Review Article

Mycobacterium avium subspecies *paratuberculosis* (MAP) and Crohn's disease: the debate continues

Michael J. Mintz[1], Dana J. Lukin[1,2]^

[1]Division of Gastroenterology and Hepatology, New York Presbyterian Hospital-Weill, Cornell Medicine, New York, NY, USA; [2]Jill Roberts Center for Inflammatory Bowel Disease, New York Presbyterian Hospital-Weill, Cornell Medicine, New York, NY, USA

Contributions: (I) Conception and design: Both authors; (II) Administrative support: DJ Lukin; (III) Provision of study materials or patients: Both authors; (IV) Collection and assembly of data: Both authors; (V) Data analysis and interpretation: Both authors; (VI) Manuscript writing: Both authors; (VII) Final approval of manuscript: Both authors.

Correspondence to: Dana J. Lukin, MD, PhD. Division of Gastroenterology and Hepatology, New York Presbyterian Hospital-Weill, Cornell Medicine, New York, NY, USA; Jill Roberts Center for Inflammatory Bowel Disease, New York Presbyterian Hospital-Weill, Cornell Medicine, 420 East 70th St, LH-508, New York, NY 10021, USA. Email: djl9010@med.cornell.edu.

Abstract: Crohn's disease (CD) in humans and Johne's disease (JD) in ruminants share numerous clinical and pathologic similarities. As *Mycobacteria avium* subspecies *paratuberculosis* (MAP) is known to fulfill Koch's postulates as the cause of JD, there has been considerable debate over the past century about whether MAP also plays a role in CD. With recent advances in MAP identification techniques, we can now demonstrate a higher presence of MAP in CD patients compared to the general population. However, it remains unclear if MAP is playing a bystander role or is directly pathogenic in these patients. Studies have shown that there may be an immune response targeting MAP in these patients, which may underlie a pathologic role in CD. Clinical studies have yielded conflicting results as to whether anti-MAP therapy improves clinical outcomes in CD, leading to the lack of its inclusion within evidence-based clinical guidelines. Additionally, many of these studies have been small case series, with only a few randomized controlled trials published to date. In this article, we will discuss the historical context of MAP in CD, review clinical and laboratory data surrounding detection of MAP and possible pathogenesis in human disease, and suggest future directions which may finally provide some clarity to this debate.

Keywords: *Mycobacterium avium* subspecies *paratuberculosis* (MAP); Crohn's disease (CD); Johne's disease (JD)

Received: 28 February 2023; Accepted: 10 July 2023; Published online: 25 July 2023.

doi: 10.21037/tgh-23-16

View this article at: https://dx.doi.org/10.21037/tgh-23-16

Introduction

Crohn's disease (CD) is a chronic disease of the gastrointestinal tract characterized by intestinal inflammation, diarrhea, abdominal pain, and weight loss. In some cases, CD may cause intestinal stricture, fistulization, or perforation. The cause of CD is unknown, but is hypothesized to result from an inappropriate immune response to intestinal microbes within a genetically predisposed host (1). Similar to CD in humans, Johne's disease (JD) in ruminants is known to cause granulomatous intestinal inflammation, resulting in diarrhea and wasting (2). Since the late nineteenth century, *Mycobacterium avium* subspecies *paratuberculosis* (MAP), is known to be involved in the pathogenesis of JD (3). The clinical similarities of CD in humans and JD in ruminants raises the question if MAP plays a role in the pathogenesis of CD. This question has been debated over the past century, with conflicting data suggesting MAP may play a causal role or rather may be a

^ ORCID: 0000-0003-4581-2267.

bystander or possibly play no role in CD. This manuscript will review the historical context, scientific evidence, and recent clinical data that fuel this ongoing debate.

Historical origins

The first suggestion that mycobacteria could cause chronic enteritis came in 1895, when acid fast bacilli were first discovered in the bovine intestine by Johne and Frothingham (4). Twort and colleagues associated MAP with JD in 1912. In 1936, G.W. Dunkin further described MAP-associated intestinal disease in cattle as "progressive wasting, diarrhea, emaciation, with an enlarged and edematous ileocecal valve which may be much inflamed, and considerable thickening of the intestinal mucosa" (3). Chronic granulomatous, stricturing enteritis was concurrently being described in humans by Thomas Dalziel, which would later become known as CD (5). Clinical and pathologic similarities of CD and JD led many in the medical community to hypothesize that MAP was culpable in CD. However, the technology had not yet been developed to reliably culture MAP in human subjects. Therefore, this hypothesis did not gain traction for several decades.

