

National Association for Colitis and Crohn's disease
(NACC)

**2nd Report (2009) on the evidence linking *Mycobacterium avium*
subspecies *paratuberculosis* (MAP) and Crohn's disease**

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Introduction

Few controversies in clinical medicine excite as much passionate debate as the putative relationship between *Mycobacterium avium* subspecies *paratuberculosis* (MAP) and Crohn's disease. This is an old story. A mycobacterial cause was considered when the condition was first described over a century ago and again when it was rediscovered by Crohn and colleagues. By far, the greatest impediment to resolving the controversy is the lack of a reliable, validated and reproducible biomarker of MAP infection in humans.

Over the millennia, many chronic afflictions of mankind have turned out to have an infectious basis. The infectious cause for peptic ulceration was an unexpected discovery that eluded generations of biologists and epidemiologists and is a continual reminder that other chronic disorders, such as Crohn's disease, might be due to a known organism, such as MAP, or an infection waiting to be discovered. However, the complexity of host-microbe interactions in Crohn's disease and the heterogeneity of the syndrome have led many to suspect that a single cause is unlikely to account for all forms of the disease.

In 2002-3 the National Association for Colitis and Crohn's disease (NACC) in the United Kingdom convened an expert review group to consider the available evidence linking MAP with Crohn's disease and to recommend research that might help answer unresolved questions.¹ The key conclusions at that time may be summarised as follows:

- MAP is widespread in the environment including the human diet
- MAP is detectable in a proportion of patients with Crohn's disease and in a smaller proportion of controls
- The hypothesis that Crohn's disease is a collection of different conditions with differing causes, either genetic or environmental, is likely to be correct. If MAP is causative, it may affect only one subset of the disease and perhaps only one genetic sub-type.

- If MAP is associated with Crohn's disease, it is not acting as a conventional infective agent.
- There is no proof at present that MAP causes Crohn's disease.
- Further research should include studies that will identify which sub-sets of Crohn's patients, if any, appear to have MAP as a primary cause of their disease.
- Precautionary measures to reduce the incidence of MAP in the food chain are welcome.

In the 5 years that have elapsed since the first NACC report, studies of both MAP and Crohn's disease have continued apace. The genome of the organism has been sequenced,² and important insights into the genetics and pathogenesis of the disease have emerged.^{3,4} Has any of this helped resolve their controversial relationship? Arguments and counter-arguments for a putative role for MAP in Crohn's disease have been summarized in several reviews,⁵⁻⁷ and available evidence has been comprehensively assessed by expert groups including the International Life Sciences Institute (Europe) and the American Society of Microbiology.^{8,9} None has been able to confirm or definitively refute a significant role for MAP in Crohn's disease. However, because of the public health implications of the debate and understandable concern amongst patients, NACC re-commissioned an expert panel to re-examine the conclusions of the first report in light of research advances, to identify gaps in knowledge, and to recommend research priorities.

The current review panel has found no reason to alter the content or conclusions of the first NACC report. Therefore, the following is an overview and perspective on new information since the publication of that report.

On the linkage of MAP and Crohn's disease

The first NACC report on the link between Crohn's disease and MAP accepted that MAP is detectable in a proportion of patients with Crohn's disease at a higher frequency than that found in controls. Since then, there have been further case-control studies looking for evidence of MAP exposure in patients with established Crohn's Disease. These studies have used in situ hybridisation and PCR assays for the IS900 sequence and enzyme-linked immunosorbent assays (ELISA) for MAP antibodies. There have been two separate systematic reviews and meta-analyses of all the case-control studies, which date from 1991.^{10, 11} The review by Feller *et al*, using only a search of Medline identified 56 different studies of which 28 were included in the meta-analysis. Of those excluded they were unable to include 16 as there was insufficient information reported to allow calculation of an odds ratio (OR) – this included 13 studies with no positive tests in cases or controls.¹⁰ The pooled OR for the 18 PCR based comparisons was 7.01 (95% CI 3.95-12.4) and for the 13 ELISA based comparisons was 1.72 (95% CI 1.02-2.90). The exclusion of the negative studies is likely to account, at least in part, for this difference in risk estimates.

