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Alimentary Tract

Anti-mycobacterial therapy in Crohn's disease heals mucosa with longitudinal scars

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Abstract

Background. A possible causative link between Crohn's disease and *Mycobacterium avium ss paratuberculosis* has been suggested.

Aim. To report unique scarring in Crohn's disease patients treated with anti-*Mycobacterium avium ss paratuberculosis* therapy.

Patients. A retrospective review of 52 patients with severe Crohn's disease was conducted. Thirty-nine patients who had at least one follow-up colonoscopy during treatment were included.

Methods. Patients received rifabutin (up to 600 mg/day), clofazimine (up to 100 mg/day) and clarithromycin (up to 1 g/day) – anti-*Mycobacterium avium ss paratuberculosis* therapy – for 6 months to 9 years. Ramp-up dosing was used. Colonoscopies and histological analyses monitored progress.

Results. Twenty-two patients (56.4%, 22/39) healed with unusual scarring, which appeared as branched, ribbon-like, elevated lines. In 2/6 patients (33.3%) who had >3 years of treatment after scarring occurred, scars receded, becoming imperceptible as full healing occurred. Histologically, a marked reduction in inflammation occurred in 15/39 patients (38.5%). Of these, 6/15 patients (40%) displayed restoration of normal mucosa. Longitudinal scarring occurred in 12/15 patients (80%) with improved histology.

Conclusions. The presence of scarring fading to normal mucosa on anti-MAP therapy implies a more profound healing not seen with standard anti-inflammatory and immunosuppressant drugs. Longitudinal scarring and consequent healing with normal histology should become a standard treatment goal for Crohn's disease.

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1. Introduction

Crohn's disease (CD) is an immunologically mediated, granulomatous, chronic inflammatory disorder of the gastrointestinal tract, often complicated by ulceration, stricturing and fistula formation. The cause of this inflammatory disease is unknown, but it is most likely induced by mucosal exposure to an infecting organism in genetically susceptible individuals where a persistent Th1-driven cell-mediated immunity is elicited [1]. One leading infectious candidate is *Mycobacterium avium ss paratuberculosis* (MAP), the causative agent of inflammatory bowel disease in sheep and in other ani-

mal species (Johne's disease) with which CD shares clinical and histological similarities [2–4]. Unlike leprosy, CD and MAP appear to have met the four Koch's postulates [5] yet its causative role remains surrounded by controversy. The supportive evidence for MAP in CD includes the identification of MAP DNA in Crohn's intestinal tissue [6,7] and in circulating leukocytes [8] and an antibody response to MAP-specific antigens [9,10]. However, the 'cause and effect' relationship remains controversial due in part to the difficulties in detecting MAP in human tissues using *in vitro* culture.

Despite the technical difficulties with MAP culture, the further evidence needed to support a contributory role for MAP in CD is a specific anti-mycobacterial therapy which will closely correlate total healing with MAP eradication. To date, the results from multi-drug regimens that included

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anti-tuberculosis antibiotics to treat CD have been largely unsuccessful [11–13], due to the choice of sub-optimal antibiotics ineffective against atypical mycobacteria. The continuing debate has led to trials of antibiotic combinations selected for their effect against intracellular atypical mycobacteria. The results of these published trials were much more encouraging [14,15] but no study has yet correlated efficacy of combination antibiotic therapy with the presence and eradication of *MAP*. Using more specific anti-*MAP* triple therapy combining rifabutin, clarithromycin and clofazimine, we [16] have reported a response in CD to anti-*MAP* therapy, with prolonged clinical remission in 6 of 12 of our patients with severe CD, and 3 patients remaining disease-free and off therapy for more than 2 years. In this paper, we reported a recurrent feature of unique, profound mucosal scarring and healing which we first photographed in a patient on anti-*MAP* treatment in 1997. Similar findings were photographed but not discussed in the accompanying paper by Shafraan et al. [15]. The current review was undertaken to extend these observations and describe for the first time the effect of anti-*MAP* therapy in achieving unique mucosal healing patterns in patients with ulcerating CD.