In the 1980's, advances in culturing techniques and immunoassays allowed Chiodini *et al.* to isolate MAP in CD patients and to describe its antibiotic susceptibility (6,7), reviving the debate about the causal role of MAP in CD. This group also showed that MAP isolated from patients with CD was able to be transmitted orally to healthy goats, who then went on to develop granulomatous ileal inflammation and ulceration (8), suggesting a plausible mechanism to cause human disease. While not strictly fulfilling Koch's postulates, these data provided sufficient rationale that zoonotic mycobacterium could play a role in CD. Most recently, the advent of gene sequencing technology (including polymerase chain reaction and *in situ* hybridization) has allowed investigators to identify MAP with relative ease, leading to a reinvigoration of studies looking into its role human disease (2).

Transmission, prevalence, and pathogenesis of MAP

MAP is present in the overwhelming majority of dairy herds in the United States and has increased in prevalence over time (9). The organism is transmitted through the oral-fecal route. Several studies have cultured viable MAP from products commonly ingested by humans. This includes approximately 2% of pasteurized dairy milk samples (10-12) and 9% of infant formula samples (13). It is an obligate intracellular organism that relies on its host to replicate and is a component of the *Mycobacterium avium* complex (MAC) (14). MAP is not eradicated by traditional anti *Mycobacterium tuberculosis* regimens (15) and, in some cases, is resistant to pasteurization (10). As such, MAP may be transmitted to the human population via multiple sources, including the milk supply in endemic areas. A recent population-based screening for MAP in India demonstrated positive antibody testing in 34% and positive PCR testing in 8.4% of approximately 26,000 serum samples (16). While this is a single study that did not test for active intestinal infection, it does suggest a substantial portion of the human population may have been exposed to MAP. The high prevalence of MAP within the human population, however, raises the question why would only a subset of patients harboring MAP go on to develop CD?

The mechanism by which MAP could cause intestinal disease in humans is unknown, further contributing to the uncertainty of its role in CD. Animal models have shown that MAP loosens the tight junctions between epithelial cells, thus increasing intestinal permeability and actively recruiting macrophages to the site of infection (17). One hypothesis is that MAP activates common inflammatory pathways as seen in intestinal tuberculosis, leading to granulomatous inflammation (3), which is often seen in CD. Whether it causes direct intestinal damage or causes inappropriate immune activation through mimicry of pro-inflammatory proteins is unknown (3). There is almost certainly a host genetic component that dictates the degree of the inflammatory response, which is likely why only a small proportion of humans who are infected with MAP go on to develop CD. While many mechanisms of MAP related disease have been proposed, convincing data is lacking and warrants further investigation.

Presence of MAP in CD versus non-CD patients

Although MAP is present in both CD and non-CD patients, its prevalence should be higher in CD patients if playing a causal role. Most studies suggest this is true. Bull *et al.* performed PCR on mucosal biopsies from both CD and non-CD patients, finding a significantly greater prevalence among patients with CD (92%) compared to non-CD controls (26%) (18). This difference in prevalence was replicated in two other studies, though

to a lesser degree, with CD MAP positivity rates of 23% and 47%, both greater than non-CD controls (19,20). Additional verification came from two recent meta-analyses, concluding that there is an increased prevalence of MAP in CD patients (21,22). On the contrary, CD has not been associated with increased incidence following occupational exposure to MAP (i.e., dairy farmers or veterinarians) as compared to urban residents (23-25).

While MAP may be more prevalent in CD, it is less clear whether the presence of MAP itself is directly pathogenic or represents general dysbiosis related to intestinal inflammation. A large study by Autschbach *et al.* found that MAP was significantly more prevalent in the intestine of patients with CD (52%) than ulcerative colitis (2%) (26), suggesting that MAP prevalence is likely specific to CD and less so a bystander of non-CD intestinal inflammation. Additionally refuting a bystander effect, MAP has been shown to directly elicit an immune response in CD patients. Serum MAP specific interferon release assays and ELISA have been shown to be more prevalent in CD (27,28) and MAP reactive $CD4^+$ cells have been isolated from intestinal biopsies in CD patients (29).

