Factors responsible for the high rate of kidney disease in Indigenous Australians: a multifaceted approach focusing on streptococcal disease

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October 2007

for the degree of Doctor of Philosophy in the

School of Public Health, Tropical Medicine and Rehabilitation Sciences at James Cook University

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DECLARATION

I declare that this thesis is my own work and has not been submitted in any form for another degree or diploma at any university or other institution of tertiary education. Information derived from the published or unpublished work of others has been acknowledged in the text and a list of references is given.

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DECLARATION ON ETHICS

The research presented and reported in this thesis was conducted within the guidelines for research ethics outlined in the National Statement on Ethics Conduct in Research Involving Humans (1999), the Joint NHMRC/AVCC Statement and Guidelines on Research Practice (1997), the James Cook University Policy on Experimentation Ethics; Standard Practices and Guidelines (2001), and the James Cook University Statement and Guidelines on Research Practice (2001). The proposed research methodology received clearance from the James Cook University Experimentation Ethics Review Committee (approval numbers A1037, A1017, A847-03, A1038, A1035, H2394) and The Townsville Hospital District Ethics Committee (03/04).

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Virginia Boon 30/09/2007

STATEMENT ON CONTRIBUTION OF OTHERS

I would like to acknowledge the financial support of Kidney Health Australia for providing a Biomedical Scholarship to support the research. I would also like to acknowledge the financial support of the Queensland Government with the funding of a Smart State program grant.

Additionally, I would like to acknowledge the contribution of Dr Ted Donelan for collecting the canine samples in the two Indigenous communities and Dr George Kan and Loretta Woodford for collecting the blood for Chapter 8.

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Virginia Boon 30/09/2007

ACKNOWLEDGMENTS

Firstly, I would like to thank my supervisors Rick Speare and Beverley Glass. Together they allowed me to explore my own research ideas and yet gave me the support to continue and complete this research. I would also like to thank George Kan for his support regarding the clinical research and Ted Donelan for facilitating the collection of animal swabs. I am also very grateful for the microbiological expertise provided by Annette Thomas and Judy Faulkner and the friendly staff at the Oonoonba Veterinary laboratories.

I am also very grateful to Jim Burnell and everyone in the annex for providing me with research space, laboratory advice and friendship. Thanks to everyone for putting up with those red plates and teaching me biochemistry! I would also like to thank Alan Baxter and the Baxter lab for helping me to conduct my animal experiments and particularly to Roby for always bleeding my mice for me.

I couldn't have finished this thesis if it wasn't for the constant support and motivation from Jim. This is his third PhD (his own, the house and now mine!) and I think he can't wait to be rid of it!

I would also like to thank the Boon clan, Mum, Dad, Syvi and John for supporting me through this PhD and even from the small beginnings of 'free choice'….

ABSTRACT

Indigenous communities in rural Australia have high rates of streptococcal disease including post-streptococcal glomerulonephritis (PSGN). The epidemiology of PSGN and streptococcal infection and the pathogenesis of streptococcal disease in these lower socio-economic communities is complex and not well understood. To gain an understanding of some aspects of this problem, this study tested several hypotheses. The major hypothesis underlying this project was that streptococci isolated from these communities will produce particular virulence factors (proteins) that will cause PSGN and that this can be demonstrated both in an animal model and in individuals with end-stage renal failure (ESRF). A second hypothesis was that dogs in these communities will play a role in the epidemiology of human streptococcal disease. These hypotheses gave rise to three objectives. Firstly; to determine if dogs residing in Indigenous communities carry streptococci that may be implicated in the high rate of streptococcal disease in the human residents of these communities. Secondly; to develop a murine model of PSGN to investigate the role of streptococcal inhibitor of complement mediated cell lysis (SIC) and the genetics of host in the pathogenesis of PSGN. Thirdly; to determine the association between elevated streptococcal antibody levels in patients to several streptococcal virulence factors and the presence of ESRF.