2. Materials and methods

A retrospective review of 52 patients on anti-*MAP* therapy with severe colonic CD failing conventional therapies was conducted. Of these followed up at our clinic, 39 patients (20M, 19F) had at least one follow-up colonoscopy dur-

ing the course of treatment and thus were included in the review. The median age at CD diagnosis was 36.5 years (range 15–75 years). Anti-*MAP* therapy was defined as either clarithromycin with rifabutin [$n = 1$] [14] or triple therapy of clarithromycin, rifabutin and clofazimine [16].

Both endoscopic and histologic activities of IBD were based upon terminal ileal and/or colonic inflammation. Colonoscopic findings indicating severe inflammatory changes included erosions or deep ulceration; contact bleeding; exudate; granularity; cobblestoning; friability; inflammatory nodularity, masses or fistulae; extensive erythema; and concomitant erythema/edema. Histological acute and chronic ileitis or colitis were recorded as the most severe inflammation found in available biopsies and were reported by a pathologist ranging from inactive chronic inflammation, through to mild, moderate, and severely active inflammation. Complete clinical response comprised patients who were asymptomatic and had normal levels of inflammatory markers[e.g. CRP, ESR].

Patients received rifabutin (up to 600 mg/day), clofazimine (up to 100 mg/day) and clarithromycin (up to 1 g/day) for between 6 months and 9 years. One patient received rifabutin and clarithromycin dual therapy for eight months and then continued with added clofazimine. A ramp-up dosing schedule was used to minimize adverse effects. Concomitant therapies included mesalazine, prednisone, azathioprine, bismuth subcitrate, olsalazine, zinc, iron tablets, methotrexate and *N*-acetyl glucosamine in some patients. Many of these medications were taken for variable periods concomitantly during introduction of the anti-*MAP* therapy

Table 1
Mucosal healing and scarring in 22 Severe Crohn's disease treated with anti-*MAP* therapy

Patient no.	Age (years)	Sex	Crohn's site	Duration of disease (years)	Initial endoscopic findings	Length of treatment at time of scarring (months)	Endoscopic healing with scarring	Histology grade
1	52	F	EC	20	Pseudopolyps	3	TC, DC	Normal
2	32	M	AC, TC	4	Large deep	45	AC, TC	Active
3	43	M	TI, SC, PA	9	Ulcers, Rectal abscess	35	TC, SC	Moderate
4	26	F	TC, TI	0.4	Ulcers	20	TC, TI	Normal
5	15	M	EC	1	Ulcers	8	TI	Mild
6	26	F	TC, D	4	Ulcers, TI stenosis	12	TC	Mild
7	38	M	AC, DC	20.2	Large ulcers	11	AC, DC	Mild
8	24	F	EC	4	Ulcers, Cobblestones	25	TC, DC	Mild
9	25	F	DC, SC	1.8	Ulcers Pseudopolyps Anal-rectal strictures	30	SC	Mild
10	33	M	DC, SC	2	Ulcers	13	DC	Active
11	28	M	DC, PA	11	Ulcers Pseudopolyps	18	DC	Normal
12	44	F	TC, DC SC	2.5	Ulcers	48	DC, SC	Normal
13	39	F	R, TI	9.1	Ulcers	9	R, TI	Moderate
14	54	M	TC, SC	9	Ulcers Pseudopolyps	1	EC	Normal
15	33	M	EC	0.7	Ulcers Pseudopolyps	19	DC	Mild
16	48	M	AC, PA	6	Ulcers	18	AC	Normal
17	48	M	TC	20	Ulcers	10	TC	Active
18	37	M	IC	2	Ulcers, rectal Abscess,	17	IC	Active
19	43	F	EC	14	Ulcers	26	TC, DC	Active
20	24	M	TI, SB	1.8	Ulcers cobblestones	13	TI, D	Active
21	51	M	EC	4	Large ulcers	16	EC	Mild
22	36	F	TC	4	Large ulcers	23	TC	Normal