CD directed therapy and its effect on MAP

The benefits of established CD therapies are traditionally thought to be due to immunomodulatory effects, however some studies have suggested that at least a part of therapeutic efficacy may be due to their anti-MAP properties (30). This argument is bolstered by *ex vivo* studies which demonstrate commonly prescribed CD medications having anti MAP activity. There is evidence that methotrexate (31), azathioprine (32), and 6-mercaptopurine (32) inhibit MAP grown in vitro. Furthermore, infliximab has been shown to have a direct effect on MAP growth and host immune response. One study showed that MAP was much less likely to grow in human macrophages treated with infliximab compared to controls (33). This study also demonstrated that CD patients positive for MAP-specific serum antibodies had a significant decrease in these serum antibodies following treatment with Infliximab (33).

MAP directed therapy and its effect on CD

Central to this debate is whether anti-MAP therapy leads to improved clinical outcomes in CD. For decades, antibiotics have been used as a primary or adjunctive treatment for CD. Meta-analyses of antibiotics targeting CD suggest that some of the most effective regimens, contain antibiotics effective against MAP, including clofazimine, rifamycin, and nitroimidazoles (15,34). However, published literature, including both case series and randomized controlled trials, has yielded conflicting results regarding formalized anti-MAP regimens in CD. *Table 1* provides a comprehensive summary of all of the clinical data that we found in our literature review. Early studies demonstrated no effect of MAP therapy on CD activity. In 1984, Shaffer *et al.* performed the first randomized controlled trial (RCT) for empiric mycobacteria treatment in CD patients. They compared treatment with rifampicin and ethambutol versus placebo in a small number of CD patients for a period of two years, which showed no difference in CD clinical outcomes (35). On the contrary, several case series subsequently showed that anti-MAP therapy could improve symptoms (36,37) and even lead to prolonged remission in certain subsets of CD patients, such as pediatric patients and patients with fistulizing disease (38,39). A large case series of 52 patients receiving anti-MAP therapy with rifabutin and a macrolide (40) showed clinical and biomarker activity with a steroid-sparing effect after a median of around two years of therapy. Results of additional large randomized controlled trials using various anti-mycobacterial combinations showed no long-term difference in clinical outcomes in CD patients who received anti-MAP therapy (41-43). While top line data from the Selby *et al.* study (43) were negative, it has been argued that the dose and formulation of the clofazimine used in this trial were suboptimal (15), though a post-hoc analysis based on an intention-to-treat analysis did identify short term benefit, with longer-term outcomes also favoring anti-MAP therapy (44,46). A limitation in these studies was that MAP positivity was not part of the inclusion criteria. It is possible, if not likely, that anti-MAP treatment would have had a more robust effect if the studies were limited to MAP positive CD patents. Additionally, none of these studies were able to confirm if MAP was successfully eradicated.

Randomized controlled trials that require the objective baseline presence of MAP via validated diagnostics and which report results in context of subsequent eradication of MAP following treatment are needed. As MAP is known to be associated with high levels of antibiotic resistance, requiring multi-drug regimens, it is possible that in many of these studies, the regimens studied were not sufficient to eradicate MAP. Therefore, it is possible that novel combinations of antibiotics could demonstrate greater efficacy in future trials.

