Review article: Mycobacterium avium subsp. paratuberculosis as one cause of Crohn’s disease


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INTRODUCTION

The Greeks conceived of inflammation in terms that were large, observable and descriptive. ‘Rubor’, ‘tumor’, and ‘dolor’ were used to describe the process. The era of the microscope reduced our phraseology to the level of the cells and new words such as ‘polys’, ‘lymphs’, and ‘granulomas’ were used to describe inflammation. Now we are in the age of the molecule and use words such as ‘TNF’, ‘interleukins’, and ‘cytokines’ when studying inflammation. These perspectives are all valuable in describing and understanding the immune response but do not provide insights into what initiates the process.

SUMMARY

A number of theories regarding the aetiology of Crohn’s disease have been proposed. Diet, infections, other unidentified environmental factors and immune disregulation, all working under the influence of a genetic predisposition, have been viewed with suspicion. Many now believe that Crohn’s disease is a syndrome caused by several aetiologies. The two leading theories are the infectious and autoimmune theories. The leading infectious candidate is Mycobacterium avium subsp. paratuberculosis (Mycobacterium paratuberculosis), the causative agent of Johne’s disease, an inflammatory bowel disease in a variety of mammals including cattle, sheep, deer, bison, monkeys and chimpanzees. The evidence to support M. paratuberculosis infection as a cause of Crohn’s disease is mounting rapidly. Technical advances have allowed the identification and/or isolation of M. paratuberculosis from a significantly higher proportion of Crohn’s disease tissues than from controls. These methodologies include: (i) improved culture techniques; (ii) development of M. paratuberculosis-specific polymerase chain reaction assays; (iii) development of a novel in situ hybridization method; (iv) efficacy of macrolide and anti-mycobacterial drug therapies; and (v) discovery of Crohn’s disease-specific seroreactivity against two specific M. paratuberculosis recombinant antigens. The causal role for M. paratuberculosis in Crohn’s disease and correlation of infection with specific stratification(s) of the disorder need to be investigated. The data implicating Crohn’s as an autoimmune disorder may be viewed in a manner that supports the mycobacterial theory. The mycobacterial theory and the autoimmune theory are complementary: the first deals with the aetiology of the disorder, the second deals with its pathogenesis. Combined therapies directed against a mycobacterial aetiology and inflammation may be the optimal treatment of the disease.
Crohn’s disease is an inflammatory disease or, more accurately, an inflammatory syndrome. Insights into the cytokine profiles of Crohn’s disease tissue furthers our understanding of the disease process and attempts to block or modify these cytokine profiles may result in symptomatic relief and histological improvement in patients. However, should understanding the root cause and cure of Crohn’s disease be desired, the aetiologies of the syndrome need to be unearthed. The aetiology of Crohn’s disease is thought to be multifactorial, involving an interaction between genetic susceptibility, undefined environmental triggers, and immune-mediated tissue injury. In this regard, several environmental triggers such as infections and diet, as well as genetic factors, are postulated to be involved in pathogenesis of the disease. None of these factors alone have been proven to cause the disease. However, there is considerable suggestive evidence to support an environmental (e.g. infectious) aetiology for Crohn’s disease. Some of the older observations include: (i) a concordance rate of only 44% among monozygotic twins with inflammatory bowel disease; (ii) clustering of inflammatory bowel disease cases among spouses and other members of affected families; and (iii) clustering among unmarried individuals having close contact.

For nearly 100 years following Dalzeil theorized that inflammatory bowel syndrome was caused by a mycobacterium, sceptics discounted the theory for the following reasons: (i) the agent could not reliably be grown in culture; (ii) an agent-specific immune response in the host could not be detected; and (iii) the agent could not be visualized in involved tissue specimens. Despite the similarity of the experience with tuberculoid leprosy, the inability to accomplish these tasks led to the conclusion that there was no proof that mycobacterial infection caused Crohn’s disease. Conventional wisdom evolved to the point where most accepted the view that mycobacteria did not cause Crohn’s disease. Only a few scientists around the world, who could not disprove this association, held onto the belief that mycobacterial infection might somehow be responsible for causing a portion of Crohn’s disease and continued their work on the subject. The similarities in the clinical and histological features between bovine paratuberculosis, human intestinal tuberculosis and Crohn’s disease were undeniable and the isolation of Mycobacterium avium subsp. paratuberculosis (M. paratuberculosis) from intestinal tissues of Crohn’s disease patients further supported their beliefs.

