscopy

SNOT-20	Sino-nasal Outcomes Test 20
TCR	T cell receptor
TEM	Transmission electron microscopy
Th	T helper lymphocyte
TLRs	Toll-like receptors
TOF	Time of flight
T_{reg}	Regulatory T lymphocyte
TSLP	Thymic stromal lymphopoietin
TSST	Toxic Shock Syndrome Toxin
Vβ	Variable β

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Thesis summary

The research described herein follows an extensive literature review of the role of environmental agents and the host immune system in the manifestation of CRS. There are clear deficiencies in our understanding regarding the microbial flora of CRS patients and non-diseased sinuses. Chapter two describes a detailed study of the fungal and bacterial microbiome of diseased and healthy sinuses and forms a basis on which to build the subsequent research projects. The third and fourth chapters describe the development of two animal models to determine the environmental and host factors, which are associated with sinonasal fungal biofilm formation. The final chapter seeks to determine the relevance of sinonasal microorganisms by detecting them on host surfaces and correlating these with specific host immune responses. The interaction of bacteria and host hypersensitivities to allergens is also explored.

The initial investigation focused on understanding the microbial flora in CRS patients. This study forms a foundation for the thesis, and was critical to address the many deficiencies and contradictions in the published literature regarding the microbiome of CRS patients. We used state of the art microbial detection techniques to determine the presence and abundance of fungi and bacteria on the mucosa of CRS patients, and appropriate healthy control mucosa. This highlighted some cornerstones of microbial variability between healthy and diseased sinuses. We have shown that the healthy sinus is clearly not sterile, and that prevalence, but more importantly, species composition and population

density are critical factors in determining the disease state. Comparisons between various detection techniques such as molecular analysis, Fluorescence *in situ* hybridization (FISH), and conventional culture showed FISH to be highly sensitive and specific, with a detection threshold related to organism abundance, whereas culture has a tendency to select for rapidly growing organisms.

The subsequent study is detailed in chapter three, and addresses two of the most contentious, environment versus host issues in the CRS research community – the interaction between fungal organisms, and the host with type I hypersensitivity to fungi. We developed a large animal (sheep) model of fungal sinusitis to investigate these factors and successfully sensitized 45% of animals to fungal antigens, as evidenced by positive skin prick tests. Despite the presence of fungal hypersensitivity, we were unable to produce fungal biofilms in the occluded frontal sinus. Following our clinical observations of fungi frequently co-habiting with bacteria, particularly *Staphylococcus aureus,* we co-inoculated fungi with this bacterium and florid fungal biofilm formed on sinus mucosa. Type I hypersensitivity to fungi had no correlation with fungal biofilm or inflammation. These results suggested that fungi may not be able to form biofilm on mucosa with intact immune defences and a primary insult from the bacteria was requisite for fungal adhesion and proliferation.

A follow up study addressed the factors, which contribute to fungal biofilm establishment on sinus mucosa. An animal model was again developed to determine if co-inoculation of fungi with other bacterial species would allow fungi to proliferate. Four bacterial species commonly detected in CRS patients were

utilized. We hypothesized that bacterial induced cilial injury may have a role in allowing early fungal adhesion, and a cilia toxin was utilized to assess the effect of isolated cilial impairment on fungal proliferation. Cilia were assessed using transmission electron microscopy. Again, no fungal biofilm formed when fungi was inoculated in isolation. Three of the bacterial species formed bacterial biofilms in >75% of sinuses, and this was associated with significant cilial damage, and fungal biofilm formation. One of the bacterial species did not form biofilm, and no fungal biofilm formed in co-inoculated sinuses. Cilia toxin caused significant cilial injury, and was also associated with fungal proliferation. This study demonstrates the importance of the physiochemical barrier in defence against fungal organisms. This led to the question of the role of fungi in CRS patients – are they contributing to the inflammation or merely saprophytic colonizers of the impaired mucosa?

The final study addressed this question in a human subject cohort. To determine if microorganisms have a role in inflammatory processes, we need to be able to display an organism specific immune response in the host. We measured the organism specific IgE levels in the serum and mucosa of 48 CRS patients and 10 controls. We also determined the presence of these microorganisms on the mucosa using conventional culture, and FISH using specific probes. We showed that in CRSwNP patients, the presence of *S. aureus* and fungi on the mucosa was related to elevated organism specific IgE within the mucosa. This phenomenon was specific to nasal polyp patients, and was not observed in non-polyp CRS or control patients. This demonstrates that these organisms have the capacity to incite specific immune responses in the host, potentially contributing

to mucosal inflammation in CRS. Additionally we determined that the presence of *S. aureus* on the mucosa also exacerbates mucosal fungal allergy, potentially enhancing hypersensitivity to ubiquitous airborne fungal allergens. Although this mechanism has been observed in other atopic diseases, this is the first study to document the phenomenon in CRS. It adds to the mounting evidence that *S. aureus* has an important role in the pathogenesis of CRS.