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Antimycobacterial Therapy in Crohn's Disease: Game Over?

See “Two-year combination antibiotic therapy with clarithromycin, rifabutin and clofazimine for Crohn's disease” by Selby W, Pavli P, Crotty B, Florin T, Radford-Smith G, Gibson P, Mitchell B, Connell W, Read R, Merrett M, Ee H, Hetzel D, on page 2313.

It has been almost a century since Dalziel, who first described what is at the present time known as Crohn's disease (CD), commented on its similarity to John's disease, which occurs in dairy herds and is caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP).¹ Interest in a possible infectious origin for CD was renewed in 1989 when Chiodini et al² cultured apparently identical MAP from 3 patients with CD. The controversy continues to rage; indeed, it has even increased following alarming reports on the detection of MAP in water supplies and milk,^{3,4} although this was not confirmed in a recent case-control study.⁵ Detection of the specific DNA insertion sequence *IS900* of MAP in a significant number of patients with CD, but not in controls,^{6,7} and the finding of MAP in the bloodstream of these patients⁸ has also contributed to enhancing the controversy. It is not the purpose of this editorial to review all the arguments for or against MAP as a causative agent for CD, as recently done by Sartor.⁹ It is, however, noteworthy that both advocates and detractors of this theory almost invariably conclude their demonstration by saying that the most irrefutable evidence that MAP causes CD lies in long-term remission of clinical manifestations and altered natural history of the disease following clearance of the infection with antibiotics. Antibiotic therapy is indeed effective in 80%–90% of patients with tuberculosis and nontuberculosis lung disease.^{10,11}

Fourteen studies assessing the efficacy of antimycobacterial therapy in CD, and which enrolled a total of 508 patients, have been published (Table 1).^{12–26} Two small, placebo-controlled trials of antimycobacterial therapy in

combination with a tapering course of corticosteroids demonstrated efficacy in the maintenance phase (following corticosteroid withdrawal) for clofazimine monotherapy and for a combination of ethambutol, clofazimine, dapsone, and rifampicin in patients with active CD.^{12,21} By contrast, 5 placebo-controlled trials in which antimycobacterial therapy was administered without corticosteroids failed to demonstrate efficacy.^{13,15,22,23,25,26} In 2000, a meta-analysis suggested that antimycobacterial treatment may be effective in maintaining remission achieved by corticosteroids.²⁷ However, because of the heterogeneity of the trials, which used a wide range of antibiotic combinations administered for variable periods to a small number of patients, no definitive conclusion could be drawn. Those studies were also criticized because they used earlier tuberculosis drugs, such as ethambutol and isoniazid, which are not effective against *M avium* complex infection, and <3 antibiotics (a number considered critical for preventing development of drug resistance). Clarithromycin and azithromycin, macrolide compounds that are considered to be the most effective drugs for treatment of MAP, were then used in 4 subsequent studies with encouraging results.^{14,16,17,19,24} However, the only placebo-controlled randomized trial was 3 months long, and the antibiotic regimen associated only 2 drugs, namely, clarithromycin and ethambutol.¹⁶ The general conclusion of most experts in the field was that the proof of efficacy of combination antibiotic therapy in CD remained elusive due to the absence of a properly conducted large placebo-controlled randomized trial.

In this context, results from the Australian trial published in the current issue of *GASTROENTEROLOGY* were awaited eagerly.²⁸ In a large placebo-controlled, double-blind, randomized trial which enrolled a total of 213 patients with active CD (Crohn's Disease Activity Index \geq 200), Selby et al²⁸ evaluated the efficacy of 2-year combination therapy with clarithromycin (750 mg/d), rifabutin (450 mg/d), and clofazimine (50 mg/d) in maintaining clinical remission following corticosteroid with-

Table 1. Efficacy of Antimycobacterial Therapy in CD in Open-Label and Randomized Controlled Trials