The second review by Abubakar *et al* used a wider search strategy and identified 58 studies and by using a different statistical approach (risk difference) for pooling the results, was able to include 49 comparisons in their meta-analysis.¹¹ The pooled estimate for the risk difference was 0.23 (95% CI 0.14-0.32) i.e. evidence of MAP infection was on average 23% higher in cases than controls. As with the first review, there was considerable heterogeneity in the risk estimates, only some of which could be explained by the year of study and assay type. Both reviews make play of the fact that the

association of MAP and Crohn's disease was only slightly weaker when the controls were patients with ulcerative colitis, although this comparison assumes that the effects on inflammation confined to the colon in ulcerative colitis are equivalent to what is seen in Crohn's disease.

These systematic reviews confirm that there is a moderately strong and specific association between Crohn's disease and evidence for MAP infection but, as both reviews emphasise, they are unable to demonstrate whether MAP exposure precedes the development Crohn's disease. In addition, the scale of the relationship between MAP and Crohn's disease is unclear from these reports because they are limited by exclusion of studies failing to detect MAP in any patient and by publication bias favouring positive results.¹²

On persistent epidemiologic hurdles for the MAP hypothesis

It is acknowledged that host-microbe interactions can be complex and evidence for causality in chronic disease, including evidence for vertical or horizontal transmission, may elude traditional epidemiologic scrutiny. This was one of the lessons of the *Helicobacter pylori* and peptic ulcer disease story. However, there are features of Crohn's disease which appear to run counter to the MAP hypothesis.

Firstly, environmental conditions expected to favour spread of a transmissible disease, such as poor domestic hygiene, crowding, and large families, actually seem to protect against development of Crohn's disease.¹³ Secondly, the incidence of Crohn's disease in Scandinavian countries is comparable with that of other developed countries, whereas detection rates for MAP in cattle are low in Scandinavia.¹⁴⁻¹⁶ Thirdly, there

appears to be no increased risk of Crohn's disease in people exposed to bovine paratuberculosis.¹⁷

Fourthly, the most consistent epidemiologic feature of Crohn's disease is the acute rise in incidence and prevalence when societies undergo transition from developing to developed status.¹⁸ This modernisation usually involves increased urbanisation and a shift from an agriculture-based economy toward manufacturing or a knowledge economy. None of these conditions would appear to pose an increased risk of infection with MAP. Furthermore, migration studies suggest that the environmental risk factors for Crohn's disease (other than smoking) are operative at an early stage in life, with little effect if exposure occurs first in adulthood.¹⁸ While this does not exclude a causative role for a single pathogen, it suggests an alternative or additional explanation for the epidemiologic trends with Crohn's disease. Environmental factors in early life which shape the developing immune system may exert their influence at the level of the microbiota colonising the gut. Since the microbiota is an environmental modifier of immune development and maturation,¹⁹⁻²³ it may also determine the risk of immunoinflammatory disease, particularly in those who are genetically susceptible. Thus, the link between Crohn's disease and a modern lifestyle may be by virtue of the impact of the latter on the commensal microbiota and thence immunity.¹⁸ This would be consistent with observations that the changing epidemiology of Crohn's disease is paralleled by similar trends in other chronic immune-mediated diseases.

Fifthly, as noted by many, the sustained clinical remission frequently seen with anti-TNF- α therapy does not seem to be consistent with the expected adverse effects of such therapy on a mycobacterial infection. Finally, detection of MAP in tissues of

patients with Crohn's disease is not proof of pathogenicity in the traditional sense of an infection. Therefore, the question arises whether MAP can infect humans in any other setting. Although the zoonotic potential of MAP appears to have been established in isolated case studies, the evidence is weak and the extreme paucity of reports suggests that this is a rare event, even in immunosuppressed patients.^{5, 8}

On the potential for an indirect pathogenic role for MAP

The first report by NACC (2003) found there was sufficient evidence to conclude that if MAP has any pathogenic role in CD, it is unlikely to be acting as a conventional pathogen. Recent work has since provided examples of potential mechanisms by which MAP might have an indirect pathogenic role.