Technical advances and innovative studies led to a rapid accumulation of evidence to support the theory that an intestinal infection with an ‘atypical mycobacteria’, namely M. paratuberculosis, may indeed be one of the causes of the Crohn’s disease syndrome. M. paratuberculosis was grown from diseased bowel and from the breast milk of lactating Crohn’s disease patients, and was also identified more frequently in Crohn’s disease tissues than in controls, using the polymerase chain reaction. The detection of cell wall-defective M. paratuberculosis, deep within the inflamed intestinal wall of Crohn’s disease patients by in situ hybridization, provided further support for its involvement in the pathogenic process. In addition, treatment of a few patients with anti-mycobacterial therapy that included macrolide antibiotics has led to clinical remission (and possibly cure) in these patients. Furthermore, a M. paratuberculosis-specific humoral immune response has been identified in Crohn’s disease patients. However, as with other infectious diseases, our knowledge regarding the many interacting variables, means of prevention, and specific chemotherapy, are all still inadequate. This review summarizes the recent studies implicating M. paratuberculosis as one of the aetiologic agents of this disease.

CULTURE EVIDENCE

During the first part of the twentieth century, it was theorized that the inflammatory bowel syndrome that became known as Crohn’s disease was caused by a mycobacterial infection. This theory fell out of favour with the mainstream largely due to the fact that investigators were unable to identify an offending organism. Interest in a possible mycobacterial aetiology was revived when Chiodini et al. reported the isolation of then uncharacterized mycobacteria from tissues of three Crohn’s patients. These fastidious organisms were initially isolated as cell wall-defective organisms. Using molecular approaches, the isolates were identified as M. paratuberculosis. Because M. paratuberculosis caused a chronic inflammatory bowel disease in primates and ruminants (known as paratuberculosis or Johne’s disease), its possible association with the development of Crohn’s disease was suspected. Since then, multiple reports describing the isolation of cell wall-defective M. paratuberculosis from Crohn’s disease patients have been published. These reports are of particular
interest because they provided a plausible explanation as to why culture proved so difficult in isolating *M. paratuberculosis* from involved tissues. The organism morphosed into an extremely fastidious cell wall-defective form and had an absolute dependence on the presence of mycobactin for growth.

**Cultures of *M. paratuberculosis* from intestinal tissues of Crohn’s disease patients**

Several groups demonstrated that cell wall-defective *M. paratuberculosis* could be recovered from intestinal tissues of patients with Crohn’s disease at a higher frequency than from patients with ulcerative colitis and from non-inflammatory bowel disease control patients. Until recently, it was impossible to verify the identity of the cell wall-defective organisms as mycobacteria, or to discern whether they were identical to the acid-fast bacillus (cell wall-intact) form of the bacteria. Based on a *M. paratuberculosis*-specific polymerase chain reaction assay and DNA hybridization techniques for the detection of a specific 1.4-kb insertion sequence (IS900) in *M. paratuberculosis*, the identification of some of these two forms as *M. paratuberculosis* was confirmed in Houston and in London. It was confirmed that the cell wall-defective forms and the reverting acid-fast bacilli were in fact different forms of *M. paratuberculosis*. In Houston, cell wall-defective isolates were obtained from 15 Crohn’s disease patients. Using the IS900 probe, 40% were shown to be *M. paratuberculosis*. Similarly, using an IS900-specific polymerase chain reaction assay, Wall *et al.* identified the IS900 in six of 17 isolates (35%, Chiodini’s cell wall-defective isolates) in long-term cultures of intestinal tissue from patients with Crohn’s disease. None of their five control cultures were positive for *M. paratuberculosis*. However, the majority of the remainder of their cultures contained mycobacterial DNA of unknown specific origin. The cell wall-defective isolates that tested positive with the IS900-specific polymerase chain reaction came only from Crohn’s patients (i.e. *M. paratuberculosis* cell wall-defective forms were not isolated from patients with other diseases or from normal controls).

