

Crohn's disease

## Does *Mycobacterium avium* subspecies *paratuberculosis* cause Crohn's disease?

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### Reassessing this persistent theory in light of advances in molecular microbial detection and genetic pathogenesis of disease

Similarities between chronic idiopathic granulomatous ileocolitis and mycobacterial infections have been noted since the original descriptions of the clinical syndrome now called Crohn's disease.<sup>1-4</sup> Interest in a possible infectious origin of this disorder was renewed in 1989 when Chiodini *et al* cultured apparently identical *Mycobacterium avium* subspecies *paratuberculosis* (MAP) from three patients with Crohn's disease.<sup>5</sup> This controversy increased in intensity following the detection of the specific DNA insertion sequence, IS900, of MAP in relatively high numbers of patients with Crohn's disease relative to ulcerative colitis and normal controls,<sup>6</sup> and is now raging as several different groups have detected this organism in the food chain<sup>7</sup> and water supply,<sup>8</sup> proposed maternal-fetal transmission in human milk,<sup>9</sup> reported long term responses to antimycobacterial antibiotic combinations,<sup>10</sup> and even cultured viable *M paratuberculosis* in blood samples of Crohn's disease patients.<sup>11</sup>

Additional data to support an association of MAP with Crohn's disease is provided by Autschbach and colleagues<sup>12</sup> in this issue of *Gut* (see page 944). This carefully performed and well controlled study used nested polymerase chain reaction (PCR) to detect the IS900 insertion element of MAP in 52% of Crohn's disease resected tissues versus 2% of ulcerative colitis and 5% of mostly non-inflammatory control tissues. This study provides novel data regarding the prevalence of MAP in various phenotypes of Crohn's disease by showing slightly higher detection of IS900 DNA in colonic (66.7%) compared with distal ileal (40.5%) tissues and decreased detection rates with corticosteroid use. In addition, these authors reported weak associations with perianal involvement and a shorter duration of disease but no correlation with patient sex, age at diagnosis, stricturing versus penetrating phenotype, or presence of granulomas.

Data from this study help address some of the controversies that have fuelled the vigorous debate between committed advocates and confirmed sceptics that is receiving increasing attention in the scientific literature, lay press, and internet chat rooms. The arguments in favour or opposed to this theory (table 1) have some merit but many are flawed by incomplete data and lack of rigorous reflection. There is no doubt that a potential source of zoonotic infection exists, with widespread MAP infections in the dairy herds of Europe, North America, and Australia,<sup>13, 14</sup> excretion of MAP in milk from infected cows,<sup>15</sup> relative resistance of intracellular MAP to widely used pasteurisation techniques,<sup>16</sup> and recovery of viable MAP from the water supplying Los Angeles.<sup>8</sup> Moreover, the vast majority of studies using diverse techniques have detected MAP DNA or cultured this organism in higher frequency from tissues of patients with Crohn's disease than from those with ulcerative colitis and other disorders, although the reported frequency of recovery in both Crohn's disease and ulcerative colitis have ranged from 0% to 100%.<sup>17</sup> These results are consistent with two possibilities: either MAP infection could cause Crohn's disease in a subset of patients that are either selectively exposed to this organism or who are genetically susceptible to infection or, alternatively, this relatively common dietary organism may selectively colonise (or a dead organism selectively lodge in) the ulcerated mucosa of Crohn's disease patients but not initiate or perpetuate intestinal inflammation. Molecular fingerprints show that genotypes of bovine and human isolates are not similar but instead indicate that human and ovine (sheep) strains are more closely related.<sup>18</sup> Maternal/fetal transmission of MAP has been proposed following culture of MAP from breast milk of two patients with Crohn's disease.<sup>9</sup> However, the frequency of positive cultures in human milk is uncertain,

this observation has not been replicated by other investigators, and there is no evidence of increased frequency of Crohn's disease in the offspring of mothers versus fathers with Crohn's disease. Even if transmission of viable MAP occurs, a plausible mechanism of tissue injury and induction of chronic intestinal inflammation has not been proposed. Even advocates of the theory that MAP causes Crohn's disease concede that infection, if present, consists of a low bacterial load and that no histochemical evidence of acid fast staining in Crohn's disease tissues is seen. This could be explained by a paucibacillary infection with an obligate intracellular, cell wall deficient bacterial form.<sup>19</sup> In this setting, inflammation and tissue injury must be mediated by a cell mediated immune response. However, a cellular immune response to MAP has not been documented in Crohn's disease patients,<sup>20</sup> despite increased serological responses to MAP antigens in the same patients. Another serious flaw in the MAP pathogenesis of Crohn's disease theory is the observation that these patients respond to chronic immunosuppressive therapies<sup>21</sup> and acquired immunosuppressive infections decrease disease activity as CD4 T cell counts fall.<sup>22</sup> In contrast, *M tuberculosis* massively proliferates with anti-tumour necrosis factor or steroid treatment and *M avium intracellulare* thrives in the intestine as CD4 counts fall in human immunodeficiency virus infected patients. It is possible that intracellular cell wall deficient MAP may not replicate well despite immunosuppression, but this issue has never been studied by in vitro investigation or in animals with Johne's disease. In the study by Autschbach *et al*, corticosteroid therapy was associated with lower MAP detection rates.<sup>12</sup>

