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Is There A Map (*Mycobacterium Avium Subspecies Paratuberculosis*) For Treating Crohn's Disease?

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The 1998 NIH workshop: "Crohn's Disease—Is there a microbial etiology? Recommendations for a research agenda" reviewed the hypothesis that *Mycobacterium avium subspecies paratuberculosis* (MAP) is the cause of Crohn's disease (CD). Their conclusions were that insufficient evidence proved or disproved that MAP is a human pathogen or the cause of CD. We have evaluated new knowledge and data involving MAP in CD by examining relevant publications from a literature search. Over 145 new clinical and laboratory studies support an association between MAP and CD. Due to improved techniques, the majority of recent studies have shown a significantly higher frequency of MAP in CD. Eleven detection studies indicate that up to 95% of all CD patients are infected with MAP. Five clinical trials using MAP specific drugs demonstrate a mean remission rate of 65% (range: 50%–88%). These newer results support the notion that treatment of MAP should be considered an option for therapy of Crohn's disease.

INTRODUCTION

Crohn's disease is a chronic inflammatory disease of the gastrointestinal tract. The origin of Crohn's disease remains unknown and there is no curative therapy, either medical or surgical, for this chronic gut disorder. Three major subtypes of Crohn's disease have been proposed: an inflammatory subtype, a fibrostenotic or stricturing subtype, and a fistulizing subtype. The etiology of Crohn's disease is incompletely understood and is likely heterogeneous in origin. Multiple factors are likely to be involved in the pathogenesis of this chronic disorder, including genetic factors and disturbances in intestinal immunoregulation.

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(continued on page 42)

(continued from page 40)

In the United States and in other countries, the development of Crohn's disease demonstrates a birth-cohort effect (1). Briefly, temporal changes in the prevalence of a disease can be produced by changing the exposure of consecutive birth cohorts to an environmental influence. The effect requires exposure early in life and an environmental factor should exert its effect during a limited time interval. A similar effect had been identified in populations of patients with peptic ulcer disease, and it was hypothesized that the change was due to a decrease in salt consumption. We now know that there was a decrease in the prevalence of *Helicobacter pylori* and that this effect altered the rate of development of peptic ulcer disease.

The epidemiology of Crohn's disease (1) therefore demonstrates the importance of environmental factors in the development of this chronic disease and supports the importance of research directed at identification of potentially causative agents. Crohn's disease was defined as a unique entity in 1932 by the seminal work of Crohn and associates (2). This allowed the disease to be viewed as a separate entity rather than as intestinal tuberculosis. The identity of environmental factors involved in the development of Crohn's disease remains a mystery, although for decades individuals have suspected that an infectious agent is involved.

Over the years, many causative agents have been proposed as the etiology of Crohn's disease. These have included infectious organisms such as Chlamydia, *Listeria monocytogenes, Pseudomonas* species, and the measles virus. Indeed, ever since the first description of this disorder by Dalziel, et al (3), a mycobacterial origin for Crohn's disease has been considered.

Over the past 75 years, multiple associations have been reported between Crohn's disease and the granulomatous processes seen with mycobacterial illnesses. This notion is supported by: 1) shared pathological features between Crohn's disease and intestinal tuberculosis, and 2) the resemblance of Crohn's disease to a form of granulomatous ileitis in animals termed "Johne's disease." Johne's disease is an inflammatory bowel disease in ruminants and primates that is caused by *Mycobacterium avium subspecies paratuberculosis* (MAP) (4). Clinical manifestations in animals include weight loss, diarrhea, and swelling around the jaw. Infected animals eventually develop protein loss via the digestive tract, and subsequently death is likely. Viable human MAP has been isolated and cultured from some patients with Crohn's disease. Given its fastidious nature, it can take months to years to grow the organism. Nevertheless, successful culture of the organism is not a frequent finding.

The December 14, 1998 NIH/NIAID workshop: "Crohn's Disease—Is there a microbial etiology? Recommendations for a research agenda" was organized to review evidence for and against the hypothesis that the bacterium *Mycobacterium avium subspecies paratuberculosis* is the cause of Crohn's disease. The workshop conclusions stated that there is insufficient evidence to prove or disprove that MAP is a human pathogen or that it is the cause of Crohn's disease. It recommended further research into the etiology and pathogenesis of MAP in CD through an extensive list of research requirements designed to contribute sufficient knowledge and data so that a firm conclusion could then be achieved. The workshop stressed the need to define a potential infectious etiology, to characterize the host immune and inflammatory responses, and to conduct crucial epidemiological and familial genetic research. Our present manuscript examines both progress that has been made since the 1998 NIH consensus conference in the linkage between MAP and Crohn's disease and newer evidence that antibiotic therapy directed against MAP can be an effective treatment for Crohn's disease.