Based on the above culture data, Schwartz *et al.* inoculated processed tissue specimens from patients with Crohn’s disease into modified mycobacterial media. The cultures developed amorphous residues, which were later identified as *M. paratuberculosis*, by applying the IS900-polymerase chain reaction assay.

The organisms from resected tissues and biopsy specimens were identified after 10–12 and 40 weeks of incubation, respectively. *M. paratuberculosis* was present in six out of seven (86%) resected tissues and in four out of 20 (20%) biopsy specimens vs. two of 36 (5.6%) control biopsy specimens. None of the three surgically resected control specimens were positive for *M. paratuberculosis*. It should be noted and emphasized that the high degree of *M. paratuberculosis* positives in the surgically resected tissues of Crohn’s disease patients are possibly due to the selected sub-population of Crohn’s disease patients and to the quantity of the tissue specimens processed. It was apparent that larger specimens which included the deeper portions of the bowel wall were more apt to yield positive results.

Of significance, *M. paratuberculosis* has recently been shown to be a human pathogen. Hermon-Taylor *et al.* identified *M. paratuberculosis* by IS900-specific polymerase chain reaction analysis in the cervical lymph nodes of a 7-year-old boy with scrofula. Five years later the boy developed classic ileal Crohn’s disease. He received treatment with clarithromycin and rifabutin and developed a fibrotic stricture of his distal ileum which subsequently required surgical resection for a small bowel obstruction. The resected specimen contained DNA from *M. paratuberculosis*, as tested by polymerase chain reaction analysis (Hermon-Taylor, personal correspondence).

**Cultures of *M. paratuberculosis* from milk of lactating mothers with Crohn’s disease**

In April 2000, using the same culture techniques that were described earlier, Naser *et al.* reported the presence of *M. paratuberculosis* in milk samples obtained from lactating mothers diagnosed with Crohn’s disease. The milk was separated into a cream layer and a pellet layer by centrifugation. Following 12 weeks of incubation in MGIT media, microbial growth was observed. Only the pellet layers, which contained the heavier cellular component of milk, from the two mothers with Crohn’s disease were positive for *M. paratuberculosis* by the IS900-specific polymerase chain reaction assay. The five control samples obtained from healthy individuals were negative for *M. paratuberculosis*.

The practical protocol of the short-term cultivation of *M. paratuberculosis* from clinical specimens has been described in detail. These latest culture studies underscore the similarities of Crohn’s disease to Johne’s disease.
IDENTIFICATION OF M. PARATUBERCULOSIS IN CROHN’S DISEASE TISSUES BY POLYMERASE CHAIN REACTION

Using the IS900-specific polymerase chain reaction amplification, the frequency of M. paratuberculosis in Crohn’s disease was found in a significantly greater proportion than from ulcerative colitis or non-inflammatory bowel disease control tissue specimens; five out of 40 (12.5%) controls and one out of 23 (4.3%) ulcerative colitis vs. 26 out of 40 (65%) Crohn’s disease, \( P < 0.01 \). Similarly, Quirke et al. identified M. paratuberculosis in two out of five patients with Crohn’s disease and in one out of five patients with ulcerative colitis. In agreement with these findings, and more recently, a group from Denmark also found the IS900 in 11 out of 24 (45%) patients with Crohn’s disease, two out of 10 (20%) patients with ulcerative colitis, and seven out of 24 (29%) children with other gastrointestinal diseases. Another group from France identified M. paratuberculosis in 13 out of 18 (72%) tissue samples from children with Crohn’s disease, one out of five (20%) children with ulcerative colitis, and seven out of 24 (29%) children with other gastrointestinal diseases. A group from the USA also found the IS900 in 11 out of 24 (45%) patients with Crohn’s disease, two out of 10 (20%) patients with ulcerative colitis, and three out of 28 (10%) patients with non-inflammatory bowel disease. Additionally, a group from the USA also found the IS900 in eight out of eight patients with Crohn’s disease, two out of two patients with ulcerative colitis and none of two non-inflammatory bowel disease controls. When summarized, these data show a frequency of M. paratuberculosis in Crohn’s disease that is substantially higher [52 positive out of 87 patients tested (63%); \( P < 0.01 \)] than ulcerative colitis [seven positive out of 45 patients tested (15.5%)] and non-inflammatory bowel disease controls [15 positive out of 94 individuals tested (16%)].

