FRI Briefings

Mycobacterium paratuberculosis — Another Emerging Pathogen of the Human Gastrointestinal Tract?

first published April 1997
prepared by M. Ellin Doyle, Ph.D.
Food Research Institute, UW-Madison

Crohn's Disease (CD) in humans and Johne's disease in cattle both involve a chronic granulomatous enteritis primarily in the small intestine. Symptoms of these diseases are similar to those of intestinal tuberculosis (caused by *Mycobacterium tuberculosis*), and the agent responsible for Johne's disease is known to be *Mycobacterium paratuberculosis*. The etiology of CD has so far defied explanation but a great deal of recent research has been devoted to a search for *M. paratuberculosis* in patients with CD, sarcoidosis, and ulcerative colitis. If this bacterium is associated with any human diseases, then there is the question as to whether people can acquire *M. paratuberculosis* from animals with clinical or subclinical Johne's disease. It is believed that CD is multifactorial in origin, possibly involving some genetic predisposition and other environmental factors as well as an infectious agent (*1*). This update will present current information on the possible role of *M. paratuberculosis* in CD and on potential vehicles for its transmission.

Mycobacterium paratuberculosis was first associated with an intestinal disease of cattle about a hundred years ago. At first it was difficult to distinguish this bacterium from tubercle bacilli but eventually they were isolated and characterized and Johne's disease was distinguished from intestinal tuberculosis. Although a number of diagnostic tests are available, it is not always possible to detect these bacteria in sick cattle. The bacteria penetrate the intestinal mucosa, are phagocytized by macrophages, and continue to multiply inside the macrophages. Granulomas, compact foci of inflammatory cells, form at these sites and the animals develop a chronic nonresponsive diarrhea which causes progressive weight loss, debilitation, and death (*2*).

Ruminants, including cattle, sheep, goats, deer, and South American camelids, are infected with *M. paratuberculosis* more commonly than other animals. Calves may acquire these bacteria early in life—either by ingestion of grass or hay contaminated with feces from infected animals or through their mother's milk. After an extended incubation period of several months to years, a persistent, non-responsive diarrhea develops. Other kinds of animals are occasionally infected: wild rabbits in Scotland (*3*), a strain of laboratory mice (*4*), and a colony of stumptail macaques (*Macaca arctoides*) in the USA (*2*). Clinical symptoms and pathology of paratuberculosis in the macaques were similar to those observed in ruminants and also resembled Crohn's disease in humans. Mycobacteria were detected in intestinal tissue specimens and in feces, and these were identified as *M. paratuberculosis* after growth on standard media and examination of their rRNA. Antibodies to *M. paratuberculosis* were present in many, but not in all, of the monkeys with clinical signs of disease. It appeared that immunological reactions were suppressed during the clinical phases of infection. Antimycobacterial agents were effective in treating disease symptoms.

A number of granulomatous infections occurring in humans are known to be caused by mycobacteria. These include tuberculosis, leprosy, some skin ulcers, and some opportunistic infections in AIDS patients (*5*). It is suspected, but by no means proven, that mycobacteria are involved in some other granulomatous diseases, including Crohn's and sarcoidosis. Sarcoidosis is a disease primarily affecting the lungs whereas CD affects the ileum (part of the small intestine) and the colon. In both diseases, lymph nodes and other organs are sometimes involved.

Mycobacteria are fastidious and slow growing organisms, and this has made their culture very difficult. Most strains of *M. paratuberculosis* require the iron-binding siderophore mycobactin J in culture media and they grow very slowly

even in its presence. In addition, mycobacteria form cell wall–deficient forms, spheroplasts, and this changes their staining properties (no longer acid-fast) and makes it extremely difficult to culture them. When *M. paratuberculosis* was first isolated from patients with CD, it took up to 18 months for the primary isolation (*6*). Later attempts to isolate *M. paratuberculosis* from clinical specimens of CD patients have not been very successful, with only six strains of DNA probe–confirmed *M. paratuberculosis* isolated from patients with Crohn's disease.

