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# MAPping the Cause of Crohn's Disease or Simply Along for the Ride?

by L. Campbell Levy and Kim L. Isaacs

## INTRODUCTION

**C**rohn's disease (CD) is a chronic inflammatory disease of the intestines without a known etiology or a cure. Similarities between Crohn's disease and Johne's disease, a mycobacterial infection of cattle, were first observed nearly one hundred years ago (1). There are over 100 species within the mycobacteria genus. Most are ubiquitous in soil and water, and, with the exception of *M. tuberculosis* and *M. leprae*, mycobacteriae do not cause disease in humans. *Mycobacterium avium* subspecies *M. paratuberculosis* (MAP) is a slow growing, intracellular organism that can exist in a cell wall deficient ("spheroplast") form. It has been theorized that Crohn's disease, like Johne's disease, is a dysregulated immune response to invasion by MAP. When Chiodini, et al cultured identical MAP from three patients with Crohn's disease in 1989, their observation spawned a renewed interest in MAP as a possible infectious cause of CD (2).

The association between CD and mycobacteria has since evolved into intense debate. Advocates of the MAP theory propose that in select population patients, MAP exposure causes the classic findings of Crohn's disease: ulceration, inflammation, edema, and stenoses (3). Detractors have argued that it is not an etiologic agent but represents an epiphenomenon. This common organism either colonizes or lodges within

the ulcerated mucosa (4), and, due to the immune dysregulation of CD, intracellular killing of the bacteria may be impaired (5). We will review the epidemiologic and clinical associations between *M. paratuberculosis* and CD and the data available regarding the treatment of CD with anti-MAP directed therapies.

## EPIDEMIOLOGIC ASSOCIATIONS

CD has marked geographic and socio-demographic variability, which implies that environmental factors influence the disease incidence (6). Similarly, a birth-cohort pattern has been observed in CD (6). A birth cohort effect is the phenomenon of successive generations exhibiting rapidly changing rates of disease, and it suggests an exposure early in life that increases with time. The candidacy of MAP as the possible key environmental exposure is supported by widespread infections of dairy herds across Europe, Australia, and North America. In the US, the estimated prevalence of MAP infection among large dairy herds is at least 40% (7). However, infiltration of MAP is not limited to dairy herds. MAP has pervaded food and water sources increasing the possibility of a pandemic zoonotic infection. Though reports have been conflicting, resistance to pasteurization techniques have been described, and MAP has been detected in retail supplies of milk and cheeses (8,9). MAP has also been recovered from the water supplying Los Angeles (10).

While MAP has been detected from multiple sources and while epidemiologic trends support the importance of a possible environmental exposure in the pathogenesis of CD, neither is sufficient to establish a causal role of MAP in CD. Theories of other possible

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exposures have been propagated as the trigger in inflammatory bowel disease (IBD), including, among others, the “hygiene hypothesis,” which suggests that a decrease in childhood infection explains the rising prevalence of CD. A recent population-based case control study by Bernstein, et al found two factors which are often associated MAP infection (11), a history of living on a farm and a history of consuming raw milk, decreased the odds of CD by 38% and 33% respectively as compared to controls (12). While these data neither prove the “hygiene hypothesis” nor disprove the MAP hypothesis, they, along with data from other recent studies (13), offer a plausible alternate explanation for observed epidemiologic trends in IBD. Other epidemiologic data are also inconsistent with the MAP infectious theory. Clusters of Crohn's disease imply an infectious etiology though such clusters are uncommon (14). In areas where Johne's disease and presumably MAP is widespread, CD has a patchy distribution (14). Furthermore, genetic testing of bovine MAP from diverse geographic locales show remarkable homogeneity, while there is a high degree of genetic heterogeneity among human and ovine MAP genotypes (15).

### DETECTING MAP IN CD PATIENTS

Since Chiodini, et al cultured MAP from three patients with CD, detection by numerous techniques has been reported in close to one hundred publications (16). MAP is fastidious and grows slowly making culture difficult. In 1992 Sanderson, et al used PCR to detect a specific DNA insertion sequence, *IS900*, of MAP in 65% (26/40) of CD patients, 4.3% (1/23) ulcerative colitis patients, and 12.5% (5/40) of controls. Since, molecular and serological methodologies, including immunocytochemistry, in situ hybridization, PCR techniques, and ELISA, have been widely used (5). The majority of studies have identified MAP in CD tissues with a higher frequency than that observed in UC or controls. However, rates are highly variable ranging from 0% to 100% (17,18). Two recently published meta-analyses underscore both the increased association of MAP and CD and also the variability of these studies. Feller, et al conducted a meta-analysis of 18 case-control studies that used PCR techniques and calculated an OR 7.01 (95% CI, 3.95-12.4) favoring a positive test (5). However,