The first objective was achieved by sampling dogs residing in two geographically distinct Australian Indigenous rural communities to reduce the possibility of location bias. Both pharyngeal and skin swabs were obtained from the study animals. Utilising routine microbiological tests and culturing methods, presumptive streptococcal species were isolated. Following this, the isolate was identified to the species level with biochemical tests and Lancefield serological grouping was also performed. The study isolated streptococcal species of Lancefield's group A, B, C, D, G and L from the dogs. The specific species isolated were *Streptococcus orisratti* (Lancefield group A), *Streptococcus dysgalactiae* subsp. *equisimilis* (Lancefield's group A, C, G or L), *Streptococcus agalactiae* (Lancefield's group B), *Streptococcus dysgalactiae* subsp. *dysgalactiae* (Lancefield's group C), *Streptococcus equi* subsp. *equi* (Lancefield's group C), *Streptococcus canis* (Lancefield's group G), *Streptococcus bovis I* (Lancefield's group D), *Streptococcus minor* (no Lancefield's group identified) and *Streptococcus suis sp.* (no Lancefield's group identified) . In this study the fact that the two species that carried the Lancefield's group A antigen were not biochemically identified as *Streptococcus pyogenes* highlights the need to assign species based on biochemical tests and not rely solely on serological grouping systems. The overall prevalence of pharyngeal and skin carriage (including the following genera's: *Streptococcus, Enterococcus, Lactococcus and Aerococcus*) was 47 and 10.8%, respectively.

Furthermore, in the current study, ten strains of large-colony forming beta-haemolytic *S. dysgalactiae* subsp. *equisimilis* were identified from the pharynx of dogs which were taxonomically identical to 'human strains'. This species of Lancefield group C and G are important human pathogens that share virulence factors with *S. pyogenes* and pharyngeal carriage of this bacterium in Australian Indigenous communities is high. Therefore, molecular studies were undertaken on the dog isolates to determine if they share virulence factors with the 'human' strains. Specifically, bacterial isolates of Lancefield's group A, B, C, G or L were included in the molecular study and an isolate of *Streptococcus minor* which is a newly recognised species. To determine if these bacteria possessed virulence factors which have been identified in *S. pyogenes* of pathogenic origin, the isolates were screened by polymerase chain reaction using oligonucleotides that were specific for *S. pyogenes* virulence genes. The gene for the M or M-like protein was found in isolates of *S. canis*, *S. dysgalactiae* subsp. *equisimilis* and *S. equi* subsp. *equi* at a prevalence of 23.1, 90 and 100%, respectively. Tested isolates of *S. agalactiae, S. canis, S. dysgalactiae* subsp. *equisimilis* and *S. orisratti* harboured the streptokinase (ska) gene at prevalence rates of 62-100%. None of the dog isolates tested harboured a gene encoding the streptococcal C5a peptidase protein. The gene encoding streptococcal pyrogenic exotoxin type G was found in 80% of *S. dysgalactiae* subsp. *equisimilis* strains and 100% of *S. minor* strains. Ten percent of *S. dysgalactiae* subsp. *equisimilis* strains carried a gene encoding the fibronectin binding protein.

This aspect of the study found that genes for various virulence factors considered significant to human health were present in the dog isolates at varying prevalence rates. This study showed that dogs residing in Australian Indigenous communities, where socioeconomic factors such as overcrowded housing conditions and poor water quality exist, carry streptococci which may mediate human disease. Furthermore, these findings suggest that in these populations where humans and animals live in close proximity, gene-transfers have occurred between traditional human and animal streptococcal populations.

The findings of this study are highly significant and support the hypothesis that streptococci carried by dogs are important in the epidemiology of streptococcal disease in Indigenous residents of rural communities. This project has provided the justification for integrated epidemiological studies of humans and dogs in these communities to further test the hypothesis.

To investigate the pathogenesis of PSGN a murine model for PSGN was developed using the congenic variant (NOD.C-*Hc*) of the non-obese diabetic (NOD) mouse and the streptococcal virulence protein SIC. We found that the production of complement component 5 was a genetic factor that was involved in the development of PSGN. Furthermore, using this murine model for PSGN, we showed that the production of SIC protein by nephritic strains of *S. pyogenes* is related to the capacity of the strain to induce nephritis.

Finally, to investigate whether streptococci played a role in ESRF, ELISA tests were developed to detect antibodies against virulence factors in human serum and used to screen patients on haemodialysis. A significantly higher proportion of patients compared to controls were seropositive to SIC (P=0.018), CRS (P=0.0098) and DRS (P=0.0003). Regression analysis showed seroreactivity to DRS (R^2=0.85, P=0·001) predicted the development of ESRF. These results suggest that these ESRF patients were exposed to strains of *S. pyogenes* that secrete SIC, CRS and DRS and may have a pathological significance. No significant difference was observed between Indigenous patients and the non-Indigenous patients.