Recent advances in molecular methods of analysis have provided alternative procedures for the detection of these bacteria. One of the most popular uses a polymerase chain reaction (PCR) assay based on the amplification of a multicopy DNA insertion element, IS900, specific to *M. paratuberculosis*. While some investigators using this probe have not detected *M. paratuberculosis* sequences in patients with Crohn's disease (*7–9*), others have detected this specific sequence in some clinical samples from patients with Crohn's disease, ulcerative colitis, and sarcoidosis (*10–16*). In a recent analysis of DNA from the intestinal mucosa from a patient with CD, DNA sequences having a 95–98.6% homology with the IS900 element were found (*10*). At least part of the reason for inconsistent results using this technique may be due to methodological differences related to the use of fresh or frozen tissue and to the size of the IS900 fragment used in assays. PCR assays are very sensitive. Through further refinements and standardization of protocol, they may help us to answer the question of whether Crohn's patients harbor *M. paratuberculosis*.

Bacterial infection normally induces an immune response and some efforts have been devoted to detection of such a response in patients with CD. Although two research groups reported an increase in IgG antibodies against a cytoplasmic antigen from *M. paratuberculosis* (*11,17*) in patients with sarcoidosis, other groups have not found an increase in specific antigens in patients with Crohn's disease (*18,19*). Other research has demonstrated no peripheral cell–mediated immune response to mycobacterial antigens (*20*) but some evidence for an induction of suppressor cells by an *M. paratuberculosis* antigen (*21*) in patients with inflammatory bowel disease. A major problem in the interpretation of these immunological assays is that many patients with CD take immunosuppressive drugs, such as steroids, and these can interfere with the tests. Methodological differences also preclude direct comparison of these studies and the immunological evidence remains inconclusive.

If *M. paratuberculosis* is a human pathogen, then the next issue is to define possible vehicle(s) of transmission. Milk and serum samples from cows in Missouri with paratuberculosis have tested positive by ELISA for antibodies to *M. paratuberculosis* (*22*). This bacterium has also been cultured from unpasteurized colostrum and milk of infected dairy cows in Ohio (*23*). However, viable *M. paratuberculosis* has not as yet been detected in any retail milk samples in the USA. A survey, using a PCR assay for IS900, of samples of whole pasteurized milk purchased in the UK found that the incidence of positive samples varied seasonally, peaking in January–March and September–November (*24*). It appears that these organisms survived pasteurization and that at certain times of the year there is a higher likelihood that *M. paratuberculosis* will be present in retail milk in London. Unfortunately, it is not easy to predict which cows will give contaminated milk because *M. paratuberculosis* has been detected in milk from apparently healthy cows as well as from sick cows. Presence of these bacteria in milk is not well correlated with positive cultures from fecal samples or with positive results with immunoassays testing serum.

Although pasteurization effectively destroys most pathogenic bacteria, neither the standard hold method (30 min at 145°F) nor the high-temperature, short-time method (15 sec at 161°F) completely eliminated viable *M. paratuberculosis* cells in milk treated in the laboratory (*25–28*). Bacterial strains which had been isolated from humans appeared to be more heat resistant than those of bovine origin. Examination of the thermal death curve for these organisms revealed a rapid initial linear death rate followed by a slower tailing. It is believed that this tailing is due to clumping of bacterial cells. Survival was also greater when samples were cooled rapidly following the pasteurization process. It is possible that commercial pasteurization equipment would be more effective than laboratory apparatus in killing these bacteria because milk in the commercial equipment is exposed to turbulent-flow conditions rather than being static. More data are needed on realistic concentrations of *M. paratuberculosis* likely to be present in raw milk and on the effectiveness of commercial pasteurization equipment.

At this point, it is impossible to state definitively: (i) that *Mycobacterium paratuberculosis* is a causative factor in human granulomatous diseases such as Crohn's disease, or (ii) what vehicle might transmit these bacteria to humans. The symptoms of CD are certainly similar to the effects produced by this bacterium in other animals but it is still difficult to demonstrate the presence of these bacteria in human clinical specimens. It is likely that if *M.*

paratuberculosis is involved in this disease it will turn out to be one of several etiological cofactors. Genetic predisposition, other environmental factors, and the measles virus have all been suggested to have a role in the development of these disease symptoms.