Author (year)	Patients included (n)	Trial	Antibiotic combination therapy	Concomitant steroid therapy	Treatment period (mos)	Primary endpoints	Efficacy (main result): Treatment/placebo (%)
Elliott (1982) ¹⁵	51	RCT	Sulfadoxine, pyrimethamine	No	12	Changes in CDAI scores	Clinical remission: 38/50
Schaffer (1984) ²³	27	RCT	Ethambutol, rifampin	No	12	Changes in CDAI scores (or any clinical indicator of disease activity)	Clinical remission: 36/64
Basilisco (1989) ¹³	24	RCT	Rifabutin	No	6	Changes in the Harvey-Bradshaw index	Clinical remission: 29/38
Hampson (1989) ¹⁸	20	Open label	Ethambutol, rifampin, isoniazid, clofazimine (or pyrazinamide)	Yes	9	Clinical remission defined as CDAI < 150	Clinical remission: 50
Prantera (1989) ²⁰	5	Open label	Dapsone	No	1	Changes in CDAI scores and mucosal healing	Clinical remission: 40
Afdhal (1991) ¹²	49	RCT	Clofazimine	Yes	12	Clinical remission (use of modified CDAI scores)	Clinical remission: 64/50
Rutgeerts (1992) ²²	16	Open label	Rifabutin, ethambutol	No	6–12	Endoscopic healing in the neoterminal ileum	Mucosal healing: 0
Prantera (1994) ²¹	40	RCT	Clofazimine, rifampin	Yes	9	Clinical remission and mucosal healing	Clinical remission: 84/35
Swift (1994) ²⁵ , Thomas (1998) ²⁶	126	RCT	Ethambutol, rifampin, isoniazid	No	24	Changes in the Harvey-Bradshaw index and CDAI scores, steroid sparing, need for surgery, radiological change	Clinical remission: 35/38
Gui (1997) ¹⁷	46	Open label	Rifabutin, clarithromycin (or azithromycin)	Yes	19	Changes in the Harvey-Bradshaw index and serum CRP, steroid sparing, need for surgery	Induction of clinical remission: 93.5
Leiper (2000) ¹⁹	25	Open label	Clarithromycin	Yes	1–15	Changes in the Harvey-Bradshaw index and serum CRP	Clinical remission: 32/48
Goodgame (2001) ¹⁶	31	RCT	Clarithromycin, ethambutol	Yes	3	Changes in the lactulose-mannitol test and the Harvey-Bradshaw index	Changes in the Harvey-Bradshaw index in the active arm: <i>P</i> = .08 vs placebo
Shafran (2002) ²⁴	36	Open label	Clarithromycin, rifabutin	No ^a	4–17	Response defined as marked improvement in CDAI scores	Clinical response: 58.3
Borody (2002) ¹⁴	12	Open label	Clarithromycin, rifabutin, clofazimine	Yes	24	Changes in the Harvey-Bradshaw index	Clinical remission: 25

CDAI, Crohn's Disease Activity Index; CRP, C-reactive protein.

^aA probiotic supplement was given to counterbalance antibiotic-induced degradation of the intestinal flora.

drawal. During a 16-week induction period, patients were randomized to receive a tapering regimen of corticosteroids in association with these 3 antibiotics or placebo. At week 16, 122 patients who achieved remission entered the maintenance phase, during which they continued trial medications for 2 years. After 2 years of treatment, trial medications were ceased, and patients in remission were followed up for 1 more year. The primary endpoints

were the proportion of subjects experiencing ≥ 1 relapse of CD at 1, 2, and 3 years. At the end of the 16-week induction period, there was a significantly higher percentage of subjects in remission in the antibiotic arm (66%) than in the placebo arm (50%; *P* = .02). The proportion of patients who relapsed at 1, 2, and 3 years was not significantly different in the 2 arms: 39% in the antibiotic group relapsed between weeks 16 and week 52

versus 56% in the placebo group ($P = .054$); 26% versus 43% relapsed at 2 years ($P = .14$); and 59% versus 50% at 3 years ($P = .54$). The number of subjects remaining in the study fell progressively from 16 weeks, and only 32 patients completed the study. The study thus did not meet its primary end points.²⁸ The authors conclude that these results do not support a role for MAP in CD, and that the use of antibiotics with a broad spectrum of activity against luminal organisms may explain the early efficacy of antibiotic combination added to corticosteroids as induction therapy.

Results of this first long-term, large-scale, randomized, placebo-controlled trial seem to be particularly convincing for several reasons. The authors used a combination of 3 antibiotics active intracellularly, with good tissular diffusion and effectiveness against MAP, thus minimizing the risk of drug resistance. Antibiotics were given for up to 2 years. Corticosteroids, which are active against MAP (that resembles *M leprae* more than *M tuberculosis* in its response to corticosteroids²⁹), were given in association during the induction period in order to optimize antibiotic efficacy, as previously shown.²⁷ The negative message from this study was reinforced by the lack of improvement observed in important secondary parameters such as C-reactive protein and mucosal healing in a subset of patients. The low number ($n = 32$) of patients remaining at 3 years could raise the possibility of a type II error, but most withdrawals were due to progression of disease, even when repeat courses of prednisolone were used. This finding, together with high observance rates (69%–74% for weeks 53–104) favor true failure of trial medication rather than a statistical bias.

