Characterisation of markers associated with systemic inflammation in children with Chronic Kidney Disease

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Declaration

This thesis 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 by another person, except where due reference is made in the text.

I consent to this thesis being made available for photocopying and loan if accepted for the award of the degree.

Judith Nairn

Signature _____ Date _____

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Publications resulting directly from this study

Nairn J, Hodge G, Henning P. Intracellular cytokines in peripheral blood leucocytes in children with Chronic Renal Failure. Pediatr Nephrol. 2006 Feb;21(2):251-6

Nairn J, Hodge G, Henning P. Changes in leukocyte subsets: clinical implications for children with chronic renal failure. Pediatr Nephrol. 2005 Feb;20(2):190-6

Nairn J, Hodge G, Henning P. Leucocyte activation and increased IL-12: Clinical implications for children with chronic renal failure Pediatr Nephrol. 2004;19: C155

Presentations during candidature

Nairn J, Hodge G, Henning P. Characterisation of systemic inflammation in children with chronic renal failure. Renal Scientific Forum 2005. Invited Speaker

Nairn J, Hodge G, Henning P. Evidence for increased systemic inflammation in Children with Chronic Renal failure Australasian Flow Cytometry Group 2004. Oral Presentation

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Grants, awards and external appointments

Grants

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2004	Australasian Flow Cytometry Group: Secretary
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Abstract

Chronic Kidney Disease (CKD) is a progressive condition that in the majority of cases leads to End Stage Renal Failure (ESRD) and the need for dialysis, with the only cure being renal transplant. CKD affects both adults and children; however the underlying causes of the disease are different. CKD in adults is most commonly secondary to diabetes and/or hypertension while CKD in children is usually caused by congenital structural abnormalities that result directly in renal dysfunction.

There have been numerous reports of inflammatory and immunological disturbances in adult CKD that involve both the cellular and humoral immune systems. Consequences of these include an increased rate of cardiovascular disease (CVD), decreased response to vaccinations, as well as increased rates of infection, anaemia and malnutrition. Children with CKD display many of the clinical complications seen in adult kidney disease that are associated with inflammatory and immunological changes.

In adults however, many of the primary conditions associated with CKD are inherently pro-inflammatory; therefore it is not clear whether the inflammatory changes observed in adults with CKD are due to pre-existing inflammatory conditions, renal disease *per se* or a combination of both.

The majority of CKD in children is caused by conditions that are not inflammatory in nature. This presents a unique opportunity to study the inflammatory consequences of CKD alone, without the added complication of underlying inflammatory disorders.

Despite this, there has been little investigation of the inflammatory and immunological status of children with CKD. Some very recent studies have

shown that children with CKD have an increased systemic inflammatory state[1-3], however the nature of these immunological and inflammatory changes remains poorly defined. Identification of the specific inflammatory processes that occur in CKD may provide new treatment targets and the opportunity to develop urgently needed new therapies.

The purpose of this thesis is to investigate the presence of immunological changes associated with inflammation in children with CKD. This is the first study to include children with very mild disease, and the significant changes that are present in the early stages of the disease are of particular note. I have shown that CKD in children is an intrinsically inflammatory condition, with increased accumulation of markers of oxidative stress and production of pro-inflammatory cytokines. The inflammatory markers identified in this study may be applied as a foundation for more sensitive diagnostic markers of disease progression as well as provide a basis for novel treatment strategies in this group of patients.

Early identification of increased inflammation is a prerequisite for the application of preventive strategies. In addition, a better understanding of the level and mechanisms of systemic inflammation in children with CKD may enable a more accurate assessment of their risk of other inflammatory conditions such as CVD, anaemia, muscle wasting, and malnutrition. Future research that specifically focuses on the reasons and mechanisms for different rates of disease progression may emerge as a result of this study.

Importantly, the findings of this study may have implications in the long term treatment of disease and may allow identification of new treatment strategies to achieve better patient outcomes.

The outcomes of the study are:

- Better definition of inflammatory profiles in paediatric CKD and correlation with disease severity and progression, which should contribute to improved management strategies.
- Identification of new treatment targets to reduce the damage caused by chronic systemic inflammation.
- Mechanistic understanding of the relationship of the inflammatory profile in regard to source leucocytes or other contributing cell types.

Abbreviations

µg	microgram
µL	microlitre
µmol/L	micromoles per litre
ADPCKD	Autosomal Dominat Polycystic Kidney Disease
AGE	Advanced Glycation End Products
AOPP	Advanced Oxidation Protein Products
BA	Brefeldin A
CBA	Cytometric bead array
CBP	Complete Blood Picture
CCR	Chemokine Receptor
CD	Cluster Differentiation
CKD	Chronic Kidney Disease
CRP	C-reactive protein
CVD	Cardiovascular Disease
DN	Double Negative
ELISA	Enzyme Linked Immuno-Sorbent Assay
ESR	Erythrocyte Sedimetation Rate
ESRF	End Stage Renal Failure
FACS	Fluorescence Activated Cell Sorter
FACSperm	FACS permeabilising solution
FITC	Fluorescein isothiocyanate
FL	Fluorescence Channel
FSC	Forward Scatter
g	gravitational force

GFR	Glomerular Filtration Rate
HLDA	Human Leucocyte Differentiation Antigen
HMG-CoA	Hydroxymethylglutaryl-coenzyme A
hr	hours
HUS	Haemolytic Uremic Syndrome
I	Ionomycin
ICAM	Intercellular Adhesion Molecule
IFN	Interferon
IL	Interleukin
IL-12R	IL-12 receptor
LPS	*E. coli* Lipopolysaccharide
Mab	Monoclonal Antibody
MCP	monocyte chemotactic protein
MFI	Mean Fluorescence Intensity
MHC	Major Histocompatibilty Complex
MIA	malnutrition, inflammation and atherosclerosis
min	Minutes
mL	millilitre
mRNA	messenger RNA
ng	nanogram
NIH	National Institute of Health
NK	Natural Killer
o/n	overnight
PBMC	Peripheral Blood Mononuclear Cells

PC-5	PE-CY5
PE	Phycoerythrin
PHA	phytohemagglutinin
PKD	Polycytic kidney disease
PMA	phorbol 12-myristate 13-acetate
PMT	Photomultiplier tube
PTH	Parathyroid hormone
PTS	phosphatidylserine
PUJ	Pelviureteric junction
RBC	Red Blood Cells
ROS	Reactive Oxygen Species
RT	Room temperature
sE-selectin	Soluble E-selectin
SLE	systemic lupus erythematosis
SSC	Side Scattter
Tc	Cytotoxic T cells
Th	T Helper cells
TNF	Tumour Necrosis Factor
uL	microlitre
VCAM	vascular cell adhesion molecule
WBC	White Blood cells