METHODS

We examined *Mycobacterium avium subspecies paratuberculosis* and Crohn's disease in Medline searches and in abstracts of international meetings covering the period from 1998 to 2006. We completed a detailed examination of all publications identified in this literature search. Over 145 additional clinical and laboratory studies were identified that report evidence supporting an association between MAP and Crohn's disease.

RESULTS

Despite the absence of an assigned champion to follow up and ensure that this work was completed following

(continued on page 45)

(continued from page 42)

Table 1
Five treatment trials of MAP antibiotic therapy in Crohn's disease

# of Patients	Antibiotics given	Follow-up (months)	Response rate	Reference
12	Rifabutin Clarithromycin Clofazimine	54	50%	11
36	Rifabutin Clarithromycin	17	58%	15
52	Rifabutin Clarithromycin	25	88%	16
20	Rifampicin Ethambutol Isoniazid Pyrazinamide	9	50%	17
28	Rifabutin Clarithromycin Clofazimine	12	50%	18

the NIH workshop, additional basic and clinical research has been reported from multiple centers around the world, contributing to our body of knowledge regarding MAP in Crohn's disease.

Eleven detection studies indicate that up to 95% of all CD patients are infected with MAP. One study revealed antibody reactivity against specific MAP antigens in the majority of CD patients, but in very few pathologic and healthy controls (5). Due to improved methods involving PCR and DNA hybridization techniques, the majority of recent studies have shown a significantly higher frequency of MAP in CD compared to older studies. Indeed, there have been multiple reports of detection of MAP in intestinal tissue obtained from patients with Crohn's disease (6-9). These results should be more reliable since several different methods of analysis have included similar findings. MAP has also been cultured in patients with Crohn's disease (10). Recent abstracts reveal both a diminished immune response to MAP in CD patients and the potential for MAP to infect intestinal epithelial cells, supporting human pathogenicity.

As shown in Table 1, five clinical trials using MAP specific drugs have shown a mean remission rate of 65% (range: 50%–88%). A clinical trial of triple antibiotic therapy for Crohn's disease demonstrated a long-term clinical response rate of 50% at 54 months of follow-up, including reversion of terminal ileal strictures in five out of 12 patients (11). Other investigators have published in abstract form (12–13) results of triple antibiotic therapy for treatment of Crohn's disease in 265 additional patients; these results report clinical response rates ranging from 62% to 66%. As a potential pathophysiological factor, studies reveal increased levels of TNF production associated with MAP detection and then subsequent lowering of levels after anti-MAP therapy (14).

DISCUSSION

There is presently no medical or surgical cure for Crohn's disease. Repeated surgical therapy increases the risk that a patient will end up with short bowel syndrome. Biological therapy with antibodies given by infusion or by subcutaneous injection therapy leads to a clinical response in 40% to 46% of patients. Biological therapy has resulted in reports of multiple serious side effects including death, lymphoma, tuberculosis, multiple sclerosis-like illness, atypical infections, and sepsis. A new treatment for Crohn's disease with lower risk of significant side effects or adverse events would be a major improvement in the medical therapy of this chronic gut disorder, and could reduce the need for surgical intervention.

Mycobacterium avium subspecies paratuberculosis (MAP) is an obligate pathogen, i.e. it cannot multiply outside the cells of animals. It is known to cause disease in a wide variety of animals, including primates and humans. MAP resides with macrophages as a slow growing cell-wall deficient form. The clinical similarities between Johne's disease and Crohn's disease have led investigators to examine a role for MAP in Crohn's disease in humans (Table 2).

MAP DNA has been detected in dairy sources and it has been suggested that the milk supply is a route of entry to human infection. Nevertheless, direct causa-

Table 2
Relationships between *Mycobacterium Avium Partuberculosis* (MAP) and Crohn's disease (CD)

Evidence for MAP in CD	*Reference*
• Birth-Cohort effect in Crohn's disease supports a role for environmental factors	1
• Pathological resemblance to Johne's disease	4
• Patient response rates in drug treatment trials of CD	11
• Rise in incidence of CD	19
• Isolation of MAP in CD by PCR, culture, histology	7

tion has not yet been shown conclusively (Table 3). MAP is present in the milk, feces, and meat of infected cattle. There is a large body of evidence which indicates that *Mycobacterium paratuberculosis* is not killed by standard food processing techniques, such as pasteurization and cooking. MAP may also be present in water supplies in areas where the feces of infected cattle wash into the water supply, and standard water treatment methods do not kill it. Mycobacteria are at least two orders of magnitude more resistant to chlorine purification than *Escherichia coli* (20). MAP infection has a significant impact on the agricultural industry around the world. As many as 58% of dairy herds in a given geographical area may be infected with MAP (21).