VISUALIZING THE ORGANISM IN INTESTINAL TISSUE OF CROHN’S DISEASE BY IN SITU HYBRIDIZATION

Polymerase chain reaction cannot discriminate between cell wall-defective and acid fast bacilli forms of M. paratuberculosis. It was reasoned that the DNA from the cell wall-defective forms of M. paratuberculosis may be accessible to a M. paratuberculosis-specific probe by in situ hybridization that precluded staining of the DNA of the acid fast form because of its waxy and tough cell wall. Clearly, detection of any form of M. paratuberculosis in Crohn’s disease patient tissues would support its association with the disease. Hence, a novel and specific in situ hybridization method for detection of the cell wall-defective forms of M. paratuberculosis in tissues was developed. The technique was based on the IS900-specific probe labelled with digoxigenin. The procedure was tailored to be specific for the detection of cell wall-defective forms of M. paratuberculosis. When applied to clinical specimens, 40% of 15 Crohn’s disease patients with granulomas showed positive signals in myofibroblasts and in macrophages. Only 4.5% of 22 Crohn’s disease patients with non-granulomatous disease were positive, as were 9.5% of 21 patients with ulcerative colitis. None of the 22 patient controls with other diseases were positive for M. paratuberculosis. Additional control tissue specimens with cell wall-defective forms of related mycobacteria (M. tuberculosis and M. smegmatis) and unrelated organisms (H. pylori or E. coli) were tested and were negative by in situ hybridization.

This novel in situ procedure provided a definitive association of M. paratuberculosis with Crohn’s disease, as well as established a method to distinguish the acid-fast from the cell wall-defective forms of M. paratuberculosis. The demonstration of cell wall-defective forms of M. paratuberculosis in a large proportion of granulomatous Crohn’s disease tissues confirms previous reports of its association with the granulomatous type of the disease. Finding M. paratuberculosis inside macrophages within the wall of diseased bowel strongly argues against it being a mere contaminant; it suggests that M. paratuberculosis is the pathogenic organism.

REMISSION OF CROHN’S DISEASE BY ANTI-MYCOBACTERIAL THERAPY

Pathogenic mycobacteria are usually intracellular parasites that reside and thrive inside macrophages and other host cells. M. paratuberculosis cannot exist outside of mammalian hosts due to its inability to obtain iron from the environment. Hence, to be effective, antimicrobial therapy should include agents that have intracellular activity. The macrolide antibiotics, azithromycin and clarithromycin, have known intracellular drug activity and are highly suited for treating these infections. Only in recent years have these antibiotics been available. M. paratuberculosis, like other ‘atyypical’ mycobacteria, is generally resistant to standard antituberculous drugs and in vivo infection is difficult to eradicate. Therefore, multi-drug regimens that include macrolide drugs may be essential for treatment to be
The failure of these same treatments in treating some forms of Crohn’s disease with anti-mycobacterial therapy which included macrolide antibiotics. Given the evidence of these recent studies, it is likely that such a trial would demonstrate efficacy of antibiotics in Crohn’s disease may not have chosen the best combination of drugs. However, Graham et al. and Gui et al. reported clinical remission in 72% of seven and 93.5% of 56, respectively, of Crohn’s disease patients who received anti-mycobacterial therapy which included macrolide antibiotics. Given the evidence of these recent studies, it is likely that such a trial would demonstrate efficacy in treating some forms of Crohn’s disease with antibiotics. The failure of these same treatments in some cases of Crohn’s disease is unexplained, but may be attributed to the fact that Crohn’s disease syndrome may be present in at least two forms; one form is caused or triggered by M. paratuberculosis infection and the other form is induced by some other unknown aetiology. If this dichotomy exists, then a test that would discriminate between the infected and non-infected Crohn’s disease patients is essential to ensure proper grouping of M. paratuberculosis-infected Crohn’s disease patients from non-infected Crohn’s disease patients before the onset of any drug treatment. The inability to document a consistent anti-mycobacterial therapy for Crohn’s disease may be reflected by the inability to identify those patients with M. paratuberculosis infection before treatment. It is possible that the confusing and ‘less than impressive’ results of the published antibiotic trials to date are due to the choice of sub-optimal antibiotics, and to the possibility that there is more than one cause of the Crohn’s syndrome.