AC, ascending colon; D, duodenum; DC, descending colon; EC, entire colon; IC, ileocaecal valve; PA, peri-anal; R, rectum; SC, sigmoid colon; TC, transverse colon; TI; terminal ileum.

and were progressively tailed off. The most common concomitant drug was mesalazine but it was not considered to have been a contributor to the scarring phenomenon having been taken prior to commencement of anti-MAP by the majority of patients for prolonged periods without resulting in scarring until after the anti-MAP therapy was introduced. Patients underwent colonoscopic examinations at intervals to monitor progress but there was no uniformed timing of colonoscopies in individual cases to reliably measure ‘time-to-scarring’. Hence, the ‘interval when scarring was first observed’ is listed in the Table but the actual interval to its first detectable appearance may have been sooner had an earlier colonoscopic examination been performed. A descriptive visual analysis of the mucosa was obtained prior to and during treatment. Endoscopic biopsy specimens were taken for histology throughout the treatment and scored by a single pathologist.

3. Results

Of the 39 patients who had at least one follow-up colonoscopy at least 12 months after commencement of anti-MAP therapy, 22 patients (56.4%) were found to heal with unusual, longitudinal scarring of the colonic mucosa (Table 1).

In contrast, suggestion of mucosal healing with scarring was rarely observed being recognizable in only 2 of 56 files reviewed of CD patients treated continuously more than 12 months using anti-inflammatory and immunosuppressive drugs. Earliest evidence of mucosal longitudinal healing scars was observed after 1 month of anti-MAP in one patient and some evidence of resolving scarring was still present at 108 months into anti-MAP therapy in these 22 patients (Table 1). Multiple mucosal ulcerations documented on pre-treatment colonoscopies had largely healed leading to longitudinal scarring in the terminal ileum [uncommon], but particularly in different segments of the colon, over the ileocaecal valve, in the rectum and in the duodenum. In all cases, scars only devel-

oped once the inflamed mucosa began to heal. Fig. 1 shows the endoscopic changes with regression of pseudopolyps leading to scar formation and healing in a patient before and after anti-MAP therapy.

Scars appeared as long, pale, initially elevated ribbon-like patterns extending longitudinally along the mucosa, and were observed in all areas of the colon only where deep ulcers had previously been seen. Complete clinical response was achieved in six patients with mucosal healing and no visible or histological inflammation. Of these, 2/6(33%) (Table 1) were patients in whom after 3 or more years of treatment following the appearance of scarring, scars began to recede, becoming barely visible as fine white lines on the background of a fully healed mucosa (Fig. 2).

At the histological level, all 39 patients showed active disease at commencement of anti-MAP therapy. Of these patients, 38.5% (15/39) showed a marked reduction in the acute and chronic inflammatory infiltrates at 1–9 years of therapy (mean = 3.1 years). In 6 of the 15 patients (40%) with improvement in histological parameters, after 18 months to 6 years of treatment, no histological evidence of inflammation was observed with the intensity of lymphoplasmacytic infiltrate returning to normal levels. Twelve of the 15 patients (80%) with improved histology also had prominent longitudinal scarring. An example of this histological improvement is shown in Fig. 3.

In spite of the presence of healing scars seven patients had active inflammation and three had mild to moderate inflammation. Here it needs to be emphasized that biopsy sites during these clinical follow-up colonoscopies largely avoided healed areas, sampling rather visibly inflamed areas as part of routine clinical follow-up. This resulted in a clear bias which underestimates the profound histological healing in the areas of scarring.