The typical regimen for treatment of MAP involves combination therapy. Effective therapy requires use of a macrolide antibiotic as part of this multiple-drug regimen. Agents commonly used include rifabutin, clarithromycin, azithromycin, clofazimine, ethambutol, and streptomycin. Triple antibiotic therapy is the basis for new treatment regimens in Crohn's disease. MAP resides within macrophages as a slow growing cell-wall deficient form. The consensus amongst the clinicians who have treated MAP is that a prolonged treatment regimen with antibiotics is required to induce remission, suggesting that anti-MAP therapy may be targeting the cell-wall deficient slow growing intracellular mycobacteria. Of note,

(continued on page 49)

Table 3
Weakness in the relationship between MAP infection and Crohn's disease (CD)

Critique	*Proposed Response*
CD is less common in rural areas where maximal MAP exposure is expected.	Exposure through inadequately treated milk supply is not restricted to rural population.
Environmental conditions i.e. poor sanitation and overcrowding should favor transmission of infection.	Causative agent of inadequately treated milk supply should not be affected by overcrowding or sanitation.
Remarkable paucity of evidence for vertical or horizontal transmission of CD.	Infection may be transmitted via milk supply not by vertical or horizontal transmission.
Sustained clinical responses to immunosuppressive drugs and anti-TNF-α at variance with chronic infection.	A complex interaction between an infectious agent and heightened immune response could induce improvement.
There is not a strong cellular or serologic reaction against MAP in affected patients.	Intracellular location of MAP may prevent cellular or serologic systemic response.
Detection of MAP in CD is neither disease-specific nor bacterial-specific.	CD likely a response to complex interaction of host and infectious agents with protean manifestations.

(continued from page 46)

Table 4
Side effects related to use of MAP antibiotics

Antibiotic	*Side effects*
Rifabutin	Reddish orange discoloration of body fluids Rash, nausea/vomiting, headache Anemia/thrombocytopenia
Clarithromycin	Abnormal metallic taste Rash, nausea/vomiting, headache Elevated BUN, elevated Protime
Azithromycin	Diarrhea Headache, nausea/vomiting
Clofazimine	Hyperpigmentation reversible over months to years after discontinuation
Ethambutol	Myocarditis/pericarditis Headache/disorientation Exfoliative dermatitis Gout, nephritis Peripheral neuritis, optic neuritis
Streptomycin	Neurotoxicity Skin rash, arthralgia Eosinophilia Ototoxicity, nephrotoxicity

reports (11) of reversal of ileal strictures in Crohn's disease with the use of antibiotic therapy provide a major opportunity for improvement in the medical therapy of fibrostenotic Crohn's disease.

All three of the commonly proposed antibiotics, rifabutin, clarithromycin, and clofazimine, are indicated for the treatment of mycobacterial disease. As shown in Table 4, these antibiotics are known to have distinct side-effects. Rifabutin is a semisynthetic ansamycin antibiotic which has been derived from rafamycin. Its use is indicated for the prevention of disseminated *Mycobacterium avium* complex disease. Clarithromycin is a semi-synthetic macrolide antibiotic. Its use is indicated for the prevention of disseminated *Mycobacterium avium* complex disease. Clofazimine is a substituted iminophenazine red dye. Its use is indicated for the treatment of the lepromatous form of *Mycobacterium leprae*. It has been shown in vitro to inhibit the growth of *Mycobacterium avium* and *Mycobacterium bovis.* The addition of clofazimine in the antibiotic combination may help to enhance the efficacy by increasing the intracellular activity of rifabutin and clarithromycin.

As an alternative explanation for these findings, for decades, antibiotics have served as an important therapy for Crohn's disease and its associated complications. The rationale for antibiotic usage has been based upon studies suggesting that luminal bacteria play an important role in the pathogenesis of Crohn's disease. Antibiotics that are used to treat Crohn's disease may also function as immunomodulators or as antioxidants (22). Thus, antibiotics could exert their effect by mechanisms other than by their antimicrobial properties.