The most irrefutable evidence that a microbial agent causes a disease is long term remission of clinical manifestations and an altered natural history of disease following clearance of the infection. In vitro sensitivity analyses show that clinical isolates of MAP are not responsive to traditional anti-*M tuberculosis* agents, and therefore lack of efficacy with isoniazid, ethambutol, and rifampicin treatment for two years with a three year follow up<sup>23</sup> does not detract from this theory. However, reports of efficacy of combinations of clarithromycin or azithromycin, rifabutin, and a variety of other agents in 58-82% of Crohn's disease patients<sup>10, 24</sup> are also not definitive due to the uncontrolled nature of these studies, the small number of patients treated, the variable treatment regimens, and the fact that these antibiotics, particularly clarithromycin,

**Table 1** Arguments for and against a *Mycobacterium avium* subspecies *paratuberculosis* (MAP) causation of Crohn's disease

## Pros

- (1) Clinical and pathological similarities between Johne's and Crohn's diseases<sup>3, 4</sup>
- (2) Presence in food chain (milk, meat) and water supplies<sup>7, 8</sup>
- (3) Increased detection of MAP in Crohn's disease tissues by culture, PCR, FISH<sup>5, 6, 33</sup>
- (4) Positive blood cultures of MAP in Crohn's disease patients<sup>11</sup>
- (5) Increased serological responses to MAP in Crohn's disease patients<sup>20, 34</sup>
- (6) Detection of MAP in human breast milk by culture and PCR<sup>2</sup>
- (7) Progression of cervical lymphadenopathy to distal ileitis in a patient with MAP infection<sup>35</sup>
- (8) Therapeutic responses to combination antituberculosis therapy that include macrolide antibiotics<sup>10, 24</sup>

## Cons

- (1) Differences in clinical and pathological responses in Johne's and Crohn's diseases<sup>4</sup>
- (2) Lack of epidemiological support of transmissible infection<sup>36</sup>
- (3) No evidence of transmission to humans in contact with animals infected with MAP
- (4) Genotypes of Crohn's disease and bovine MAP isolates not similar<sup>18</sup>
- (5) Variability in detection of MAP by PCR (0–100% in Crohn's disease and ulcerative colitis tissues)<sup>8</sup> and serological testing<sup>37</sup>
- (6) No evidence of mycobacterial cell wall by histochemical staining
- (7) No worsening of Crohn's disease with immunosuppressive agents or HIV infection
- (8) No documented cell mediated immune responses to MAP in patients with Crohn's disease<sup>20</sup>
- (9) No therapeutic response to traditional antimycobacterial antibiotics<sup>23</sup>

MAP, *Mycobacterium avium* subspecies *paratuberculosis*; PCR, polymerase chain reaction; FISH, fluorescent in situ hybridisation; HIV, human immunodeficiency virus.

have a broad spectrum of activity against commensal enteric bacteria. Moreover, these studies and a yet to be published ongoing controlled trial in Australia using these agents in Crohn's disease patients are flawed by not assessing IS900 DNA in biopsy specimens by PCR and serological responses to MAP before and after therapy, so that clinical results can be correlated with the presence of tissue MAP and its clearance with treatment. Selective responses in those patients with detectible MAP colonisation that clear infection with antibiotic treatment would strongly imply a causal relationship of the infection.

Our evolving molecular understanding of gene/environmental interactions offers an opportunity to reassess the MAP causation theory of Crohn's disease in a new light. NOD2/CARD15 is an intracellular receptor for muramyl dipeptide (MDP), the smallest immunologically active component of bacterial peptidoglycan. Ligation of MDP by NOD2/CARD15 activates nuclear factor  $\kappa$ B. This pathway may contribute to clearance of intracellular bacterial infection<sup>25</sup> and secretion of  $\alpha$  defensins by Paneth cells, which constitutively express NOD2/CARD15.<sup>26</sup> The three most common polymorphisms of this gene are found in 25–35% of Caucasian Crohn's disease patients<sup>27</sup> and lead to defective nuclear factor  $\kappa$ B activation by MDP.<sup>28, 29</sup> Expression of the common truncation mutation of NOD2/CARD15 is associated with defective clearance of invasive salmonella infection in epithelial cells.<sup>25</sup> In addition, NOD2/CARD15 mutations in Crohn's disease are associated with diminished mucosal  $\alpha$  defensin expression.<sup>30</sup> Thus an attractive explanation linking NOD2/CARD15 to

