NK, T and NK T-cells in ageing, coeliac disease and inflammatory bowel disease

BY

RANDALL HILTON GROSE

B.Biotech (Hons)

A thesis submitted to the University of Adelaide as the requirement for the degree of Doctor of Philosophy

The Department of Medicine, the University of Adelaide;

The Basil Hetzel Institute for Medical Research and the Department of Gastroenterology and Hepatology, The Queen Elizabeth Hospital

March 2008

For Riley

TABLE OF CONTENTS

ABSTRACT

This thesis investigated the number and function of natural killer T-cells (NK T-cells) as a function of age, in coeliac disease, Crohn's disease and ulcerative colitis.

NK T-cells are a newly appreciated class of immune cells that are able to regulate the activity of the broader T-cell population. NK T-cells have been implicated in animal models of autoimmune disease and in human autoimmune disease. A subset of NK cells express the T-cell receptor (TCR) and are termed NK T-cells. In humans a further small subset of NK T-cells express an invariant TCR α chain (Vα24Jα18) and contain the immunoregulatory cell population that is distinguished from classical T-cells by promptly producing interleukin-4 (IL-4). Invariant NK T-cells (iNK T-cells) have the surface phenotype of Vα24+ Vβ11+ T-cells and express CD161+ NK markers. They are CD4+ (single positive; SP) or CD4- (double negative; DN), CD1d restricted and are α-galactosylceramide (α-GalCer) reactive.

NKT cells have been implicated in numerous autoimmune disorders. Early work showed a major deficiency of NKT cell numbers in nonobese diabetic (NOD) mice, a well-established model of spontaneous, autoimmune T-cell mediated insulin-dependent diabetes. Both the number of NKT cells and function, as assessed by IL-4 release following TCR ligation, are dramatically reduced in NOD mice. NK T-cells have been implicated in other models of autoimmunity such as, experimental allergic encephalomyelitis (EAE). They have since been investigated and shown to be deficient in a number of human autoimmune diseases including, systemic sclerosis (SSc), and systemic lupus erythematosus (SLE), multiple sclerosis, atopic asthma, atopic dermatitis, rheumatoid arthritis, type 1 diabetes mellitus and scleroderma. The basis of the work presented within this thesis originated from the deficiency of NK T-cells in models of autoimmune diseases and human autoimmune diseases.

The initial aim of this thesis was to investigate the phenotype and function of Vα24+ NK T-cells in normal healthy control subjects and with respect to age. The original aim was to investigate whether NK cells, T-cells, NK T-like cells and invariant NK T-cells (iNK T-cells) are deficient in coeliac disease, Crohn's disease and/or ulcerative colitis.

Blood was collected for flow cytometry from normal control subjects, subjects with coeliac disease, Crohn's disease and ulcerative colitis. The number of circulating NK cells, T-cells, NK T-like cells and iNK T-cells was assessed by three-colour flow cytometry. Intracellular cytokine production was measured after *in vitro* anti-CD3/ anti-CD28 antibodies, gluten fraction 3 and PMA:ionomycin stimulation. Vα24+ T-cells were quantified in ileocolonic biopsies by immunofluorescence and as mRNA by relative and real-time PCR (RT-PCR).

The number of circulating Vα24+ T-cells and iNK T-cells decrease with age in normal healthy control subjects. Cytokine production was also affected by age. The work of this thesis has identified a subpopulation of otherwise normal healthy individuals whom have normal numbers of circulating Vα24+ T-cells, reduced numbers of circulating Vα24+ Vβ11+ T-cells and consequently iNK T-cells.

