While the exact cause of Crohn’s disease (CD) is not known, it is thought that chronic inflammation in CD results from an inappropriate and chronic activation of the innate and adaptive mucosal immune systems in a genetically susceptible host, and that enteric microflora play a pivotal role in the initiation and maintenance of the disease.\(^1\) Organisms such as \textit{Pseudomonas maltophilia}, \textit{Mycobacterium kansasii}, \textit{Chlamydia trachomatis}, \textit{Bacteroides fragilis}, \textit{Listeria monocytogenes}, \textit{Escherichia coli} and \textit{Mycobacterium avium} subspecies \textit{paratuberculosis} (MAP) have been implicated and identified in the intestines of patients with CD; however, no other putative pathogenic organism except MAP causes a chronic granulomatous inflammation of the intestines in every other species in which it is present.\(^2\) Moreover, because of remarkable clinical, morphological and epidemiological similarity between intestinal tuberculosis (caused by \textit{Mycobacterium tuberculosis}) and CD in humans and Johne’s disease (caused by \textit{Mycobacterium avium paratuberculosis}) in cattle, a mycobacterial pathogen is thought to be a causative organism of CD.\(^3\)–\(^5\) An association and causality of \textit{Helicobacter pylori} with peptic ulcer disease revolutionized the treatment of peptic ulcers; similar implication of MAP as a cause of CD has raised hopes amongst physicians, microbiologists and basic scientists for the cure of this disease.\(^8\)

In the first report almost 25 years back, Chiodini and colleagues isolated cell-wall deficient cells, called spheroplasts, from tissue samples after several months of incubation from patients with CD. These spheroplasts were sub-cultured and they later developed a cell wall that stained positively with Ziehl-Neelson staining; they were classified as mycobacterial like organisms.\(^9\)–\(^11\) These isolates were later confirmed as \textit{M. avium paratuberculosis} by DNA hybridization. The unusual nature of the cell wall-deficient MAP and the challenges in culture of MAP with its fastidious and slow growing requiring months to years to culture and multiple studies reporting failure to culture MAP from patients with CD dampened the initial zeal.\(^12\)

There was then a period of silence in the 1990s. With availability of better culture techniques and use of molecular techniques to identify MAP in 2000s, there was resurgence of interest of many laboratories in this field. There has been a flurry of publications since then relating CD and MAP.\(^13\)–\(^25\)

MAP infection in humans is difficult to detect. The organisms are intracellular and minimize their own immune recognition. They are therefore difficult to isolate and propagate in culture and are relatively resistant to chemical and enzymatic lysis. Reliable access to their DNA is only achieved during sample processing by combining exposure to stringent lysis buffers with an additional optimised mechanical disruption step. Freezing samples and tissue extracts especially at -20°C substantially reduces the PCR detection rate of their GC-rich DNA. The identification of MAP in patients with CD can be done by several different techniques such as culture of MAP from the intestines and blood; PCR amplification of blood or intestinal biopsies using IS900 DNA, a sequence specific for MAP; in-situ hybridization in tissues using IS900 sequence; serological tests using MAP specific antigens and direct visualization of MAP by light microscopy.\(^4\)–\(^26\) According to a meta-analysis, compared with individuals free of IBD, the pooled odds ratio (OR) of those with CD having MAP was 7.01 (95% CI 3.95–12.4) using PCR in tissue and 1.72 (1.02–2.90) in studies using ELISA in the serum.\(^27\) According to another recent meta-analysis, MAP was detected more frequently from patients with CD compared with those with ulcerative colitis (risk difference 0.19, 95% CI, 0.10–0.28).\(^28\)
In the present issue of the *Journal*, Sasikala *et al.* have shown that none of their 81 patients with CD and 85 controls had MAP-DNA in intestinal biopsies using MAP-specific IS900. Similar results have also been reported from another center.\(^{30}\)

The inconsistency of findings across different studies, using methods that are prone to technical variability, continues to be a major impediment to a definitive assessment of the role of MAP in CD. It is worth noting that in-house PCR in the tuberculosis laboratories are prone to error, in part because of the many methodological variances between laboratories such as DNA extraction protocols, adequate removal of tissue inhibitors, variable PCR conditions (direct PCR vs. nested PCR, differing number of cycles and extension times, etc.) and amplicon detection methods.\(^{31}\) There are two other reasons which can hamper the sensitivity of PCR for MAP-related infections: (i) the cell wall is very thick and hard to lyse, and (ii) the organism is buoyant and therefore resists concentration by centrifugation.\(^{3,32}\)