M. PARATUBERCULOSIS-SPECIFIC IMMUNE RESPONSE IN CROHN’S DISEASE PATIENTS

It has been argued that if an infecting organism were to be the cause of the inflammatory disease process known as Crohn’s disease, then surely there must be evidence of a specific immune response against the organism. In the past, the failure to identify a specific immune response against M. paratuberculosis was cited as evidence that the organism could not be a cause of Crohn’s disease.

There are many ubiquitous species of mycobacteria. Because M. paratuberculosis contains high levels of cross-reacting antigens with other mycobacteria or other acid-fast organisms, it was almost impossible to demonstrate a M. paratuberculosis-specific humoral immune response in patients with Crohn’s disease. However, the use of recombinant clones expressing M. paratuberculosis antigens have provided a more sensitive and discriminating test than those employing crude antigens. A genomic library was constructed using M. paratuberculosis (strain Linda; isolated from Crohn’s patients) chromosomal DNA in an expression vector, and screened with hyper-immune rabbit antibodies. As a result of this approach, 24 recombinant clones expressing M. paratuberculosis antigen(s) or epitope(s) were purified and analysed. These clones were used to determine whether a M. paratuberculosis-specific humoral immune response could be detected in patients with Crohn’s disease compared to those with other mycobacterial diseases, and controls. Two putative recombinant clones encoding MAP-specific antigens, 35K (p35) and 36K (p36), were identified and their seroreactivities were evaluated by the immunoblotting technique. Specific reactivity was found when M. paratuberculosis-specific p35 and p36 recombinant antigens were used against Crohn’s disease patient sera compared to controls. Of 61 sera from Crohn’s disease patients, 77% reacted with both antigens, compared to 8% of 12 sera from ulcerative colitis patients and 0% of 35 samples from normal controls (P < 0.001).

The specific sero-reactivity against M. paratuberculosis p35 and p36 antigens in most Crohn’s disease patients’ sera is consistent with a causal role for the organism in the development of the Crohn’s disease syndrome. The possibility arises that these antibody profiles may allow prospective identification of those patients who have M. paratuberculosis as the cause of their Crohn’s disease syndrome and may allow separation of these individuals from patients who may have some other cause for their disease.

IS M. PARATUBERCULOSIS A POTENTIAL FOOD-BORNE PATHOGEN?

Mycobacterium paratuberculosis commonly infects dairy cows, leading to Johne’s disease, a chronic inflammatory disease of the intestine. Current data suggest that a high prevalence of infection of up to 18% of the cattle in the US has been recorded. The infection is chronic,