In summary, significant basic and clinical research has been conducted to evaluate an association between *Mycobacterium avium paratuberculosis* and Crohn's disease. Multiple techniques have identified MAP in intestinal tissue from patients with Crohn's disease. Antibiotic therapies directed against mycobacterial infection have provided prolonged response rates that are comparable to or better than the present use of biological agents in patient with active Crohn's disease. ■

References

1. Sonnenberg A, Koch TR. Period and generation effects on mortality from idiopathic inflammatory bowel disease. *Dig Dis Sci,* 1989;34:1720-1729.
2. Crohn BB, Ginzburg L, Oppenheimer GD. Regional ileitis; a pathological and clinical entity. *JAMA*, 1932;99:1323-1329.
3. Dalziel TK. Chronic intestinal enteritis. *BMJ*, 1913; 2: 1068-1070.
4. Greenstein RJ. Is Crohn's disease caused by a mycobacterium? Comparisons with leprosy, tuberculosis, and Johne's disease. *Lancet*, 2003;3:507-514.
5. Collins MT, Lisby G, Moser C, et al. Results of multiple diagnostic tests for *Mycobacterium avium subsp. Paratuberculosis* in patients with inflammatory bowel disease and in controls. *J Clin Microbiol,* 2000;38(12):4373-4381.
6. Autschbach F, Eisold S, Hinz U, et al. High prevalence of *Mycobacterium avium subspecies paratuberculosis* IS900 DNA in gut tissues from individuals with Crohn's disease. *Gut*, 2005;54:944-949.
7. Sechi LA, Mura M, Tanda F, et al. Identification of *Mycobacterium avium subsp. paratuberculosis* in biopsy speciments from patients with Crohn's disease identified by in situ hybridization. *J Clin Microbiol,* 2001;39(12):4514-4517.

8. Gearry RB, Aitken JM, Roberts RL, et al. Gastrointestinal *Mycobacterium avium paratuberculosis* and Crohn's disease. *J Gastroenterol Hepatol,* 2005;20:1943.
9. Sechi LA, Scanu AM, Molicotti P, et al. Detection and isolation of *Mycobacterium avium subspecies paratuberculosis* from intestinal mucosal biopsies of patients with and without Crohn's disease in Sardinia. *Am J Gastroenterol,* 2005;100:1529-1536.
10. Naser SA, Ghobrial G, Romero C, et al. Culture of *Mycobacterium avium subspecies paratuberculosis* from the blood of patients with Crohn's disease. *Lancet*, 2004;364: 1039-1044.
11. Borody TJ, Leis S, Warren EF, et al. Treatment of severe Crohn's disease using antimycobacterial triple therapy—approaching a cure? *Dig Liver Dis,* 2002;34:29-38.
12. Selby W, et al. Antibiotic therapy for Crohn's disease. The Gastroenterological Society of Queensland. 9th Annual Colon Update in Gastroenterology and Hepatology. June 2005, Queensland, Australia.
13. Borody TJ, et al. Australian Gastroenterology Week Meeting. October 2005, Brisbane, Queensland, Australia.
14. 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-α secretion. *Dig Liver Dis,* 2007; in press.
15. Shafran I, Kugler L, El-Zaatari FA, Naser SA, Sandoval J. Open clinical trial of rifabutin and clarithromycin therapy in Crohn's disease. Dig Liver Dis, 2002; 34(1):22-28.
16. Gui GP, Thomas PR, Tizard ML, et al. Two-year outcomes analysis of Crohn's disease treated with rifabutin and macrolide antibiotics. *J Antimicrob Chemother,* 1997;30:393-400.
17. Hampson SJ, Parker MC, Saverymutter SH, et al. Quadruple antimycobacterial chemotherapy in Crohn's disease: Results at 9 months of a pilot study in 20 patients. *Aliment Pharmacol Ther,* 1989;3(4):343-352.
18. Douglas A, Bramble MG, Silcock JC. Antimicrobial treatment for Crohn's disease: does it prevent surgery and how fast does it act? *Gut*, 2000;(suppl) 48:A360.
19. Ekbom A. In: Targan S, Shanahan F, Karp LC, eds. *Inflammatory bowel disease: From bench to bedside,* 2nd Ed. 2003.
20. Le Dantec C, Duguet JP, Montiel A, et al. Chlorine disinfection of atypical mycobacteria isolated from a water distribution system. *Appl Environ Microbiol,* 2002; 68:1025-1032.
21. Obasanjo IO, Grohn YT, Mohammed HO. Farm factors associated with the presence of *Mycobacterium paratuberculosis* infection in dairy herds on the New York State Paratuberculosis Control Program. *Prev Vet Med,* 1997;32:243-251.
22. Karp SM, Koch TR. Micronutrient supplements in inflammatory bowel disease. *Dis Mon,* 2006; 52: 211-220.