

# THE INNATE IMMUNE RESPONSE TO STAPHYLOCOCCUS AUREUS BIOFILMS ON HUMAN SINONASAL EXPLANTS

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THE UNIVERSITY  
*of* ADELAIDE

“We shall not cease from exploration,  
and the end of all our exploring will be  
to arrive where we started  
and to know the place for the first time.”

T.S. Elliot (1888-1965)

*Dedicated to my beloved wife Renata who first crossed the Andes  
Mountain Range from Brazil to be with me in Chile and now has crossed  
The Pacific Ocean to accompany me in Australia*

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## Abstract

Chronic Rhinosinusitis (CRS) is the persistent and symptomatic inflammation of the mucosa of the nose and paranasal cavities. It is a prevalent condition severely affecting the quality of life of around 10% of the population in Western countries. Its pathogenesis involves environmental factors such as viruses or bacteria on predisposed hosts triggering local mucosal inflammation. *Staphylococcus aureus* (*S. aureus*) is the most common isolated bacterium in CRS and, when forming biofilms, increases its resistance to antibiotics, being correlated with recalcitrant cases and higher rates of mucosal inflammation. The local inflammation can be explained by virulence factors from *S. aureus*, and also by innate and adaptive immune mechanisms of the host immune response. Although some researchers have explored the late or adaptive immune response associated with *S. aureus* biofilms, less is known about the initial or innate immune response that *S. aureus* biofilms trigger in the mucosa.

This thesis aimed to study part of the mucosal innate immune response to *S. aureus* biofilms. We have challenged human sinonasal tissues—from normal donors undergoing transnasal pituitary surgery—with *S. aureus* biofilms *ex vivo* using an explant model. This model mimics *in vivo* conditions because it allows biofilms to grow at the air-liquid interface. Also, the biofilm-mucosa interaction is more physiological than primary cell cultures, because the communication between different host cells is preserved in the explant model. After the interaction with *S. aureus* biofilms, explant tissues produced IL-6 and other cytokines polarised to a Th1/Th17 type of immune response. The observed Th1/Th17 immune response differs from previous reports in eosinophilic CRS patients with nasal polyps (CRSwNP) showing a predominant Th2 response. Apparently there is an evolution from early Th1/Th17 immune responses to late Th2 in *S. aureus* biofilm associated infections. The turning point between these two types of immune responses seems critical in CRS because it could explain the origin of the Th2 inflammation. In the

future, the use of long-term animal models could help to illustrate the progression from an initial Th1/Th17 to a late Th2 type of immune response in the sinonasal mucosa.

*S. aureus* biofilms also induced apoptosis in the mucosa as demonstrated by the up-regulation of cleaved caspase-3 in our settings. We also demonstrated the induction and activation of the Nod2 receptor and downstream pathway secondary to *S. aureus* biofilms. The Nod2 receptor recognises a small portion of peptidoglycan that is available during early phases of *S. aureus* biofilm formation. The role of Nod2 in CRS and biofilm infections should be evaluated in future studies.

In conclusion, we demonstrated that early *S. aureus* biofilms induce a proinflammatory response in the sinonasal mucosa. This proinflammatory response seems to be crucial for biofilm attachment and persistence, and its modulation could represent an alternative to prevent *S. aureus* biofilm infections. Secreted staphylococcal products such as alpha ( $\alpha$ ) toxin and staphylococcal protein A (SpA) are two virulence factors critical during early biofilm growth. These proteins are also able to generate immune responses and represent targets for potential therapeutic intervention during biofilm infections in the sinonasal mucosa.

## **Statement**

I certify that this thesis contains no material accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge, contains no material previously published or written by another person, except the properly referenced citations. I also testify that no part of this work will be submitted for any other degree or diploma in any other university or tertiary institution without the previous approval of the University of Adelaide.