there were moderate to high levels of heterogeneity between studies that was likely due to confounding, bias, and differences in study populations. Similarly, Abubakar and colleagues applied less stringent inclusion criteria for a similar meta-analysis of 49 studies that used PCR or in-situ hybridization. While this analysis also favored a positive test, the study was limited by a significant degree of heterogeneity and also evidence of statistically significant publication bias (16). As both authors emphasize, even if the limitations of these studies are discounted, it is impossible to establish whether the exposure to MAP is causal or an epiphenomenon based on these data alone. A lack of standardization among the studies to date has hampered the ability to form reproducible prevalence estimates and strengths of the relationship between MAP and CD.

### PLAUSIBLE MECHANISM OF PATHOGENESIS

Evidence for a plausible pathophysiologic mechanism linking the presence of MAP with inflammation and tissue damage is lacking. A recent study by Scanu, et al exemplifies the current lack of understanding of the underlying pathogenesis of CD and the role that might be played by MAP. PCR detected *IS900* DNA in 87% (20/23) of patients with CD and in a similarly high 75% (15/20) of patients with irritable bowel syndrome (IBS), which is a non-inflammatory process (15% [3/20] of controls had positive tests) (19).

A lack of histochemical evidence of acid fast bacilli and low intensity of PCR signals from intestinal tissues indicate low numbers of measurable MAP organisms (20). It is possible that MAP causes a paucibacillary infection as observed in tuberculoid leprosy or that the organisms accumulate in a reservoir such as adipocytes as has been noted in *M. tuberculosis* (21). If this is the case, tissue injury would have to be mediated by a cellular immune response. Despite elevated serologic responses to MAP antigens, Olsen, et al found no change in IL-10 or interferon gamma levels after stimulating CD patients with these antigens (22). A recent study by Clancy and colleagues described a high level of tumor necrosis factor alpha among patient with CD and MAP infection as compared to CD patients without MAP or to IBS and UC patients with MAP (23). This supports the hypothesized link

**Table 1**  
Open label and randomized trials of antimycobacterial therapy for CD

Author (year)	(n)	Trial	Therapy	Treatment Period (mos)	Primary Endpoint	Treatment/Placebo (%)
Elliot (1982) (30)	51	RCT	Sulfadoxine, pyrimethamine	12	Clinical remission	38/50
Shaffer (1984) (39)	27	RCT	Ethambutol, rifampin	12	Clinical remission	36/64
Hampson (1989) (33)	20	Open label	Ethambutol, rifampin, Clofazimine	9	Clinical remission	50
Prantera (1989) (35)	5	Open label	Dapsone	1	Clinical remission	40
Afdhal (1991) (28)	49	RCT	Clofazimine	12	Clinical remission	64/50
Rutgeerts (1992) (37)	16	Open label	Rifabutin, ethambutol	6–12	Mucosal healing	0
Prantera (1994) (36)	40	RCT	Clofazimine, rifampin	9	Clinical remission	84/35
Gui (1997) (32)	46	Open label	Rifabutin, clarithromycin	19	Induction of clinical remission	35/38
Thomas (1998) (41)	126	RCT	Ethambutol, rifampin, isoniazid	24	Clinical remission	93.5
Leiper (2000) (34)	25	Open label	Clarithromycin	1–15	Clinical remission	32/48
Goodgame (2001) (31)	31	RCT	Clarithromycin, ethambutol	3	Change in Harvey-Bradshaw index	Change in the active arm: P = .08 vs placebo
Shafran (2002) (40)	36	Open label	Clarithromycin, rifabutin	4–17	Clinical response	58.3
Borody (2002) (29)	12	Open label	Clarithromycin, rifabutin, clofazimine	24	Clinical remission	25
Selby (2007) (38)	213	RCT	Clarithromycin, rifabutin, clofazimine	24	Clinical remission	39/56

Adapted from: Peyrin-Biroulet L, Neut C, Colombel JF. Antimycobacterial therapy in Crohn's disease: game over? *Gastroenterology*, 2007;132(7):2594–2598

between CD, MAP, and a cellular immune response. Similar trials will need to replicate this study and also follow immune responses after the clearance of MAP to further strengthen this data.