The mere take-home message from this study is that the antibiotic regimen that was used has no role in maintenance treatment of CD. Does it definitely refute any therapeutic role of antimycobacterial therapy in CD? The question remains open. Selby et al²⁸ did not assess *IS900* DNA in biopsies by polymerase chain reaction and serologic response to MAP before and after therapy. There is thus no evidence that MAP, if present, was cleared by treatment. MAP may require higher doses of antibiotics or other antibiotics for eradication. Subtherapeutic doses of rifabutin (450 mg), clarithromycin (750 mg), and clofazimine (50 mg) per day²⁸ were used, whereas the optimal dose of rifabutin, clarithromycin, and clofazimine for treatment of *M avium* complex infections is 600 mg/d, 1000–2000 mg/d, and 100 mg/d, respectively.^{30,31} The minimal inhibitory concentration (MIC_{90}) of clarithromycin (at which 90% of *M avium* complex isolates are inhibited) is 4 $\mu\text{g/mL}$.³² When the drug is given orally at a dose of 400 mg, blood levels attain 2 $\mu\text{g/mL}$, and they rise to 4.4 $\mu\text{g/mL}$ at a dose of 1200 mg.³² Despite being used in combination, low-dose antibiotics may fail in the long run owing to the development of drug resistance.

Another retrospective study from Australia demonstrated mucosal healing in 22 of 39 (56.4%) patients treated with higher doses of rifabutin (up to 600 mg/d), clofazimine (up to 100 mg/d), and clarithromycin (up to 1 g/d) for 6 months to 9 years.³³ Important reservoirs of bacteria may not have been reached by antibiotics. One possible example is mesenteric fat, which is in close contact with the intestine. Mesenteric fat is hypertrophied in CD and is heavily colonized by bacteria.³⁴ It has been recently shown that *M tuberculosis* can enter into adipocytes, where it accumulates intracytoplasmic lipid inclusions and survives in a nonreplicating state that is insensitive to the major antimycobacterial drug, isoniazid.³⁵ Whether the same phenomenon occurs for MAP in the mesenteric fat of patients with CD remains unknown. A better understanding of the virulence mechanisms of MAP is required to develop more targeted therapies against this intracellular pathogen. In this setting, the recent identification of novel virulence factors, by generating a mutant library of MAP, may help us to design more effective vaccines and chemotherapies directed against animal and human infections with MAP.³⁶

Selby et al²⁸ did not consider genes or the environment in the etiology of CD. MAP may be responsible for, and anti-MAP therapy effective in, a subset of patients who are genetically predisposed. The common truncation mutation of the nucleotide-binding oligomerization domain 2 (*NOD2*) is associated with defective clearance of invasive *Salmonella* infection in epithelial cells.³⁷ *NOD2* mutations in CD are associated with diminished mucosal antimicrobial peptide expression.³⁸ Thus, an attractive explanation linking *NOD2* to CD is that of ineffective clearance of intracellular MAP infection. *NOD2* status was not assessed in Australian patients, but available phenotypic information argues against an association, because the disease site, including the ileum where *NOD2* is predominantly expressed, did not affect response rates.²⁸ Likewise, no relation was found between *NOD2* mutations and a positive MAP serology in a Canadian study.³⁹ Other susceptibility genes may influence intracellular MAP clearance and infection in CD. A genome-wide association study identified the *interleukin-23 receptor (IL-23R)* as a novel susceptibility gene for CD.⁴⁰ Mounting evidence suggests that IL-23, similar to IL-12, is critical for generation of an adaptive immune response that is protective against intracellular pathogens, including *M tuberculosis* infection.^{41,42} Finally, as stated by the authors, the aim of this trial was not to definitively prove or disprove the hypothesis that MAP plays a role in the etiology of CD.²⁸ Interestingly, in Selby et al's trial, concomitant use of immunomodulatory therapy was the only parameter that was associated with a significantly greater response in the antibiotic group. It has recently been shown that 6-mercaptopurine and methotrexate inhibit MAP

growth in vitro.⁴³ These data are compatible with the hypothesis that clinical improvement in patients with inflammatory bowel disease treated with immunomodulators could be due to treatment of a MAP infection.⁴³

In conclusion, this is a landmark study, because it is the largest randomized placebo-controlled antibiotic trial ever performed in CD. Despite some caveats, as mentioned, it should help to refute arguments favoring MAP as an etiologic agent, although it does not definitively eradicate that hypothesis. Nor does it dismiss the connection between the bacteria and the disease. Despite its broad-spectrum activity, the antimycobacterial antibiotic regimen used in the Australian study is not particularly effective against Gram-negative bacteria. New therapeutic trials should target members of the intestinal flora, such as *Bacteroides* and adhesive *Escherichia coli*, which have now been associated with CD by different groups throughout the world.^{44–48}

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