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Daniel Anibal Cantero Cajas

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## **Publications generated during the candidature**

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## **Presentations during the candidature**

### **Department Presentation, TQEH, Adelaide, August 2011**

“The host immune response to *S. aureus* biofilm”

### **BHI Postgraduate Seminar, Adelaide, September 2011**

Research Proposal: “The immune response to *S. aureus* biofilms”

### **Department Presentation, TQEH, Adelaide, February 2012**

“Antibacterial Response to *S. aureus* biofilms on sinus mucosa”

### **Research Day BHI, Adelaide, October 2012:**

“Characterization of the acute inflammatory response to *Staphylococcus aureus* biofilm on a human sinonasal explant model.”

### **BHI Postgraduate Seminar, Adelaide, November 2012**

“Initial Immune response of Explant tissues to *S. aureus* biofilms.”

### **Department Presentation, TQEH, Adelaide, November 2012**

“*Staphylococcus aureus*: Host bacterium interactions in the sinonasal mucosa. Update and future perspectives”

### **Presentation at the University of Adelaide, Discipline of Surgery Research Dinner, May 2013.**

“Early inflammatory Response to *Staphylococcus aureus* biofilm on a human nasal explant model.”

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“Innate immune response to *S. aureus* biofilms on sinonasal tissue explants.”