Circulating CD161+ NK cells, Vα24+ T-cells and the SP subset of Vα24+ T-cells were reduced in coeliac disease. The low numbers of circulating Vα24+ T-cells was independent of diet. The number of circulating Vα24+ Vβ11+ T-cells were reduced in coeliac disease, and as a consequence, the number of circulating Vα24+ Vβ11+ α-GalCer/CD1d tetramer+ and Vα24+ 6B11+ iNK T-cells were reduced. The deficiency of Vα24+ T-cells was not confined to the blood, but observed within the intestinal mucosa. Intestinal Vα24 mRNA expression from subjects with coeliac disease was reduced compared to levels in normal subjects as assessed by relative and RT-PCR. Thus, Vα24+ T-cells were deficient in coeliac disease both systemically and mucosally. Cytokine

production by Vα24+ T-cells, 6B11+ and Vα24+ α-GalCer/CD1d tetramer+ iNK T-cells after 4 h *in vitro* anti-CD3 stimulation was also impaired in subjects with coeliac disease.

Circulating CD56+, CD57+, CD94+, CD161+ NK cells were reduced in Crohn's disease and ulcerative colitis. Vα24+ T-cells and the SP subset of Vα24+ T-cells were reduced in Crohn's disease but not in ulcerative colitis. Circulating Vα24+ Vβ11+ T-cells, Vα24+ Vβ11+ α-GalCer/CD1d tetramer+ and Vα24+ 6B11+ iNK T-cells were deficient in both Cohn's disease and ulcerative colitis. The deficiency of Vα24+ T-cells was also observed within the intestinal mucosa. Intestinal Vα24 mRNA expression from Crohn's disease and ulcerative colitis was reduced compared to levels in normal subjects as assessed by relative and RT-PCR. Cytokine production by Vα24+ T-cells, 6B11+ and Vα24+ α-GalCer/CD1d tetramer+ iNK T-cells after 4 h *in vitro* anti-CD3 stimulation was impaired for subjects with Crohn's disease and ulcerative colitis.

In summary, Vα24+ T-cell number and function were affected by age. Further investigations are warranted to see if deficiency of this immunoregulatory population is associated with disease. The decrease and dysfunction in immunoregulatory cells, Vα24 T-cells and iNK T-cells could contribute to the pathogenesis of coeliac disease, Crohn's disease and ulcerative colitis. Coeliac disease, Crohn's disease and ulcerative colitis are polygenetic diseases in which environmental factors play a significant role in disease development and state. The reduced numbers of iNK T-cell along with their impaired function may only be two factors. Presumably, other factors are involved. Nevertheless, iNK T-cells offer a potential target for the therapeutic intervention of coeliac disease, ulcerative colitis and Crohn's disease.

PUBLICATIONS ARISING FROM THIS THESIS:

R H. Grose, A G. Cummins, and F M. Thompson. Deficiency of 6B11+ Invariant NK T-Cells in Celiac Disease. Dig Dis Sci. 2008 Jul;53(7):1846-51.

R H. Grose, A G. Cummins, and F M. Thompson. Deficiency of invariant NK T-cells in coeliac disease. *Gut*, 2007; 56: 790-795.

R H. Grose, F M. Thompson, A G. Baxter, D G. Pellicci and A G. Cummins. Deficiency of invariant NK T-cells in Crohn's disease and ulcerative colitis. *Dig Dis Sci*, 2007; 52: 1415-1422.

PUBLISHED ABSTRACTS

Grose RH, Thompson FM and Cummins AG. 2000. Systemic Vα24+ NK T-cell deficiency in coeliac disease. *J Gastroenterol Hepatol* **15**: J90.

Cummins AG, **Grose, RH** and Thompson FM. 2000. Vα24+ NK T-cell deficiency in blood is present in Crohn's disease but not in ulcerative colitis. *J Gastroenterol Hepatol* **15**: J103.

Grose RH, Thompson FM and Cummins AG. 2000. Deficiency of Vα24+/CD3+ (NK T) immunoregulatory cells in coeliac disease. *J Pediatr Gastroenterol Nutr* **31**: 53.