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progressive and incurable. Eradication of the disease is difficult because of its insidious nature, long incubation period, and inadequate methods for early detection. Cattle with sub-clinical infections shed *M. paratuberculosis* in their faeces onto pastures from where it may find its way into water supplies. Excretion of *M. paratuberculosis* in milk also occurs and the bacterium spreads through the blood to multiple internal organs. Consequently, raw products originating from infected cattle may harbour *M. paratuberculosis*. Indeed, recent studies reported that *M. paratuberculosis* is found in milk, water and meat. It has also been found that this organism is relatively resistant to heat and may survive the pasteurization process. In a study of the retail milk supply in Britain between 1990 and 1994, a litre of milk from a supermarket contained about 2000–3000 *M. paratuberculosis* organisms per ml. Grant et al. also isolated viable *M. paratuberculosis* from 19% of 31 cartons of pasteurized cows’ milk obtained from 16 supermarkets. Hence, it is conceivable that *M. paratuberculosis* may be transmitted to humans by the consumption of contaminated food. This again emphasizes the importance of developing a diagnostic test to detect this organism in animals with sub-clinical Johne’s disease and humans with Crohn’s disease. The detection of *M. paratuberculosis* is extremely important for anti-mycobacterial therapy in humans and disease prevention in animals. Elimination of *M. paratuberculosis* from our dairy herds is not a difficult task but would be expensive and has been resisted by the USDA and the dairy industry based on their belief that *M. paratuberculosis* is not a human pathogen.

**DISCUSSION**

As early as 1913, Dalziel in Scotland wondered whether the terminal ileitis seen in patients, which looked like intestinal tuberculosis, but in whom no evidence of the *M. tuberculosis* could be found, was related to Johne’s disease. We have learned much about the microbiology of *M. paratuberculosis* since bovine Johne’s disease was first described. We now know that *M. paratuberculosis* also infects a wide variety of mammalian species. Thus far, *M. paratuberculosis* is known to induce inflammatory bowel disease in cattle, sheep, deer, alpacas, bison, monkeys, rabbits and chimpanzees. The spectrum of clinical and pathological manifestations amongst the various species varies, but the differences are not as great as those described for *M. leprae* in humans, where the clinical manifestations of the infection ranges from the sub-clinical, to the mild tuberculoid form, and to the severe lepromatous form. The clinical manifestations of leprosy depend on the host’s immune response towards the invading organism and the same could be true for an infection with *M. paratuberculosis*.

The pathogenesis of *M. paratuberculosis* infection in animals and in humans is conjectured to occur through a cascade of events that lead ultimately to overt disease. *M. paratuberculosis* may be found in two forms. It may possess a cell wall and can take up the acid-fast stain or it may lose most of its cell wall and exist as an intracellular cell wall-defective form. This trait would explain the difficulties associated with staining the organism in human tissues or in the sub-clinical infections in animals using traditional stains.

*M. paratuberculosis* is an extremely slow growing *Mycobacterium*. In cattle and most likely in humans, there is usually a long delay from the time that the host becomes infected to the time that clinical disease becomes manifest. The clinical manifestations result from the host’s immune response against the organism’s antigens. If the immune response was totally effective, the bacteria would be eradicated and there would be no disease. The disease results from the activated immune system that is not able to effectively control the infection. Inflammatory symptoms result from the cytokine cascades that are triggered. Local tissue destruction results in ulceration and fistulas. Attempts at repair and healing cause collagen deposition and scarring. The result is a chronic inflammatory disease of the bowel. As with other mycobacteria, granulomas are common.

*M. paratuberculosis* is a member of the *Mycobacterium avium* complex and has evolved so that it can live in a wide range of mammalian host intestines. Thus, it has been suspected that *M. paratuberculosis* enters through the gastrointestinal tract; and indeed, they have been shown to invade intestinal epithelial cells in vitro. The *M. avium* complex consists of 28 serovars of two mycobacterial species, *Mycobacterium avium* and *Mycobacterium intracellulare*. The first of these can be divided into three subspecies, known as *Mycobacterium avium* subsp. *avium*, *Mycobacterium avium* subsp. *silvaticum* and *Mycobacterium avium* subsp. *paratuberculosis*. The main
difference between *M. avium* avium and *M. avium* paratuberculosis is the IS900 insertion complex and the fact that it is a facultative intracellular parasite. Although usually considered to be opportunistic pathogens, some of the *M. avium* complex organisms including *M. paratuberculosis* that have been isolated from diseased patients have been shown to be virulent and resistant to most antibiotics. Strains of *M. avium* complex including *M. paratuberculosis* have also been shown to be more resistant to heat than *M. bovis*. Therefore, if *M. paratuberculosis* causes Crohn’s disease, food would be the most likely vehicle for transmission.