## List of Abbreviations used in this thesis

**ACP:** Antrochoanal Polyp

**ADAM10:** A disintegrin and metalloproteinase domain-containing protein 10

**ADEPs:** Acyldepsipeptides

**AERD:** Aspirin-Exacerbated Respiratory Disease

**AFRS:** Allergic Fungal Rhinosinusitis

**AIM2:** Absent in melanoma protein 2

**AIP:** Auto inducing peptide

**AKT1:** V-akt murine thymoma viral oncogene homolog 1

**AMPs:** Antimicrobial peptides

**APCs:** Antigen Presenting Cells

**$\alpha$ -toxin:** Alpha toxin or alpha haemolysin

**BAI:** Biofilm Associated Infections

**$\beta$ -haemolysin:** beta haemolysin or beta toxin

**BPE:** Bovine Pituitary Extract

**CA-MRSA:** Community-acquired MRSA

**Can:** Collagen binding surface protein

**CASP1:** Caspase 1

**CASP3:** Caspase 3

**CBD:** Calgary Biofilm Device

**CCL2:** Chemokine (C-C) ligand 2

**CCL5:** Chemokine (C-C motif) ligand 5 (RANTES)

**cdNA:** Complementary DNA

**CHIPS:** Chemotaxis inhibitory protein of staphylococci

**CF:** Cystic Fibrosis

**CFTR:** Cystic Fibrosis Trans-membrane Conductance Regulator

**CFU:** Colony Forming Unit

**CifA:** Clumping factor A

**CifB:** Clumping factor B

**CLRs:** C-type Lectin Receptors

**CpG:** Cytosine-phosphate-Guanine. Areas of DNA where these sequences occur.

**CRS:** Chronic Rhinosinusitis

**CRSsNP:** Chronic Rhinosinusitis Without Nasal Polyps

**CRSwNP:** Chronic Rhinosinusitis With Nasal Polyps

**COPD:** Chronic Obstructive Pulmonary Disease

**CSF:** Cerebrospinal Fluid

**CSLM:** Confocal Scanning Laser Microscope

**CT:** Computer Tomography

**CVID:** Common Variable Immune Deficiency

**CXCL1:** Chemokine (C-X-C) motif ligand 1

**CXCL2:** Chemokine (C-X-C) motif ligand 2

**Cy3:** Cyanine dye number 3

**C5aR:** C5a receptor

**DAB:** Diaminobenzidine

**DAP:** Diaminopimelic acid

**DAPI:** 4', 6-diamidino-2-phenylindole

**DCs:** Dendritic Cells

**DMEM:** Dulbecco's Modified Eagle's Medium

**dsRNA:** Double-Stranded RNA

**dsDNA:** Double-Stranded DNA

**δ toxin:** Delta toxin or delta haemolysin

**ECP:** Eosinophilic Cationic Protein

**eDNA:** extracellular DNA

**EGFR:** Epidermal growth factor receptor

**ELISA:** Enzyme-Linked Immunosorbent Assay

**EM:** Eosinophilic Mucus (Mucin)

**EPOS:** European Position paper on Rhinosinusitis and Nasal Polyps

**EP(3)OS:** European Position paper on Rhinosinusitis and Nasal Polyps, 1997

**EPS:** Extracellular Polymeric Substances

**ESS:** Endoscopic Sinus Surgery

**Fab:** Antigen-binding fragment

**FACS:** Fluorescence-activated cell sorting

**FADD:** Fas (TNFRSF6)-associated via death domain

**Fc:** Fragment crystallizable or gamma fraction of immunoglobulins

**FOXP3:** Forkhead box P3

**FPR:** Formyl peptide receptor

**FnbpA:** Fibronectin binding protein A

**FnbpB:** Fibronectin binding protein B

**GATA-3:** Transcription factor differentiating to Th2 responses. GATA is a family of

transcription factors. "GATA" represents a DNA sequence that these proteins bind.