The benefit of anti-MAP therapy may be as an adjunctive

Table 1 Anti-mycobacterial therapy trials in Crohn's disease

Author	Year	Study design	Treatment	Treatment duration	Study Population	Endpoints	Treatment results	Authors conclusion if anti-MAP therapy was effective
Shaffer *et al.* (35)	1984	Randomized controlled trial	Rifampicin + ethambutol versus placebo	24 months	5 patients in the treatment arm; 8 in the placebo arm	CDAI, hospital admission, need for steroids	No significant difference	No
Borody *et al.* (36)	2002	Prospective open label cohort	Rifabutin + clarithromycin + clofazimine	24 months (extended if clinical response)	12 patients received treatment	HBI	Significant improvement in HBI compared to baseline	Yes
Leiper *et al.* (37)	2000	Prospective open label cohort	Clarithromycin	4 weeks (extended to 12 weeks if clinical response)	25 patients received treatment	HBI, CRP	Significant improvement in HBI and CRP	Yes
Agrawal *et al.* (38)	2020	Retrospective case series	Variable; some combination of anti-MAP therapy combined with infliximab and/or fecal transplant	Variable; 70% received treatment for 3 years	7 pediatric patients in durable clinical and endoscopic remission following MAP therapy	Maintenance of remission off all therapy	Maintenance of remission for median of 8.5 years	Yes
Agrawal *et al.* (39)	2020	Retrospective case series	Variable; anti-MAP combination therapy plus standard Crohn's disease therapy	Variable	16 pediatric patients	CDAI, SESCD, CRP	Significant improvement in all endpoints	Yes
Gui *et al.* (40)	1997	Prospective case series	Rifabutin plus clarithromycin or azithromycin	Mean of 18.7 months (635 months)	52 patients	Steroid dependence; HBI, ESR, CRP	HBI, ESR, CRP all improved significantly; only two of patients remained dependent on steroids	Yes
Afdhal *et al.* (41)	1991	Randomized controlled trial	Steroids + clofazimine versus steroids + placebo	At least 18 months	25 treatment arm; 24 placebo	CDAI	No significant difference	No
Swift *et al.* (42)	1994	Randomized controlled trial	Rifampicin, isoniazid, ethambutol versus placebo	24 months	63 in treatment arm; 63 placebo	CDAI, radiologic assessment, steroid use, surgery	No significant difference in all endpoints	No
Selby *et al.* (43)	2007	Randomized controlled trial	Clarithromycin + rifabutin + clofazimine versus placebo	16 weeks; extended to 104 weeks if initial clinical response	102 in treatment arm; 111 placebo	CDAI	Significant difference at 16 weeks; no significant difference at 104 weeks	No
Agrawal *et al.* (44)	2015	Prospective case series	Variable combination of anti-MAP therapy + infliximab + hyperbaric oxygen therapy	Variable disease refractory to standard therapy	9 pediatric patients with severe fistulizing	Fistula healing	Complete fistula healing in all participants	Yes
Graham *et al.* (45)	2019	Randomized controlled trial	RHB-104 (clarithromycin, rifabutin, clofazimine) versus placebo	26 weeks	331 subjects randomized 1:1 to treatment arm versus placebo	Clinical remission (CDAI <150); clinical response (>100 point decrease in CDAI)	Significantly improved clinical remission and response rates	Yes

CDAI, Crohn's Disease Activity Index; MAP, Mycobacterium avium subspecies paratuberculosis; HBI, Harvey-Bradshaw Index; CRP, C-creation protein; SESCD, Simple Endoscopic Scoring System for Crohn's Disease; ESR, erythrocyte sedimentation rate.

therapy in a certain subsets of CD patients. A recent case series published by Agrawal et. al demonstrated that CD patients refractory to conventional therapies could obtain prolonged deep remission when anti-MAP therapy and fecal microbiota transplant was used in combination with anti-tumor necrosis factor medication (39). The same group also published a case series showing that medically refractory fistulizing CD may particularly be responsive to anti-MAP therapy when used in combination with hyperbaric oxygen therapy as well as anti-TNF therapy (37). Designing studies that look at anti-MAP therapy as an adjunctive therapy, primarily to concurrent biologic and/or small molecule therapies, should be considered in the future.

Additionally, new data have emerged from a recently completed large randomized controlled trial (45) that provides some encouragement that anti-MAP therapy may have a role in treating CD. The MAP US Trial was a large randomized, placebo controlled, multi-national study looking at the efficacy of RHB-104 in CD. RHB-104 is a combination pill containing clarithromycin, clofazimine, and rifabutin in a formulation shown in culture to have synergistic inhibitory properties on MAP growth (47). This study included 331 participants with active CD who were randomized 1:1 to receive RHB-104 or placebo, along with their standard CD-directed therapy as prescribed by their physician. The study met its primary endpoints with significantly more participants receiving RHB-104 therapy achieving clinical remission (CDAI <150) compared to those receiving placebo (week 16: 42.2% *vs.* 29.1%, P=0.015; week 26: 37% *vs.* 23%, P=0.007). It also met several secondary endpoints such as clinical response at week 26 (44.0% *vs.* 30.9%, P=0.016) and sustained clinical remission at week 52 of therapy (25.3% *vs.* 12.4%, P=0.003). The subset of patients receiving tumor necrosis factor alpha antagonists and immune modulators in combination with anti-MAP therapy were observed to have a more robust clinical response. Notably, while this study did report biochemical improvement in CRP or fecal calprotectin favoring RHB-104, endoscopic disease response was only assessed in a small subset of patients. Furthermore, the study did not perform subgroup analysis stratified by baseline MAP positivity. Therefore, it is not clear whether response correlated with MAP eradication following therapy. An open-label extension (MAP US2) (48), evaluating open-label RHB-104 among week 26 non-responders, has completed enrollment. Among 38 patients switching from placebo to RHB-104, 14 (36.8%) achieved clinical remission by CDAI, in contrast to 3 (18.8%) of those with a primary non-response to RHB-104 completing an additional 16 weeks of therapy (n=16).