Further strengthening the link between *M. paratuberculosis* and Crohn’s syndrome is the work recently carried out by Naser et al. Using bovine Johne’s disease as a model, they examined human breast milk taken from mothers suffering from Crohn’s disease in attempts to recover viable *M. paratuberculosis*. They reasoned that if *M. paratuberculosis* were to be found in human breast milk, then the association between *M. paratuberculosis* and Johne’s disease in ruminants, and the association between *M. paratuberculosis* and Crohn’s disease in humans would be established. Indeed, *M. paratuberculosis* was cultured from milk of mothers with Crohn’s disease, just as *M. leprae* was detected in milk from mothers with leprosy.

**AUTOIMMUNE OR MYCOBACTERIAL DISEASE?**

There is still much to be learned about *M. paratuberculosis* and the diseases that it may cause in humans. Crohn’s disease may actually be a syndrome with multiple aetiologies that result in the same clinical, endoscopic, radiologic and pathologic findings that define the syndrome. *M. paratuberculosis* may be one of the aetiologies of this syndrome. The percentage of Crohn’s disease patients infected with *M. paratuberculosis* is not known but the current data suggest that infection with this organism accounts for a large proportion of Crohn’s disease: 35% to 40% by culture and 40% of the granulomatous category of the disease by *in situ* hybridization. The development of a *M. paratuberculosis*-specific ELISA based assay will help resolve this question.

*M. paratuberculosis* causes a zoonotic infection that results in an inflammatory bowel disease in a wide range of mammalian species. The full spectrum of the clinical manifestations of *M. paratuberculosis* infection in man is yet to be defined. *M. paratuberculosis* is a very slow growing bacterium that causes an intracellular infection. Disease results from the interplay of the organism and the immune response that it elicits. The clinical manifestations of infection depend on many variables that include the host’s genetic inheritance, the status of the immune system, and perhaps prior exposure to cross-reacting antigens from other mycobacteria. Some strains of *M. paratuberculosis* may be more pathogenic than others. Suppressing the host’s immune response will modify the clinical manifestations of the disease. Due to the inherently slow rate of bacterial reproduction and the low bacterial load, immune suppression does not result in obvious worsening of disease. The host response is more akin to leprosy than infection with *M. tuberculosis*.

We hypothesize that the mode of transmission for *M. paratuberculosis* infection is for the organism to enter the host as acid fast bacilli and/or as the cell wall-deficient mutant via consumption of food or contaminated water (e.g. in a free-living environmental amoeba). In genetically predisposed individuals, the body is unable to clear the infection and infected macrophages may serve as vehicles to distribute *M. paratuberculosis* to other areas of the body: the bacteria may reside in the macrophage component of human milk. Once established, the infection persists and triggers an ineffective, ‘abnormal’ immune response that damages tissue and causes the disease.

The two leading theories for Crohn’s disease pathogenesis are the autoimmune theory and the mycobacterial theory. The autoimmune theory suggests that the underlying defect in Crohn’s disease is one of feedback controls on the immune response. It implies that Crohn’s disease is a result of immune disregulation, where an excessive TH1-driven, cell-mediated immune response is elicited and persists. The mycobacterial theory implies that the chronic intracellular infection with *M. paratuberculosis* elicits a persistent TH1-driven, cell-mediated response. The mycobacterial theory and the autoimmune theory of Crohn’s disease should be seen as complementary and not mutually exclusive. The mycobacterial theory deals with the aetiology of the disorder, whereas the autoimmune theory deals with its pathogenesis. Support for this viewpoint will be found in studying the cytokine profiles and the cell-mediated immune responses of tuberculosis, leprosy and other atypical mycobacterial infections. Chronic intracellular *M. paratuberculosis* infection may be the trigger for an
excessive TH1 immune response that results in the clinical, endoscopic, radiologic and histological manifestations known as Crohn's disease. Therapy needs to be directed against a mycobacterial aetiology rather than merely suppressing inflammation. A combination of these two approaches may be the optimal strategy.

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