Firstly, it has been shown that MAP expresses the mannose alpha 1, 3 mannose epitope for the anti-saccharomyces cerevisiae antibody (ASCA) that is found in sera of about two thirds of patients with Crohn's disease and this has the capacity to inhibit the bacteriocidal function of macrophages.²⁴ This is particularly noteworthy because of the occurrence of Crohn's-like lesions in patients with metabolic disorders affecting phagocytic function and evidence for defective phagocytic function in Crohn's disease.²⁵ Thus, a microbial-driven acquired defect in phagocytic function, may adversely affect the host's capacity to clear intracellular bacteria from the mucosa, and thereby contribute to or act as an alternative to genetic defects in autophagy that have been described in Crohn's disease.²⁶

Secondly, a variety of metabolites or components of mycobacteria may have additional modifying influences on the inflammatory process. For example, bacterial

DNA (hypomethylated CpG DNA) has immunostimulatory properties attributable to its role as a natural ligand for host toll like receptors (TLR9). Therefore, the presence of mycobacterial DNA or other bacterial DNA within granulomas can be anticipated to modify cytokine production within the inflammatory microenvironment, and thereby a potential impact on the clinical course of the disease.²⁷

Thirdly, the possibility that MAP antigens might mimic peptides of host origin and excite autoimmune cross-reactivity has been explored.²⁸ Whether immunologic cross-reactivity could act as an initiator or perpetuator of tissue damage is unclear, but this scenario could explain the widespread prevalence of MAP in the environment, a harmless passenger in most people, possibly having a bearing on disease only in those who are genetically susceptible.

On the impact of anti-mycobacterial treatment

Given the difficulty of culturing MAP and the lack of a convenient and reproducible diagnostic biomarker, progress in understanding the potential role of this organism in human disease will continue to be logistically difficult. For example, in addition to conflicting results from different laboratories on the detection of MAP either by culture or by molecular methods in Crohn's disease and a lack of sequential testing within the same patients, there is no evidence that the presence or absence of MAP can be correlated with the clinical course of the disease. However, the achievement of sustained remission of Crohn's disease following treatment with anti-mycobacterial drugs would represent convincing evidence for a role for MAP. As acknowledged in the first NACC report, a clinical trial with anti-MAP therapy might help establish the proportion of cases where

this organism has a contributory role, particularly if supported by evidence for a reduction in MAP load.

To date, results of clinical trials with anti-mycobacterial drugs in Crohn's disease have been disappointing. The outcome of the Australian trial, alluded to in the first NACC report, has since been reported.²⁹ It does not provide support for a role for MAP in the pathogenesis of the disease in the majority of patients, although does not definitively exclude it. Sustained remission was not achieved with clarithromycin, rifabutin and clofazimine for up to two years, although there was a short-term improvement when the combination was added to corticosteroids. The latter effect was attributed by the investigators to non-specific antibacterial effects. Notwithstanding various caveats including the antibiotic dosage and the lack of data on the presence or absence of MAP before or after the treatment, this was an important study. It was the largest randomised controlled trial of antibiotics in Crohn's disease and the only one to assess therapy directed against MAP. Additional studies with more effective anti-MAP regimens are desirable and should incorporate methods to monitor the MAP status of the patients in each treatment limb. It is also desirable that the economic impact of MAP, worldwide, in dairy herds should drive innovative control strategies, including new drug development.

MAP in the context of other hypotheses and other microbiota

While acknowledging the importance of research attention directed toward the putative linkage of MAP with CD, the first NACC (2003) report cautioned that this should not detract from the pursuit of other hypotheses of equal merit. One of the difficulties in attempting to prove a cause and effect relationship in Crohn's disease for

any single organism or environmental factor is the heterogeneity of the disorder. In addition to genetic heterogeneity of the host, heterogeneity is also evident at the level of the commensal microbiota and the environmental factors influencing the microbiota. Therefore, the role of MAP should be considered in the wider context of other host-microbe interactions.