Crohn's disease is that defective function of this gene results in ineffective clearance of intracellular MAP infection and in decreased luminal  $\alpha$  defensin secretion that permits increased mucosal adherence and epithelial invasion of ingested organisms. Defective clearance of intracellular MAP by innate immune cells, including macrophages, could explain the seemingly paradoxical therapeutic response of some Crohn's disease patients to granulocyte-macrophage colony stimulating factor.<sup>31</sup> However, the phenotypic information provided by Autschbach and colleagues<sup>12</sup> argues against an association of NOD2/CARD15 with MAP infection, as MAP was more commonly detected in colonic than ileal disease and was not more frequently found in early onset or stricturing disease.<sup>12</sup> Likewise, patients with extensive ileocolitis responded better to macrolide antibiotics and rifabutin than did those with isolated ileal disease.<sup>10</sup> These results directly contrast with the strong association of NOD2/CARD15 polymorphisms with early onset ileal Crohn's disease with a stricturing phenotype.<sup>32</sup>

Crohn's disease certainly has environmental and host genetic influences that interact to cause clinically evident disease. It is equally clear that MAP is widely present in our food chain and that the DNA of this organism can be recovered from the intestine of Crohn's disease patients. Although existing data do not compellingly implicate MAP as a causal agent in Crohn's disease, neither do they definitively exclude this possibility. We must determine whether MAP infection causes human disease, which is unlikely in my opinion, or whether this environmental contaminant innocently lodges in ulcerated mucosa. Is

MAP analogous to *Helicobacter pylori* in peptic ulcer disease, gastritis, and gastric cancer, where host genetics and microbial virulence factors determine immune responses that mediate clinical disease in a small minority of patients exposed to a widespread infectious agent? Are we repeating the mistake of *H. pylori* where the scientific establishment resisted a new theory that challenged established paradigms of peptic ulcer disease until overwhelming clinical evidence made such resistance untenable? Well designed clinical, microbiological, and mechanistic experiments are urgently needed to definitively settle this still unresolved debate.

To establish a causal relationship between MAP and Crohn's disease, we need to determine if clearance of MAP selectively changes the natural history of disease in an infected subset of patients, perform definitive investigations of cellular immune responses to this organism in Crohn's disease and control patients, determine if NOD2/CARD15 and other microbial signalling pathways influence intracellular MAP infection and clearance, and review results of ongoing large multi-institutional studies to detect MAP in shared coded tissues by various molecular and culture methods. These studies need to be designed and conducted by established investigators who bring no predetermined biases to this contentious topic. If MAP is responsible for a subset of Crohn's disease, public health measures must be implemented to eliminate the source of infection in our food chain and food processing practices must be modified. In addition, the medical community must develop ways to efficiently and cost effectively screen for MAP infection and develop methods to efficiently clear this organism from infected tissues, possibly through a combination of effective antibiotics and immunostimulants that enhance innate clearance responses. If there is no evidence of a causal association of MAP and Crohn's disease, we need to direct resources to other avenues of research. This controversy has persisted far too long and needs to be expeditiously resolved.

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## Colitis

## Probiotics and barrier function in colitis

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### Probiotic administration may exert a protective effect in colitis by preventing mucosal barrier disruption and influencing the extent of mucosal injury

There is strong evidence of a role for the indigenous flora in driving inflammatory responses in inflammatory bowel disease (IBD) in genetically predisposed individuals.<sup>1</sup> For years, researchers have tried in vain to identify a specific pathogen as the cause of these chronic intestinal inflammatory disorders but the possibility that one or more bacterial agents are responsible cannot be ruled out. Considering the implications of a pathogen in IBD, as yet

undiscovered due to technical limitations, it was hypothesised that modulation of an abnormal microflora in these patients by introducing high titres of "protective" bacteria might overwhelm the "aggressive" strain(s) and inhibit its deleterious effects. On this basis, probiotic treatment was proposed as a therapeutic approach.<sup>2</sup>

Probiotics are defined as "living organisms which, on ingestion in certain numbers, exert health benefits

beyond inherent basic nutrition".<sup>3</sup> Bacteria associated with probiotic activity are most commonly lactobacilli, bifidobacteria, and streptococci but other non-pathogenic bacteria such as some strains of *Escherichia coli* and microorganisms such as the yeast *Saccharomyces boulardii* have been used in IBD.

Encouraging results have been obtained with probiotics in several experimental animal models of IBD.<sup>4–7</sup> In humans, probiotics are effective in the prevention of pouchitis onset and relapse.<sup>8–10</sup> Results in ulcerative colitis are promising, both in prevention of relapse and treatment of mild to moderate attacks.<sup>11–13</sup> Results in Crohn's disease are not yet clear because of conflicting data and the limited number of well performed studies.<sup>14–16</sup>

Efforts are being made by many researchers to unravel the precise mechanisms by which probiotic bacteria and their metabolic products (short chain fatty acids, vitamins) exert their