Discussion

For more than a century, MAP has been associated with intestinal inflammation in ruminants and humans. While Koch's postulates have been fulfilled in JD, direct causation has been difficult to prove in CD. While MAP is detected in more CD patients than non-CD patients, MAP-directed therapy has not conclusively been demonstrated to improve clinical disease. There have been a number of case series and controlled trials over the past four decades using anti-MAP therapy. The results have been conflicting with a large heterogeneity in study design. The most recent RCT using RHB-104 provides the most encouragement that anti-MAP therapy may have a therapeutic role in CD, but that role remains unclear, and further investigation will be required. Specifically, future randomized controlled trials reporting on the objective baseline presence of MAP via validated diagnostics as well as response stratified by subsequent eradication of MAP following treatment are needed.

Most notably, we do not have a clear sense of the mechanism by which anti-MAP therapy may modify the clinical history of CD. While direct elimination of the organism could potentially reduce molecular mimicry or direct immune activation, it is possible that the antibiotic cocktail is down-modulating the abnormal immune activation characteristic of CD without directly eradicating a specific organism. Further analysis of longitudinal microbiota, proteomic, and metabolomic changes following anti-MAP therapy could help explore these hypotheses. Additionally, not all patients with CD harbor intestinal MAP. Furthermore, it is difficult to estimate the percentage of cattle harboring MAP which develop JD due to herd surveillance and pre-emptive culling of infected cattle (49,50), and it is similarly not clear what percentage of humans positive for MAP develop CD. Therefore, it is possible that MAP colonization is responsible for CD within a subset of patients, but separate inflammatory triggers may drive disease in others. Subgroup analysis of randomized controlled trials, such as MAP US, analyzing clinical response according to MAP positivity may shed some light on association *vs.* causality. This could theoretically lead to a more personalized approach to CD management, where MAP testing may help to risk-stratify patients and incorporate MAP therapy as a component of CD treatment regimens. However, given the lack of conclusive data

anti-MAP therapy cannot be included in evidence-based treatment guidelines for CD at present.

Conclusions

Since the discovery of MAP, the scientific community has sought to elucidate its potential role in the development of CD. While there does seem to be a higher prevalence of MAP infection in CD patients compared to the healthy population, it is still unclear if MAP plays a bystander role or is directly pathogenic. More recent case series and randomized controlled trials investigating anti-MAP therapy are encouraging but do not provide definitive evidence for clinical benefit. Acknowledging the heterogeneity of CD, future directions should include determining if there is a specific subset of CD patients who may have the most benefit from anti-MAP treatment, and possibly using anti-MAP treatment as adjunctive therapy in this population. Additionally, if MAP is proven to cause zoonotic disease, we will likely see further development and trials of candidate MAP vaccines, some of which are already underway (51,52). In sum, there remain many basic, translational, and clinical questions unanswered. The debate will continue.

Acknowledgments

Funding: None.

Footnote

Provenance and Peer Review: This article was commissioned by the Guest Editor (Keith Sultan) for the special series "Controversies and Updates in Inflammatory Bowel Disease" published in *Translational Gastroenterology and Hepatology* for the series. The article has undergone external peer review.

Peer Review File: Available at https://tgh.amegroups.com/article/view/10.21037/tgh-23-16/prf

Conflicts of Interest: Both authors have completed the ICMJE uniform disclosure form (available at https://tgh.amegroups.com/article/view/10.21037/tgh-23-16/coif). The series "Controversies and Updates in Inflammatory Bowel Disease" was commissioned by the editorial office without any funding or sponsorship. DJL receives educational grants for fellowship support and research grants for investigator-initiated research, receives speaker's bureaus Non-branded disease state awareness. DJL is also in the consulting or advisory boards of companies listed below: Abbvie, Abgenomics, Boehringer Ingelheim, BMS, Eli Lilly, Fresenius Kabi, Janssen, Magellan, Palatin Technologies, Pfizer, Prometheus Labs, Takeda, and sits on DSMB for one clinical trial. No funds were utilized in the writing of this manuscript. The authors have no other conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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doi: 10.21037/tgh-23-16
Cite this article as: Mintz MJ, Lukin DJ. *Mycobacterium avium* subspecies *paratuberculosis* (MAP) and Crohn's disease: the debate continues. Transl Gastroenterol Hepatol 2023;8:28.