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

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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 (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 CD1d+ 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 \( V\alpha 24+ \) 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 \textit{in vitro} anti-CD3/anti-CD28 antibodies, gluten fraction 3 and PMA:ionomycin stimulation. \( V\alpha 24+ \) T-cells were quantified in ileocolonic biopsies by immunofluorescence and as mRNA by relative and real-time PCR (RT-PCR).

The number of circulating \( V\alpha 24+ \) 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 \( V\alpha 24+ \) T-cells, reduced numbers of circulating \( V\alpha 24+ V\beta11+ \) T-cells and consequently iNK T-cells.

Circulating CD161+ NK cells, \( V\alpha 24+ \) T-cells and the SP subset of \( V\alpha 24+ \) T-cells were reduced in coeliac disease. The low numbers of circulating \( V\alpha 24+ \) T-cells was independent of diet. The number of circulating \( V\alpha 24+ V\beta11+ \) T-cells were reduced in coeliac disease, and as a consequence, the number of circulating \( V\alpha 24+ V\beta11+ \alpha\text{-GalCer/CD1d tetramer}^+ \) and \( V\alpha 24+ 6B11+ \) iNK T-cells were reduced. The deficiency of \( V\alpha 24+ \) T-cells was not confined to the blood, but observed within the intestinal mucosa. Intestinal \( V\alpha 24 \) mRNA expression from subjects with coeliac disease was reduced compared to levels in normal subjects as assessed by relative and RT-PCR. Thus, \( V\alpha 24+ \) T-cells were deficient in coeliac disease both systemically and mucosally. Cytokine
production by $\alpha 24+$ T-cells, 6B11+ and $\alpha 24+$ $\alpha$-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. $\alpha 24+$ T-cells and the SP subset of $\alpha 24+$ T-cells were reduced in Crohn’s disease but not in ulcerative colitis. Circulating $\alpha 24+$ $\beta 11+$ T-cells, $\alpha 24+$ $\beta 11+$ $\alpha$-GalCer/CD1d tetramer+ and $\alpha 24+$ 6B11+ iNK T-cells were deficient in both Cohn’s disease and ulcerative colitis. The deficiency of $\alpha 24+$ T-cells was also observed within the intestinal mucosa. Intestinal $\alpha 24+$ 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 $\alpha 24+$ T-cells, 6B11+ and $\alpha 24+$ $\alpha$-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, $\alpha 24+$ 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, $\alpha 24$ 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:


PUBLISHED ABSTRACTS


Cummins AG, **Grose, RH** and Thompson FM. 2000. V\(\alpha\)24+ NK T-cell deficiency in blood is present in Crohn's disease but not in ulcerative colitis. *J Gastroenterol Hepatol* 15: J103.


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

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<td>γ</td>
<td>Gamma</td>
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<td>~</td>
<td>Approximately</td>
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<td>&lt;</td>
<td>Less than</td>
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<td>&gt;</td>
<td>More than</td>
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<td>±</td>
<td>Plus or minus</td>
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<tr>
<td>µg</td>
<td>Microgram</td>
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<td>µl</td>
<td>Microlitre</td>
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<td>µm</td>
<td>Micrometer</td>
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<tr>
<td>Aa</td>
<td>Amino acid</td>
</tr>
<tr>
<td>AGA</td>
<td>Anti-gliadin antibody</td>
</tr>
<tr>
<td>ARA</td>
<td>Anti-reticulum antibody</td>
</tr>
<tr>
<td>Bp</td>
<td>Base pairs</td>
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<td>BSA</td>
<td>Bovine serum albumin</td>
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<td>CD</td>
<td>Cluster defined antigen</td>
</tr>
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<td>cDNA</td>
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<td>Cm</td>
<td>Centimetre</td>
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<tr>
<td>C_{t}</td>
<td>Threshold temperature</td>
</tr>
<tr>
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<td>Double distilled water</td>
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<td>Deoxyribonucleic acid</td>
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<td>dNTP</td>
<td>Dinucleotide triphosphate</td>
</tr>
<tr>
<td>DTT</td>
<td>Dithiothreitol</td>
</tr>
<tr>
<td>EAE</td>
<td>Experimental autoimmune encephalomyelitis</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylene diamine tetra acetic acid</td>
</tr>
<tr>
<td>EMA</td>
<td>Endomysial antibody</td>
</tr>
<tr>
<td>ESPGAN</td>
<td>European Society for Paediatric Gastroenterology and Nutrition</td>
</tr>
<tr>
<td>FACS</td>
<td>Fluorescence activated cell sorter</td>
</tr>
</tbody>
</table>
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
<table>
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<tr>
<th>Abbreviation</th>
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<td>NK T-cell</td>
<td>Natural killer T-cell</td>
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<tr>
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<tr>
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<td>Standard error of mean</td>
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<tr>
<td>SLE</td>
<td>Systemic lupus erythematosus</td>
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<tr>
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<td>Systemic sclerosis</td>
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<td>TBE</td>
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