Grose RH, Thompson FM and Cummins AG. 2001. $V\alpha24^{+}$ NK T-cells are deficient and have defective interleukin-4 and γ-interferon production in Crohn's disease. *J Gastroenterol Hepatol* **16** (Suppl) A28.

Grose RH, Cummins AG, Thompson FM. 2002. Systemic deficiency of Vα24 Vβ11 and Vα24 Vβ13 double positive NK T-cells in celiac disease. *American Gastroenterology Association, San Francisco,* May 2002. ***(Poster of Distinction)***

Thompson FM, **Grose RH**, Cummins AG. 2002. Vα24 positive NK T-cell deficiency in Crohn's disease and ulcerative colitis. *Mucosal Immunology Update*, 10: 6012 (Supplement).

Cummins AG, **Grose RH,** Thompson FM. Deficiency of NK T-cells in inflammatory bowel disease. Broad Medical Program, Investigator's Meeting, Los Angeles, 2003, 43.

Grose RH, Thompson FM, Cummins AG. 2003. Deficiency of Vα24 T-cells in coeliac disease. J Gastroenterol Hepatol 18:(Suppl) B26.

Grose RH, Thompson FM, Cummins AG. 2003. Vα24+ T-cells are deficient and defective in interleukin-4 and γ-interferon production in IBD. J Gastroenterol Hepatol 18 (Suppl) B43.

Grose RH, Cummins AG, Thompson FM. Deficiency of NK and CD1d specific Valpha 24+ NK T-cells in Crohn's disease and ulcerative colitis. Gastroenterology 2004;126:A566 (supplement).

Grose RH, Thompson FM and Cummins AG. Decrease of CD1d specific invariant NK T-cells with age. Australasian Society for Immunology, Adelaide, 2004.

Grose RH, Thompson FM, Cummins AG. Deficiency of NK and CD1d restricted Vα24+ NK T-cells in Crohn's disease and in ulcerative colitis. Investigators Meeting, Broad Medical Research Program, Los Angeles, 2005.

DECLARATION BY STUDENT

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

I acknowledge that copyright of published works contained within this thesis resides with the copyright holder/s of those works.

Randal Hilton Grose

Signature……
Date……/……/…….

ACKNOWLEDGMENTS

I would like to start by thanking my supervisors Fiona Thompson and Adrian Cummins for their constant support, guidance and patience. Thank you for letting me have a long enough leash particularly during the difficult writing up period. Adrian you are a brilliant doctor, keeping a busy schedule with patients, surgery and research, yet still being very approachable. Thank you for providing me with the opportunity to travel overseas and interstate to present this work during my PhD candidature.

I would particularly like to thank Professor Ian Roberts-Thompson, the head of the Gastroenterology and Hepatology Department for allowing me to undertake this work within the Department at The Queen Elizabeth Hospital (TQEH).

I am indebted to TQEH Research Foundation for my PhD scholarship. I would also like to thank the Broad Medical Research Program in IBD and for recognizing the important work presented in this thesis in the form of further funding.

I would like to thank my colleagues and friends at TQEH, WCH and RAH, Department of Gastroenterology, Rheumatology, Transplantation Immunology, Child Health Research Institute (CHRI) and Mucositis Laboratories; including Gary Goland, Wendy Uylaki, Ravi Krishnan, Henry Betts, Lan Lan Zhang, Rachel Gibson, Joanne Bowen, Ashley Newland, Madelyn Zamitkowski, Wai Lim, Shilpa Prasad, Emma Leedham, Julie Johnston, Svjetlana Kireta, Xanthe Strudwick, Michaela Sherer and not to forget all the Honours and 3rd year Physiology students that have passed through TQEH, including Kimberley Camac, Laura Watson, Rebecca Skinner, Nick Mabarrack, Boris Fedoric, Joshua Woeing, Simon Fallon, Arlyn Gamache, Georgia Bradtke, and Emmy Bauer.

I would like to thank my fellow PhD student Nicola Leung, with whom I briefly shared an office. I am confident that she will do well.