The studies described in this thesis have resulted in significant advances in our understanding of streptococcal disease, particularly as it relates to renal disease in Indigenous communities in rural Australia. The project has contributed significantly to knowledge in the three study topics. Firstly, carriage of streptococci by dogs has been demonstrated. Furthermore, the horizontal transfer of genes for virulence factors present in human streptococcal strains to canine streptococci is a major advance. It highlights the necessity to consider dogs in the epidemiology of streptococcal disease in Indigenous communities. Secondly, the rodent model for PSGN appears to be arguably the best developed so far and will allow testing of more virulence factors. Finally, the demonstration of an association between ESRF and antibody levels to particular virulence factors supports the hypothesis that chronic streptococcal infection plays a role in this disease and justifies further studies to test the hypothesis, even antibiotic prophylaxis to prevent progression of PSGN.

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LIST OF ABBREVIATIONS

°C	degrees Celsius
A_{260}	absorbance at 260 nm
A_{280}	absorbance at 280 nm
A_{320}	absorbance at 320 nm
A_{414}	absorbance at 414 nm
A_{600}	absorbance at 600 nm
ABS	Australian Bureau of Statistics
ABTS	2,2'-azino-di(ethylbenzthiazoline-6-sulphonic acid)
Anti-DNase B	anti-deoxyribonuclease B
ARF	acute rheumatic fever
ASO	antistreptolysin O
ATCC	American Type Culture Collection
AUD	Australia
BCA	bicinchoninic acid
BCS	Purina body condition score
BLIS	bacteriocin-like inhibitory substance
BLAST	Basic Local Alignment Search Tool
C1	complement component 1
C3	complement component 3
C5	complement component 5
CDC	Centers for Disease Control and Prevention
CFA	complete Freund's adjuvant
CRS	closely related to SIC
DAB	3,3'-diaminobenzidine tetrahydrochloride
DNA	Deoxyribonucleic acid
DPX	di-n-butylphethalate polystyrene xylene
DRS	distantly related to SIC
EDTA	ethylenediamine tetraacetic acid
ELISA	enzyme linked immunosorbent assay
ESRF	end stage renal failure
ET-1	endothelin-1
G	Gauge
g	gravity
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
GAS	group A Streptococcus

GBM	glomerular basement membrane
GBS	group B Streptococcus
GCS	group C Streptococcus
GGS	group G Streptococcus
GFR	glomerular filtration rate
HBA	horse blood agar
HRP	horseradish peroxidase
IDDM	insulin-dependent diabetes mellitus
IFA	incomplete Freund's adjuvant
IPTG	Isopropyl β-D-1-thiogalactopyranoside
JCU	James Cook University
kb	kilobase
KW	Kruskal Wallis statistic
LB	Luria Burtani
LPC	lipidic polylysine core
M	molar
MAC	membrane attack complex
Mga	multiple gene regulator
MHC	major histocompatibility complex
mM	millimolar
mmol	millimole
MSCRAMMS	microbial surface components recognizing adhesive matrix molecules
mw	molecular weight
NAPlr	nephritis-associated plasmin receptor
NATSISS	National Aboriginal and Torres Strait Islander Survey
NOD	non obese diabetic
NSW	New South Wales
NT	Northern Territory
OF	opacity factor
ORF	open reading frame
PA-Ag	pre-absorbing antigen
PBS	phosphate buffered saline
PCR	polymerase chain reaction
pmp	per million population
PSGN	post streptococcal glomerulonephritis
PTSAgs	pyrogenic toxin superantigens

PVDF	polyvinylidene fluoride
RALP	RofA-like protein
RAPD	random amplification of polymorphic DNA
RFLP	restriction fragment length polymorphism
RFR	renal functional reserve
Rgg/RopB	transcriptional regulator
RHD	rheumatic heart disease
rpm	revolutions per minute
rSIC	recombinant SIC
rRNA	16S ribosomal ribonucleic acid
SalA	salivaricin A
SBA	sheep blood agar
SCPA	C5a Peptidase
SCS	skin condition score
SD	standard deviation
SDS	sodium dodecyl sulphate
SDS-PAGE	sodium dodecyl sulphate polyacrylamide gel electrophoresis
SEM	structural equational modeling
SIC	streptococcal inhibitor of complement mediated cell lysis
Ska	streptokinase
SLE	systemic lupus erythematosus
SpeA	superantigens
SpeB	streptococcal pyrogenic exotoxin type B
SpeG	streptococcal pyrogenic exotoxin type G
SPSS	statistical package of social sciences
TAE	tris/acetate/EDTA
TBS	tris buffered saline
TE	tris/EDTA
TH	Todd Hewitt
THY	Todd Hewitt Yeast
US	United States
UV	ultraviolet
V	volts
VRE	vancomycin resistant enterococci
WHO	World Health Organization