Firstly, MAP is not the only bacterium linked with Crohn's disease. At least eight research groups have independently found an increase in mucosa-associated adherent invasive *E. coli* (AIEC) in Crohn's disease.³⁰ *E. coli* antigens and DNA have been detected within the granulomas of Crohn's disease. As alluded to earlier, MAP and AIEC may even be interactive in some patients because of the potential for inhibition of the bactericidal function of phagocytes by MAP, which could account, in part, for disturbed handling of mucosal bacteria in Crohn's disease.²⁴

Secondly, the increased bacterial numbers in the mucosa of patients with Crohn's disease may be a non-specific consequence of defective innate immunity increasingly linked with this condition. Genetic risk factors for Crohn's disease include those which code for intracellular sensors of bacterial cell walls (NOD2/CARD15) and for processing and clearance of intracellular bacteria by autophagy (ATG16L1 and IRGM). Recent studies, reported only in abstract so far, have shown that NOD2 is also important in the autophagy pathway and is dependant on Atg16L1 as well as Atg5 and Atg 7.³¹ Dendritic cells from patients with NOD2 or ATG16L1 mutations are unable to activate the autophagy pathway, and hence fail to form autophagosomes. In addition there is a failure to express HLA Class 2 molecules on the surface of the dendritic cells which has implications for antigen processing. Therefore, Crohn's disease may arise from an

abnormal host-microbe interaction due to genetically determined immune responses to bacteria or impaired handling of bacteria within the mucosa.^{25, 26} The identity of the predominant bacteria may vary and whether the host genotype can be correlated with the presence of absence of MAP remains to be determined.

Thirdly, there is increasing evidence for reduced bacterial diversity of the commensal microbiota in patients with inflammatory bowel disease, including reductions in lactobacilli, bidobacteria, and the anti-inflammatory commensal, *Faecalibacterium prausnitzii*.^{32, 33} Since the commensal microbiota influences the maturation and function of the developing immune system,¹⁹⁻²³ disturbances in microbial biodiversity might contribute to individual variations in immunologic behaviour during and after childhood. The challenge will be to link specific microbiota with individual variations in the immune response, a strategy that already appears to be promising.³⁴

Finally, several lines of circumstantial evidence suggest that an environmental influence on the commensal microbiota may underpin much of the changing epidemiology common to several immune-mediated chronic inflammatory disorders, including Crohn's disease.¹⁸ Many of the elements of a modern lifestyle can be viewed as proxy markers of microbial exposure during early life.^{13, 18} These include changes in domestic hygiene, antibiotic usage, family size, birth order, endemic crowding, decline in parasitism, diet, sedentary lifestyle, and obesity. Non-culture-dependent metagenomic strategies can provide a molecular profile of complex microbial populations, and this promises to show the linkages amongst individual variations in diet, alterations in gut microbiota, and perhaps, risk of disease.³⁵

Conclusions

- The conclusions of the first NACC review (2003) remain valid and without need for alteration.
- Recent research findings have not strengthened the theory that MAP plays a primary role in Crohn's disease.
- The context of the debate should be broadened to include other components of the microbiota and current concepts of host-microbe interactions.

Recommendations

- The MAP status of the host should be assessed at diagnosis and correlated with clinical course
- MAP status should also be correlated with host genotype
- Research to identify suitable growth conditions for MAP is highly desirable. The inability to culture the organism within a reasonable time frame is a significant impediment to progress. For the same reason, a reliable, reproducible biomarker for identifying MAP in humans is needed, and would greatly facilitate clinical research on the role of this organism in human disease.
- The use of non-culture-dependent metagenomic approaches should be encouraged to profile bacterial populations in lesions of Crohn's disease.
- Comparative studies of the genetic fingerprints of strains in the local animal reservoir and patients with Crohn's disease may shed light on the sources of MAP in such patients.

- While increased attention to hygiene and rigor of pasteurisation is welcome in the dairy food industry, clinicians should dissuade patients from abandoning dairy products, the nutritional benefits of which are real and far outweigh risks in relation to MAP, which remain theoretical
- In areas with high levels of MAP exposure in population controls, the outcome of those control subjects with evidence of MAP exposure should be determined.

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