4. Discussion

In this retrospective review we describe a novel yet frequent appearance of linear scarring associated with mucosal

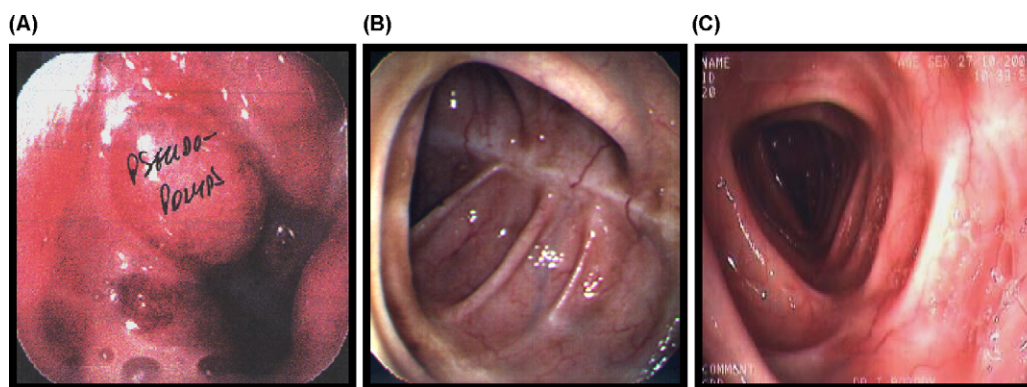


Fig. 1. Regression of pseudopolyps in the left descending colon in a patient on anti-MAP therapy. (A), Before anti-MAP therapy – severe inflammation with contact bleeding and pseudopolyps; (B), 1 year on anti-MAP therapy – elevated longitudinal scarring with haustrations pointing to the scar; (C), 2 years on anti-MAP therapy – The scar has now become a faint line lacking distinct elevation from the mucosal wall, suggesting progressive softening of scar tissue.

