

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

BY

RANDALL HILTON GROSE

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The Department of Medicine, the University of Adelaide;
*The Basil Hetzel Institute for Medical Research and the Department of
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For Riley

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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 (V α 24J α 18) 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 V α 24⁺ V β 11⁺ 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 V α 24⁺ 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. V α 24⁺ T-cells were quantified in ileocolonic biopsies by immunofluorescence and as mRNA by relative and real-time PCR (RT-PCR).

The number of circulating V α 24⁺ 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 V α 24⁺ T-cells, reduced numbers of circulating V α 24⁺ V β 11⁺ T-cells and consequently iNK T-cells.

Circulating CD161⁺ NK cells, V α 24⁺ T-cells and the SP subset of V α 24⁺ T-cells were reduced in coeliac disease. The low numbers of circulating V α 24⁺ T-cells was independent of diet. The number of circulating V α 24⁺ V β 11⁺ T-cells were reduced in coeliac disease, and as a consequence, the number of circulating V α 24⁺ V β 11⁺ α -GalCer/CD1d tetramer⁺ and V α 24⁺ 6B11⁺ iNK T-cells were reduced. The deficiency of V α 24⁺ T-cells was not confined to the blood, but observed within the intestinal mucosa. Intestinal V α 24 mRNA expression from subjects with coeliac disease was reduced compared to levels in normal subjects as assessed by relative and RT-PCR. Thus, V α 24⁺ T-cells were deficient in coeliac disease both systemically and mucosally. Cytokine

production by V α 24⁺ T-cells, 6B11⁺ and V α 24⁺ 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. V α 24⁺ T-cells and the SP subset of V α 24⁺ T-cells were reduced in Crohn's disease but not in ulcerative colitis. Circulating V α 24⁺ V β 11⁺ T-cells, V α 24⁺ V β 11⁺ α -GalCer/CD1d tetramer⁺ and V α 24⁺ 6B11⁺ iNK T-cells were deficient in both Crohn's disease and ulcerative colitis. The deficiency of V α 24⁺ T-cells was also observed within the intestinal mucosa. Intestinal V α 24 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 V α 24⁺ T-cells, 6B11⁺ and V α 24⁺ 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, V α 24⁺ 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, V α 24 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.

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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.

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Randal Hilton Grose

Signature.....

Date...../...../.....

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ABBREVIATIONS AND SYMBOLS USED IN THIS THESIS

| | |
|--------------------|--|
| α | Alpha |
| β | Beta |
| γ | Gamma |
| ~ | Approximately |
| < | Less than |
| > | More than |
| \pm | 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 ₂ O | 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 |
| °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 |