The strength of the association as shown by two meta-analyses and consistent detection of MAP-DNA by many groups does not prove causality, and may arise from prior ingestion of nonviable organisms in water or milk. However, the detection of MAP-DNA in blood of patients with CD may suggest that a viable form of the organism is present in them.\(^{33,34}\) The presence of MAP in blood may also be a reflection of an increase in intestinal permeability which is seen in 40% of patients with CD.\(^{35}\) However, the more frequent detection of MAP in CD compared with UC argues against increased gut permeability as the sole explanation.\(^{27}\)

Before we say that MAP is the cause of CD, MAP has to meet the Koch’s postulates for microbial causation.\(^{36}\) There are some preliminary data that suggest that MAP has partially fulfilled the Koch’s postulates. There are also evidences that we are exposed to MAP through our environment. MAP has been isolated from pasteurized milk infant formula made from pasteurized milk, breast milk from women with CD, surface water, soil, cow manure “lagoons” that can leach into surface water, and municipal tap water, providing multiple routes of transmission to humans.\(^{4,37–39}\)

While there are many protagonists who believe that MAP may be a cause of CD, there are many who argue against the association. First, farmers and rural dwellers should be at increased risk of a livestock-associated pathogens, yet there is no evidence that they have increased rates of CD.\(^{40}\) Secondly, environmental conditions, such as poor sanitation and overcrowding which should favor transmission of an infection, actually appear to protect against CD. Thirdly, immunosuppressive drugs and anti-tumor necrosis factor-α should be associated with increased rates and severity of mycobacterial diseases, rather than improvement in the disease.\(^{41}\)

The protagonists defend objections in many ways. We are not aware of the best staining technique for MAP. The organism that causes cat scratch disease, *Bartonella henselae*, was not seen under the microscope until researchers finally thought to try the Warthin-Starry silver stain on diseased lymph nodes almost 30 years after the disease was first described.\(^{42}\) While now “easily” seen by the usual Hematoxylin & Eosin stain, *Helicobacter pylori* were visualized by the Warthin-Starry silver stain in Warren and Marshall’s first report.\(^{43}\) All the available histochemical stains have not been tried to stain MAP in histological specimens of patients with CD. Most studies have used mucosal biopsies from the lesions or blood for showing MAP using various techniques described above. Mucosal biopsies generally are superficial and surrounded by necrosis and exudation; MAP may not be present in sufficient numbers in the superficial layers of intestine and may thus skip detection. It is possible that MAP is more often present in deeper layers of the intestine, and the fistulous tracts. One of the very important pathological features of CD is creeping fat in the mesentery which is not seen in other inflammatory conditions. The mesenteric fat may also be a good site to look for MAP.\(^{44}\) To say that MAP is somehow “harmless to man” we will have to prove that and they are present in human as a bystander, once it is well known that MAP causes chronic inflammation of the intestines in many cattle, and even in subhuman primates.

The present study has included relatively large number of patients with CD. A negative association between CD and MAP as reported by the authors raises a question that in a tuberculosis endemic country, is MAP less prominent? Does *M. tuberculosis* or any other intestinal infection inhibit MAP? Before we believe this, such data needs to be replicated at other centers. There is another concern related with the duration of the disease included in this study. Most patients with CD reporting to tertiary care centers have a disease spanning over a long period of time.\(^{45,46}\) the duration of symptoms in this study is relatively short (mean 8 months). It may not be wise to conclude, as the authors of this study have concluded, that MAP is not the cause of CD in India, especially once there are evidences from other parts of the world. If MAP is the cause or not the cause of CD, it will not vary according to country or even continent.

The failure of the anti-MAP drug trial (clarithromycin, rifabutin, and clofazimine) from Australia beyond sixteen weeks may not be taken as the final evidence against an association between MAP and CD.\(^{47,48}\) We do not exactly know the anti-MAP drugs, the best combination regimen, and the duration of the therapy.\(^{49}\) It is just a beginning of the process, the evidences either for or against have started pouring in. We will have to work and connect evidences into a complete story.

The remarkable work by Barry Marshall and Robin Warren led to a change in the concept and more importantly treatment of peptic ulcers. Despite many arguments against it, MAP stands tall as a candidate pathogen for CD. Gastroenterologists, microbiologists, pathologists, surgeons, and
basic scientists should join hands and try find out the exact cause of this crippling disease.

References


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