# Regulatory T Cells, Th17 Effector Cells and Cytokine Microenvironment in Inflammatory Bowel Disease and Coeliac Disease

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**“Complete happiness is nothing else than exceptional harmony in the digestive tract”**

Lunatcharsky (1875 – 1933)

## Dedication

This thesis is dedicated to my Great Aunt Krystyna Luzny, who taught me courage, strength, compassion and resilience. This thesis could not have been completed without her support.

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# Abstract

Inflammatory bowel disease (including Crohn's disease and ulcerative colitis) and coeliac disease are debilitating gastrointestinal diseases that seriously affect the quality of life of those affected. Under normal circumstances, the intestinal immune system is maintained in a state of controlled inflammation, whereby balance exists between protective immunity, mediated by effector cells, and tolerance mediated by cells with regulatory function. However, an aberrant immune response is believed to contribute to the intestinal inflammation present in individuals afflicted by these diseases.

This thesis investigated the involvement of $CD4^+$ $CD25^{high}$ $Foxp3^+$ Regulatory T cells (Treg) and Th17 Effector cells in both inflammatory bowel disease (IBD) and coeliac disease. The reciprocal relationship between Treg and Th17 cells under certain cytokine conditions, has prompted the exploration of these two cell types in IBD and coeliac disease. Previous studies have examined these factors individually in a range of diseases, however, to our knowledge the study of both Treg and Th17 in IBD and coeliac disease subjects represents a novel area of research.

Crohn's disease (CD), ulcerative colitis (UC) and coeliac disease subjects were recruited through the Department of Gastroenterology and Hepatology at The Queen Elizabeth Hospital (QEH) in Adelaide, South Australia. In total, one-hundred and seventeen subjects were enlisted in this study to donate blood samples. In addtion, intestinal biopsy samples were collected from fifty-six subjects undergoing colonoscopy at the QEH Department of Gastroenterology and Hepatology. All subjects participated, with informed consent and ethics approval.

Treg and Th17 cell numbers were investigated in the peripheral blood of Crohn's disease, ulcerative colitis, coeliac disease and control subjects using multi-colour, intracellular flow cytometry. A decrease in Treg cell numbers and an increase in Th17 cell numbers was observed in IBD, but not in coeliac disease. Closer investigation into the ratio of Treg and Th17 cells within patients identified a near 1:1 Treg/Th17 ratio in control subjects, but a lower Treg/Th17 ratio in IBD patients. This suggested a disturbance in regulatory and effector cell equilibrium. Furthermore, the excess of Th17 cells and deficiency of Tregs could contribute to the pathologies observed in IBD.

The discovery of an imbalance in Treg and Th17 cell numbers in IBD prompted further investigation of these cells in intestinal biopsies collected from IBD, coeliac and control subjects. Real time RT-PCR of intestinal biopsy samples demonstrated increased expression of the Th17 cytokine, IL-17a, in both IBD and coeliac disease. Elevated levels of the Treg transcription factor Foxp3 were also identified in intestinal biopsies from IBD subjects. It was therefore hypothesised that Treg cells may have been actively recruited from the periphery in an attempt to control inflammation in the gut; however, the intestinal cytokine microenvironment may have restricted the regulatory function of these cells.

Cytokines known to promote human Th17 differentiation, namely IL-1β, IL-6, TGF-β, IL-21 and IL-23, were explored in intestinal biopsy samples from IBD, coeliac and control subjects. High levels of IL-1β and IL-6 were detected in IBD patient samples, however, no change in levels of IL-21 or IL-23 were observed in IBD or coeliac disease subjects. Elevated levels of TGF-β were only identified in UC. No changes in cytokine

expression were observed between control and coeliac subjects, except a significant decrease in IL-6 levels was identified in coeliac disease sufferers.

The pro-inflammatory microenvironment identified in intestinal biopsies from IBD subjects may have promoted the continual differentiation and development of Th17 cells, whilst restricting Treg activity. Moreover, the observed deficiency of Treg in IBD patients may have impaired the ability of the immune system to limit excessive pathogenic Th17 driven immune responses in the intestinal mucosa. Therefore, therapeutic approaches that aim to re-establish regulatory and effector cell homeostasis by increasing Treg numbers in IBD patients, and specifically targeting Th17 cells, may prove effective in the treatment of IBD. Approaches such as these could provide greater focus to treatment strategies for IBD management compared to current broad-spectrum immunosuppressive therapies that could increase susceptibility to cancer or infection in IBD patients. In addition, the imbalance of regulatory and effector cells demonstrated in the peripheral blood of IBD patients may potentially provide new options for a non-invasive diagnostic tool.