I would especially like to thank the nurses and staff in the Department of Gastroenterology, IMVS and Manse who were responsible for collecting blood and intestinal biopsy samples.

Thank you also to the Grose, Zarucki, Hendry and Jessup families who have always shown an interest in my work. Thank you everyone for knowing when to and not to ask me about my thesis. Thank you everyone, especially Tara for taking an interest and for always sounding convinced when I told you "I was nearly finished".

Thanks Chris Mavrangelous, "*an all round good guy*", who was always only a phone call away. Thanks for answering all my concentration questions and allowing me to 'borrow' reagents and antibodies. Thanks for including me in all your 'mid life crisis' sports activities, including; squash, baseball, gym and cycling. No excuses now, I must finally join the Southern Veteran Cycling Club.

Finally, the work presented within this thesis would not have been possible if it weren't for those patients with coeliac disease, Crohn's disease and ulcerative colitis that have generously provided their time, and ultimately blood and intestinal biopsy samples. Without their generosity my sample size would be very small (n=1).

ABBREVIATIONS AND SYMBOLS USED IN THIS THESIS

α	Alpha
β	Beta
γ	Gamma
~	Approximately
<	Less than
>	More than
±	Plus or minus
µg	Microgram
µl	Microlitre
µm	Micrometer
Aa	Amino acid
AGA	Anti-gliadin antibody
ARA	Anti-reticulum antibody
Bp	Base pairs
BSA	Bovine serum albumin
CD	Cluster defined antigen
cDNA	Complementary DNA
Cm	Centimetre
C_t	Threshold temperature
DDH_2O	Double distilled water
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
dNTP	Dinucleotide triphosphate
DTT	Dithiothreitol
EAE	Experimental autoimmune encephalomyelitis
EDTA	Ethylene diamine tetra acetic acid
EMA	Endomysial antibody
ESPGAN	European Society for Paediatric Gastroenterology and Nutrition
FACS	Fluorescence activated cell sorter

FCS	Foetal calf serum
FITC	Fluorescein isothiocyanate
G	Gram
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
GFD	Gluten free diet
H	Hours
HLA	Human leukocyte antigen
IDDM	Insulin-dependent diabetes mellitus
IEL	Intraepithelial Lymphocyte
IFN-γ	Interferon gamma
Ig	Immunoglobulin
iGb3	Isoglobotrihexosylceramide
IL	Interleukin
iNK T-cell	invariant Natural Killer T-cell
Kb	Kilobase
L	Litre
LGL	Large granular lymphocytes
M	Molar
mAb	Monoclonal antibody
Mg	Milligram
MHC	Major histocompatibility complex
Ml	Millilitre
Mm	Millimetre
mM	Millimolar (10^{-3} M)
mRNA	Messenger ribonucleic acid
MW	Molecular weight
N	Sample size
NaCl	Sodium chloride
Ng	Nanogram
NK cell	Natural killer cell

NK T-cell	Natural killer T-cell
Nm	Nanometres
o/n	Overnight
^{o}C	Degree Celsius
PBMC	Peripheral blood mononuclear cell
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PMA	Phorbol 12-myristate 13-acetate
Rpm	Revolutions per minute
RPMI	Roswell Park Memorial Institute
RT	Room temperature
RT-PCR	Real time polymerase chain reaction
SAPE	Streptavidin phycoerythrin
SD	Standard deviation
SEM	Standard error of mean
SLE	Systemic lupus erythematosus
SSc	Systemic sclerosis
TBE	Tris borate EDTA
TCR	T-cell receptor
TGF-β	Transforming growth factor-beta
TNF	Tumour necrosis factor
TTG	Tissue transglutaminase
UV	Ultraviolet light
V	Volts
v/v	Volume per volume
w/v	Weight per volume
Y	Year
Δ	Delta
α-GalCer/CD1d	α- galactosylceramide /CD1d
IL-2R	Interleukin-2 receptor