The discovery of a NOD2/CARD15, a major Crohn's susceptibility gene (24), has advanced our understanding of the etiopathogenesis of CD and may shed light on the role of environmental exposures. The most common polymorphisms of NOD2/CARD15 in Caucasian Crohn's patients lead to defective activation of nuclear factor B pathways, which play a key role in the clearance of intracellular bacteria (25). Given that defective clearance of other bacteria have been associated with NOD2/CARD15 mutations in CD (25), ineffective clearance of intracellular MAP is an attractive theory explaining the associations between CD, MAP,

and NOD2/CARD15. Importantly, the vast majority of people with NOD2/CARD15 risk alleles do not develop Crohn's despite the demonstration of functional deficits (26). Similarly, individuals with loss of function mutations in genes of the interleukin-12-interferon- $\gamma$  axis, suffer severe disseminated mycobacterial disease when exposed to the attenuated BCG vaccine or to environmental bacteria (26). Despite functional gene deficits, these individuals lead healthy lives unless exposed. An overlap between CD and mycobacteria susceptibility genes has not yet been established, and, while provocative, these observations do not establish a causal relationship between MAP and CD. However, they may offer a window into future investigations beyond epidemiologic data and into the pathogenesis, *(continued on page 21)*

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when investigations will endeavor to determine if NOD2/CARD15 polymorphisms and other cellular signaling pathways affect MAP infection or clearance.

### TREATMENT OF CD PATIENTS WITH ANTI-MAP DIRECTED THERAPY

Long-term remission and an alteration of the natural history of CD after treatment with antibiotics active against MAP has been advocated as the strongest evidence supporting MAP as a cause of CD (4,27). At least fourteen treatment trials evaluating the efficacy of MAP in CD have been published (Table 1) (28–41). Like the case-control trials evaluating the association between MAP and CD, the treatment trials are diverse with widely variable treatment regimens, endpoints, treatment periods (one-to-24 months), sizes (five-to-213 subjects), and methodologies. Half were open label uncontrolled trials, and every trial except for two contained fewer than 51 patients. Many of these trials included anti-*M. tuberculosis* agents (31,33,37,39) which have been proved ineffective against clinical isolates of MAP in previous sensitivity analyses. Ten trials used fewer than three antibiotics which is thought to increase the risk of drug resistance (27). Six trials used agents with a broad spectrum of activity against commensal bowel flora (29,31,32,34,38,40). Results of the trials also were varied. Two trials (28,36) demonstrated efficacy for maintenance after corticosteroid induction, but five randomized trials that did not administer steroids did not. Selby, et al recently published the first long term, large scale, randomized, placebo-controlled trial (38). In this multi-center, double blind study, 213 patients with active CD (CDAI  $\geq$  200) were treated with clarithromycin, rifabutin, and clofazimine or placebo for two years. Thirty-nine percent of patients on antibiotics and 56% on placebo experienced at least one relapse between weeks 16 and 52 of the study, which was the primary endpoint. The difference did not reach statistical clinical significance (OR 2.04; 95% 0.84–4.93). Nearly 66% of the patients withdrew by week 104; the majority withdrew due to treatment failure or persistent disease activity. The authors concluded that their results did not support a pathogenic role for MAP in the majority of Crohn's patients. Assessment of *IS900* DNA from intestinal biopsies before and after treatment would likely have offered

more definitive evidence for or against a causal role of MAP. However, Selby and colleagues report in their discussion that methods for PCR testing of MAP were not reliable when the trial started (38). Conceivably, a subgroup of patients could have benefited from MAP treatment. Perhaps, a more understated conclusion would be more appropriate, but it is clear that this anti-MAP directed antibiotic regimen was ineffective for the maintenance of remission in CD patients.

### CONCLUSION

There is little doubt that environmental exposures and host genetic susceptibilities play a significant role in the pathogenesis of Crohn's disease. Some type of environmental exposure, which could include MAP, likely explains the increasing prevalence of CD and the birth cohort effect. While MAP has been identified in patients with CD, it is not clear whether it is pathogenic or simply an epiphenomenon. Plausible mechanisms of chronic intestinal inflammation caused by MAP have been theorized, but none has been proved or convincingly advanced. Treatment trials of CD with anti-MAP antibiotic therapy have been inconsistent, and the best data available does not support a pathogenic role for MAP in the majority of CD patients. Despite years of debate and mounting data from epidemiologic studies, diagnostic studies, and antibiotic treatment trials, the MAP theory of causation in Crohn's disease remains neither definitively proved nor refuted. Before widespread public health measures are proposed to eradicate MAP and before long courses of anti-MAP antibiotics are endorsed as mainstream therapies for CD, a causal relationship needs to be established. This will likely require well-designed studies that incorporate sophisticated MAP molecular detection technology, investigate genetic susceptibility to both CD and mycobacterial disease, and determine if clearance of MAP alters the immune response or, more importantly, the natural course of the Crohn's disease. ■

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