# Declaration

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 to Nicola Eastaff-Leung 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, when deposited in the University Library, being made available for loan and photocopying, subject to the provisions of the Copyright Act 1968. I also give permission for the digital version of my thesis to be made available on the web, via the University's digital research repository, the Library catalogue, the Australasian Digital Theses Program (ADTP) and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

**Date:** **Nicola Eastaff-Leung**

# Publications, Presentations and Awards

**PUBLICATIONS:**

**Eastaff-Leung N**, Mabarrack N, Barbour A, Cummins A and Barry S. (2009) Regulatory T Cells, Th17 Effector Cells and Cytokine Microenvironment in Inflammatory Bowel Disease. Accepted for publication. Journal of Clinical Immunology.

**AWARDS RECEIVED:**

- Australian Crohn's and Colitis Young Researcher Award (2007)
- Queen Elizabeth Hospital Research Day Award for Best Presentation for Higher Degree Research (2007)
- Health Science Research Committee Postgraduate Travelling Fellowship (2007)
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# Abbreviations

| | |
|---|---|
| α | Alpha |
| β | Beta |
| γ | Gamma |
| Δ | Delta |
| ± | Plus or minus |
| μg | Microgram |
| μl | Microlitre |
| ACCA | Australian Crohn's and Colitis Association |
| AGA | Anti-gliadin antibody |
| APC | Antigen presenting cell |
| BSA | Bovine serum albumin |
| CD | Cluster defined antigen |
| cDNA | Complimentary DNA |
| CBE | Complete Blood Exam |
| CIA | Collagen induced arthritis |
| Ct | Cycle threshold |
| DC | Dendritic cell |
| $dH_20$ | Distilled water |
| DMSO | Dimethly sulfoxide |
| DNA | Deoxyribonucleic acid |
| dNTP | Dinucleotide triphosphate |
| EAE | Experimental autoimmune encephalitis |

| | |
|---|---|
| EATL | Enteropathy associated T cell lymphoma |
| EDTA | Ethylene diamine tetra-acetic acid |
| FACS | Fluorescence activated cell sorter |
| FCS | Foetal calf serum |
| FITC | Fluorescein isothiocyanate |
| Foxp3 | Forkhead box p3 |
| G | Gram |
| g | Gravitation force |
| GALT | Gut associated lymphoid tissue |
| GATA3 | GATA binding protein 3 |
| H | Hours |
| HLA | Human leukocyte antigen |
| IBD | Inflammatory bowel disease |
| IEL | Intraepithelial lymphocyte |
| IFN | Interferon |
| Ig | Immunoglobulin |
| IL | Interleukin |
| IL-R | Interleukin receptor |
| IPEX | Immune Dysregulation Polyendocrinopathy Enteropathy X-linked. |
| L | Litre |
| LPS | Lipopolysaccharide |
| M | Molar |
| Mg | Milligram |
| MHC | Major histocompatability complex |
| Ml | millilitre |

| | |
|---|---|
| mRNA | Messenger ribonucleic acid |
| MW | Molecular weight |
| N | Sample size |
| NaCl | Sodium Chloride |
| NFκβ | nuclear factor kappa-light-chain-enhancer of activated B cells |
| NK | Natural Killer |
| NLR | NOD-like receptors |
| NOD2 | nucleotide-binding oligomerization domain containing 2 |
| ns | not significant |
| NSAID | Non-steroidal anti-inflammatory |
| o/n | Overnight |
| °C | Degrees Celsius |
| PBMC | Peripheral blood mononuclear cells |
| PBS | Phosphate buffered saline |
| PCR | Polymerase chain reaction |
| PE | Phycoerythrin |
| PE-Cy5 | Phycoerythrin-cyanin-5 |
| PMA | Phorbol 12-myristate 13-acetate |
| QEH | Queen Elizabeth Hospital |
| RA | Rheumatoid arthritis |
| RORγ | Retinod related orphan receptor gamma |
| RORC | Retinoic acid related receptor C |
| Rpm | Revolutions per minute |
| RPMI | Roswell Park Memorial Institute |
| RT | Room temperature |

| | |
|---|---|
| RT-PCR | Reverse transcription real time polymerase chain reaction |
| SCFA | Short chain fatty acid |
| SD | Standard deviation |
| SEM | Standard error of the mean |
| STAT | Signal transducer and activator of transcription |
| TBE | Tris borate EDTA |
| T-bet | T box expressed in T cells |
| TCR | T cell receptor |
| TGF-β | Transforming growth factor beta |
| Th | T helper |
| TLR | Toll-like receptor |
| TNF | Tumour necrosis factor |
| TGA | Tissue transglutaminase |
| tTGA | anti-tissue transglutaminase |
| Treg | Regulatory T cell |
| UC | Ulcerative Colitis |
| UV | Ultra violet |
| V | Volts |
| v/v | volume per volume |
| w/v | weight per volume |
| Y | years |