**G-CSF:** Granulocyte Colony Stimulating Factor (CSF3)

**GM-CSF:** Granulocyte Macrophage Colony Stimulating Factor (CSF2)

**γ hemolysin:** Gamma haemolysin or gamma toxin

**HAMsF12:** Ham's nutrient mixture number 12

**HE:** Hematoxylin and Eosin

**H1a:** Alpha haemolysin

**H1g:** Gamma hemolysin

**ICD-9:** Ninth Revision codes

**IFN-γ:** Interferon gamma

**Ig:** Immunoglobulin

**IgE:** Immunoglobulin E

**IgG:** Immunoglobulin G

**IHC:** Immunohistochemistry

**IL-1:** Interleukin 1

**IL-1β:** Interleukin 1 beta

**IL-2:** Interleukin 2

**IL-4:** Interleukin 4

**IL-5:** Interleukin 5

**IL-6:** Interleukin 6

**IL-7:** Interleukin 7

**IL-8:** Interleukin 8 (or CXCL8)

**IL-10:** Interleukin 10

**IL-12:** Interleukin 12

**IL-13:** Interleukin 13

**IL-17:** Interleukin 17

**IL-17A:** Interleukin 17A

**IL-17C:** Interleukin 17C

**ILC:** Innate Lymphoid Cell

**INCSs:** Intranasal corticosteroids

**IRF5:** Interferon regulatory factor 5

**IRF7:** Interferon regulatory factor 7

**IPAF:** Ice protease-activating factor

**INCSs:** Intranasal corticosteroids

**ITF:** Insulin Transferrin Selenium

**LPS:** Lipopolysaccharide

**LAS AF:** Leica Application Suite Advance Fluorescence Software

**LTA:** Lipoteichoic acid

**MAPK3:** Mitogen-Activated Protein Kinase 3

**MASP-1:** Mannose Associated Serine Protease 1

**MASP-2:** Mannose Associated Serine Protease 2

**MBL:** Mannose Binding Lectin

**MCP-1 (MCAF):** Monocyte Chemoattractant Protein 1

**MDP:** Muramyl Dipeptide

**MDSCs:** Myeloid-Derived Suppressor Cells

**MFI:** Mean Fluorescence Intensity

**MFU:** Mc Farland Unit

**MIC:** Minimum Inhibitory Concentration

**MIP-1 $\beta$** : Macrophage Inflammatory protein 1 $\beta$

**MLST**: Multilocus Sequence Typing

**mRNA**: Messenger RNA

**MRSA**: Methicillin-resistant *S. aureus*

**MSCRAMM**: Microbial Surface Components Recognising Adhesive Matrix Molecules

**MyD88**: Myeloid Differentiation primary response gene (88)

**NADPH**: Nicotinamide Adenine Dinucleotide Phosphate (reduced form)

**NALT**: Nasal Associated Lymphoid Tissue

**NCBI**: National Centre for Biotechnology Information

**NF $\kappa$ B**: Nuclear factor kappa light chain enhancer of activated B cells

**NF $\kappa$ B1**: Subunit 1 of NF $\kappa$ B. The gene encodes a 105 kD protein

**NLRs**: Nucleotide binding and oligomerization domain (NOD)-like receptors

**NLRP1**: NOD-, LRR- and pyrin domain-containing 1

**NLRP3**: NOD-, LRR- and pyrin domain-containing 3

**NO**: Nitric Oxide

**Nod1**: Nucleotide-binding Oligomerization Domain containing 1 protein

**Nod2**: Nucleotide-binding Oligomerization Domain containing 2 protein

**NSAID**: Non-steroidal anti-inflammatory

**ODN**: Oligodeoxynucleotides

**OME**: Otitis Media with Effusion

***P. aeruginosa***: *Pseudomonas aeruginosa*

**PAMPs**: Pathogen Associated Molecular Patterns

**PBS**: Phosphate-Buffer Saline Solution

**PBP2a**: Penicillin Binding Protein 2a

**PCR:** Polymerase Chain Reaction

**PGN:** Peptidoglycan

**PIA:** Polysaccharide Intercellular Adhesin

**PMN:** Polymorphonuclear

**PNAG:** Poly-N-Acetylglucosamine

**PRRs:** Pattern Recognition Receptors

**PROMS:** Patient Reported Outcome Measures

**PSM:** Phenol soluble modulins

**PVL:** Panton Valentine leukocidin

**qRT-PCR:** Quantitative Reverse-Transcriptase Polymerase Chain Reaction

**RIPK2:** Receptor-interacting serine/threonine-protein kinase 2

**RLRs:** Retinoic Acid-Inducible Gene Receptors

**ROR- $\gamma$ t:** RAR-related orphan receptor gamma

***S. aureus:*** *Staphylococcus aureus*

**SAGs:** Superantigens

**SCCmec:** Staphylococcal chromosome cassette *mec*

**SCV:** Small Colony Variants

**SDHA:** Succinate Dehydrogenase Complex, subunit A

**SEA:** Staphylococcus enterotoxin A

**SEB:** Staphylococcus enterotoxin B

**SEC:** Staphylococcus enterotoxin C

**SED:** Staphylococcus enterotoxin D

**SEE:** Staphylococcus enterotoxin E

**SEG:** Staphylococcus enterotoxin G

**SEM:** Scanning Electron Microscopy

**SET:** Staphylococcus enterotoxin T

**SEIs:** Staphylococcal enterotoxin-like proteins

**SEIj:** Staphylococcal enterotoxin-like protein J

**SEIV:** Staphylococcal enterotoxin-like protein V

**SLE:** Systemic Lupus Erythematosus

**SLPI:** Secretory leukocyte proteinase inhibitor

**SNOT:** Sino-Nasal Outcome Test

**SpA:** *Staphylococcus aureus* protein A

**SP-D:** Surfactant protein D

**ssRNA:** Single-Stranded RNA

**STAT3:** Activator of Transcription 3

**Tc:** T cytotoxic cell

**T-bet:** T-box transcription factor differentiating to Th1 responses

**TCR:** T cell receptor

**TEM:** Transmission Electron Microscopy

**TGF- $\beta$ 1:** Transforming growth factor beta 1

**Th:** T helper cell

**Th1:** T helper cell 1

**Th2:** T helper cell 2

**Th17:** T helper cell 17

**TIR:** Toll/Interleukin (IL)-1 receptor

**TLRs:** Toll Like Receptors

**TLR2:** Toll Like Receptors number 2

**TLR3:** Toll Like Receptors number 3

**TNF:** Tumor Necrosis Factor (formerly known as TNF-alpha)

**TNF- $\beta$ :** Tumor Necrosis Factor beta

**TNFR1:** Tumor necrosis factor receptor 1

**TSST:** Toxic Shock Syndrome Toxin

**Treg:** T regulatory cells

**VAS:** Visual analogue scores

**VRSA:** Vancomycin Resistant Staphylococcus Aureus

**vWF:** Von Willebrand factor

**USA:** United States of America

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