The Role of *Mycobacterium avium* ss *paratuberculosis* (MAP) in patients with Crohn's Disease.

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Statement of Originality

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 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.

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analysed serum samples for me using techniques he has developed to identify MAP infected patients using *M. paratuberculosis* antigens as serological markers (p35 and p36).

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Lastly, I must thank my family…my husband Richard, my beautiful children Flynn and Claire, and my parents Sandra and Ian Turton. They have put up with me over the course of this thesis and without them I wouldn't have been able to continue.....Love you all xx

It hasn't been smooth sailing for me during this thesis…I got married, gave birth to two beautiful, amazing kids who I love more then anything. In January 2006 we almost lost our son from undiagnosed Type 1 Diabetes at 14 months, which completely turned our world upside down. But I am proud to say I've finally finished!!

Dedication

I dedicate this thesis to my two beautiful children Flynn and Claire….I love them with all my heart.

List of publications

Clancy R, Ren Z, Turton J, Pang G, Wettstein A. Molecular evidence for Mycobacterium avium subspecies paratuberculosis (MAP) in Crohn's disease correlates with enhanced TNF-alpha secretion. . Dig Liver Dis. 2007 May;39(5):445-51.

Ren Z, Turton J, Borody T, Pang G, Clancy R. Selective Th2 pattern of cytokine secretion in Mycobacterium avium subsp. paratuberculosis infected Crohn's disease. J Gastroenterol Hepatol. 2008 Feb;23(2):310-4.

Foell D, Wittkowski H, Ren Z, Turton J, Pang G, Daebritz J, Ehrchen J, Heidemann J, Borody T, Roth J, Clancy R. Phagocyte-specific S100 proteins are released from affected mucosa and promote immune responses during inflammatory bowel disease. J Pathol. 2008 Oct;216(2):183-92.

ABSTRACT

The cause of chronic inflammation in the gut of subjects with Crohn's Disease (CD) is unclear; however most would agree that 3 interacting factors are critical to mucosal inflammation: genetic susceptibility, enteric microflora and the host immune system. The most controversial theory is whether or not a particular microbe(s) infects and maintains in intestinal tissues, resulting in chronic inflammation. The most discussed microbe is *Mycobacterium avium* ss *paratuberculosis* (MAP). The aim of this thesis was to better document the natural history of MAP infection in subjects with Crohn's disease, IBS, "non-Crohn's colitis" and normal subjects, correlating clinical status with parameters relating to MAP, to the mucosal T cell response and to the genetic susceptibility gene CARD15/NOD2.

MAP is an obligate intracellular pathogen, which causes chronic inflammation in the intestine of many species, including primates. MAP was first identified as the causative agent in Johnes Disease, a gastrointestinal disease in ruminants and primates. The infection of domestic livestock with MAP is now widespread, which has increased the risk of transmission of MAP to humans via milk products. The involvement of MAP in human patients with Crohn's disease (CD) has been difficult to prove, due to the difficulties in isolating and detecting this organism.

By using nested PCR (Polymerase Chain Reaction) on DNA extracted from fresh human intestinal mucosal biopsy samples the presence of MAP can be verified. Bacteria contain unique insertion elements, which play a role in virulence, pathogenicity and antibiotic resistance. Within MAP a unique 1.4 kb insertion element was identified, IS900. By using DNA primers for PCR within this unique insertion element we were able to identify MAP in tissue. There was found to be no statistical significance among the disease groups tested.

The next objective was to determine whether the presence of MAP in gut biopsies was associated with a different cytokine secretion profile as observed in organ and whole blood culture, using an ELISA assay. Significantly higher levels of TNF-α

were found in culture supernatants from organ culture for Crohn's Disease when compared to ulcerative colitis ($p<0.05$), irritable bowel ($p<0.01$) and controls ($p<0.0001$). When TNF-α levels were correlated with the presence of MAP, significantly greater concentrations were only found in MAP positive Crohn's Disease patients ($p<0.05$). In whole blood culture significantly higher levels of IL-4 ($p<0.05$) and IL-2 ($p<0.05$) were found in MAP positive Crohn's Disease patients compared to MAP negative CD, which is consistent with a skewed Th2 immune response. This data provided the first evidence of an abnormal macrophage handling of MAP and an altered T cell function linked to MAP infection.

Finally, the co-existence of MAP infection and NOD2/CARD15 mutation status was investigated. SNP analysis of the three most common NOD2/CARD15 variants: missense mutations R702W (2104C→T, SNP8) and G908R (2722G→C, SNP12) and the frameshift mutation 1007fs (3020insC, SNP13); was performed on trial participants and compared to MAP status to determine if genetic susceptibility to CD predisposes them to MAP infection. Analysis of CD patients NOD2 status and cytokine profile found no correlation. Given that there is no clear link between NOD2 gene mutation, presence of MAP and cytokine secretion in our CD patients the suggestion that defective handling of MAP is due to NOD2/CARD15 gene mutations is not relevant to our group.

We can conclude that

1. MAP is not essential for CD,
2. That MAP is present in IBD and non-IBD patients,
3. There is a defect in the cellular handling of MAP in CD and
4. MAP in CD has the capacity to enhance drive a Th2 response, which in turn down regulates the protective Th1 response and enhances mucosal permeability which leads to increased inflammation.

Table of Contents