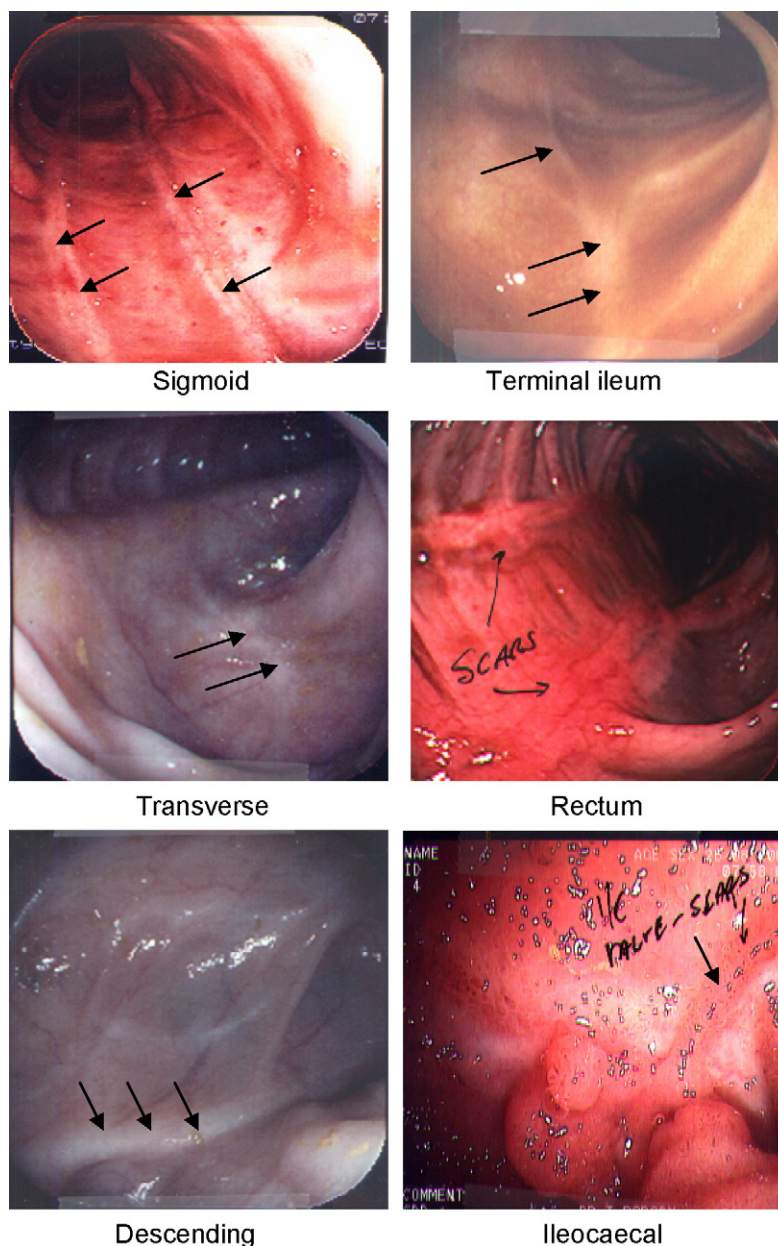


Fig. 2. Endoscopic examples showing completely healed mucosa and longitudinal scarring (see arrows) in Crohn's disease treated with anti-MAP therapy in all areas of the colon.

healing when patients with severe, ulcerating CD are treated with anti-MAP therapy. To our knowledge, this healing process, seen with such high frequency in patients receiving this new treatment, has not been previously reported as a feature in CD patients treated with anti-inflammatory and immunosuppressive agents. Scarring occurred in all areas of the colon and the duodenum but particularly in the transverse and descending colon replacing previous ulcerated mucosa. The scars appeared to recede over time with the resolution of mucosal inflammation and ulceration. Mucosal healing and normalisation in histological pattern can be achieved during treatment of CD using anti-inflammatory agents, [17,18] and antibodies directed against tumour necrosis factor (TNF- α) [19]. Gener-

ally, the mucosa appears to heal with disappearance of ulcers, regression of mucosal friability, and disappearance of contact bleeding and oedema. However, mucosal healing leading to longitudinal scar formation is not a common endoscopic feature in patients treated with anti-inflammatory and biological agents even in well-controlled trials [20], reporting on azathioprine [18], methotrexate [21] or infliximab [22]. Even the Crohn's Disease Endoscopic Index of Severity (CDEIS), an established standard measure of mucosal healing during anti-inflammatory therapy, does not even include this category of healing [23].

An inherent selection bias may exist in our retrospective clinical practice review but this is largely ruled out on the

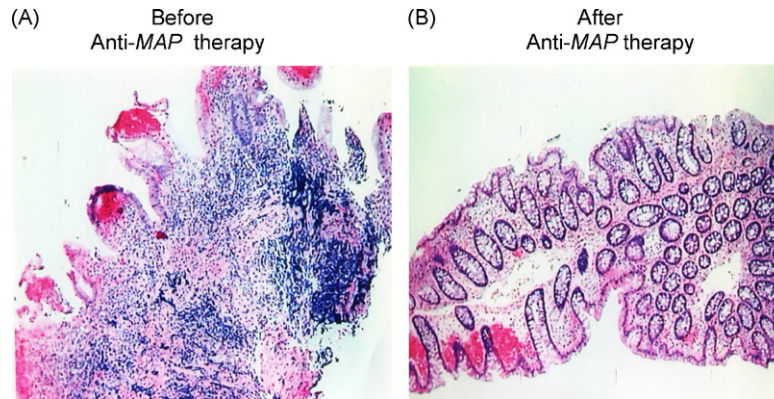


Fig. 3. Histologic changes in a patient before (A) and >3 years after anti-*MAP* therapy (B). Endoscopic full-thickness biopsy of the colonic mucosa demonstrates a dramatic improvement in mucosal architecture and a marked reduction of the inflammatory cell infiltrate accompanied by restoration of normal epithelial mucin.

grounds that scar formation occurred generally in patients treated with anti-*MAP* therapy for a considerable length of time and there was no focus on biopsying the healed areas. Rather, biopsies were directed at those areas that were still inflamed for clinical reasons. We and others have previously reported a similar, preliminary finding of mucosal healing in patients treated for a long periods with anti-*MAP* therapy [15,16] but in those reports we were not aware of its significance. In this retrospective study, patients excluded were those who did not have ulceration, who were treated for too short a period of time and those with no follow-up colonoscopy to date. We would like to suggest that this unusual finding in a proportion of our CD patients on long-term anti-*MAP* therapy may represent an ultimate endpoint of mucosal healing in patients on CD therapy.

The apparent failure to achieve total histological healing in some patients with longitudinal scarring is at first surprising. However, on review of our patient files the data showed that when biopsies were taken of areas with ongoing, resolving inflammation there is a lack of histological healing as scar areas were not biopsied. On the other hand, those patients whose biopsies were taken from the scarred area had total histological healing. Because this is a retrospective analysis of clinical practice data the results need to be confirmed by a well-designed prospective study addressing sites of biopsy, mucosal healing rates, and scarring mechanisms with anti-*MAP* therapy.

Unlike surgical scars in which fibroblasts play a key role in tissue repair, the colonic healing associated with scarring achieved during anti-*MAP* therapy could reflect transmural healing of full-thickness inflammation, a process most likely to involve smooth muscle cells. Indeed, it has been reported that endoscopic assessment of inflammation in CD better reflects transmural histopathology and the severity and extent of inflammation than mucosal biopsies [24]. Comparison of the biopsy specimens taken before and after anti-*MAP* therapy showed a restored mucosal architecture with a complete disappearance of inflammatory cell infiltrate

in the patients with complete mucosal healing. Furthermore, patients with resolving histological parameters also showed signs of greater mucosal healing, prominent longitudinal scarring and complete clinical response. This suggests that the depth of healing achieved and consequent scarring in patients with deep colonic ulceration could be related to an underlying etiology which is specifically addressed by anti-*MAP* therapy.

The precise mechanism of scar formation as a consequence of mucosal healing with anti-*MAP* treatment is unclear. It is known that granuloma formation where infectious agents are sequestered and resist rapid immune destruction is a normal process leading to tissue repair. Ultimately, scar tissue replaces inflammation which clears the offending agent. Such sequence of events is seen in intestinal inflammation due to bacterial infection [25–27]. Granulomas are a common microscopic feature of CD in which an etiologic agent including DNA specific for some microorganisms including *MAP* have been identified [28–31]. Thus the progressive nature of mucosal healing in patients treated with anti-*MAP* therapy and the consequent scarring that leads to complete normalisation of the mucosa could represent a novel healing process associated with a persistent infection in the intestinal mucosa. The scar tissue may indicate that anti-*MAP* therapy eradicates the offending organism to achieve a more complete and full-thickness healing of the intestinal wall, a therapeutic outcome ostensibly not generally attained by other therapies for CD. However, we have no direct evidence that our CD patients were *MAP*-infected since an antigen-specific serological assay was not available and, moreover, such assay is not generally used due to poor sensitivity and specificity. In addition, culture and PCR capability was not yet available. Thus our assumption is based on recent evidence that *MAP* is detected in a majority of patients with CD [3,31].

In conclusion, it seems that anti-*MAP* therapy can have a profound effect on mucosal healing and the effect is perhaps demonstrated by longitudinal scarring and histological

repair. Provided other studies confirm our findings this healing appearance, rarely seen otherwise in IBD clinical practice nor reported in the literature, could argue for the use of anti-*MAP* treatment to achieve complete healing with normalisation of the mucosa in Crohn's disease.

Practice points

- Anti-*MAP* therapy in severe Crohn's disease can lead to healing.
- Unique longitudinal healing scars are a feature of *MAP* healed Crohn's.
- Profound healing is achievable in severe Crohn's disease with antibiotics.

Research agenda

- Independent confirmation of profound healing is now needed.
- Prospective studies documenting 'time to healing', correlation with histology, and duration of scar persistence are needed.

Conflict of interest statement

Thomas J Borody has a pecuniary interest in Giaconda Ltd., the licensor of Myoconda (anti-*MAP* therapy).

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