References

1. McFadden, J.J., and H.M. Fidler. Mycobacteria as possible causes of sarcoidosis and Crohn's disease. *J. Appl. Bacteriol*. 81:47S–52S (1996).

2. McClure, H.M., R.J. Chiodini, D.C. Anderson, R.B. Swenson, W.R. Thayer, and J.A. Coutu. *Mycobacterium paratuberculosis* in a colony of stumptail macaques (*Macaca arctoides*). *J. Infect. Dis.* 155:1011–1019 (1987).

3. Grieg, A., K. Stevenson, V. Perez, A.A. Pirie, J.M. Grant, and J.M. Sharp. Paratuberculosis in wild rabbits (*Oryctolagus cuniculus*). *Vet. Rec.* 140:141–143 (1997).

4. Veazey, R.S., H.W. Taylor, D.W. Horohov, J.L. Krahenbuhl, J.L. Oliver, and T.G. Snider. Histopathology of C57BL/6 mice inoculated orally with *Mycobacterium paratuberculosis*. *J. Comp. Pathol.* 113:75–80 (1995).

5. Zumla, A., and D.G. James. Granulomatous infections: etiology and classification. *Clin. Infect. Dis.* 23:146–158 (1996).

6. Chiodini, R.J., and C.A. Rossiter. Paratuberculosis: a potential zoonosis? *Vet. Clin. N. Am.: Food Animal Pract.* 12:457–467 (1996).

7. Rowbotham, D.S., N.P. Mapstone, L.K. Trejdosiewicz, P.D. Howdle, and P. Quirke. *Mycobacterium paratuberculosis* DNA not detected in Crohn's disease tissue by fluorescent polymerase chain reaction. *Gut* 37:660–667 (1995).

8. Frank, T.S., and S.M. Cook. Analysis of paraffin sections of Crohn's disease for *Mycobacterium paratuberculosis* using polymerase chain reaction. *Modern Pathol*. 9:32–35 (1996).

9. Dumonceau, J.M., A. Van Gossum, M. Adler, P.A. Fonteyne, J.P.Van Vooren, J. Devière, and F. Portaels. No *Mycobacterium paratuberculosis* found in Crohn's disease using the polymerase chain reaction. *Dig. Dis. Sci*. 41:421–426 (1996).

10. Mishina, D., P. Katsel, S.T. Brown, E.C. Gilberts, and R.J. Greenstein. On the etiology of Crohn's disease. *Proc. Natl. Acad. Sci. USA* 93:9816–9820 (1996).

11. El-Zaatari, F.A.K., S.A. Naser, D.C. Markesich, D.C. Kalter, L. Engstand, and D.Y. Graham. Identification of *Mycobacterium avium* complex in sarcoidosis. *J. Clin. Microbiol*. 34:2240–2245 (1996).

12. Suenaga, K., Y. Yokoyama, K. Okazaki, and Y. Yamamoto. Mycobacteria in the intestine of Japanese patients with inflammatory bowel disease. *Am. J. Gastroenterol*. 90:76–80 (1994).

13. Dell'Isola, B., C. Poyart, O. Goulet, J.F. Mougenot, E. Sadoun-Journo, N. Brousse, J. Schmitz, C. Ricour, and P. Berche. Detection of *Mycobacterium paratuberculosis* by polymerase chain reaction in children with Crohn's disease. *J. Infect. Dis*. 169:449–451 (1994).

14. Fidler, H.M., W. Thurrell, N. McI. Johnson, G.A.W. Rook, and J.J. McFadden. Specific detection of *Mycobacterium paratuberculosis* DNA associated with granulomatous tissue in Crohn's disease. *Gut* 35:506–510 (1994).

15. Lisby, G., J. Andersen, K. Engbæk, and V. Binder. *Mycobacterium paratuberculosis* in intestinal tissue from patients with Crohn's disease demonstrated by a nested primer polymerase chain reaction. *Scand. J. Gastroenterol*. 29:923–929 (1994).

16. Murray, A., J. Oliaro, M.M.T. Schlup, and V.S. Chadwick. *Mycobacterium paratuberculosis* and inflammatory bowel disease: frequency distribution in serial colonoscopic biopsies using the polymerase chain reaction. *Microbios* 83:217–228 (1995).

17. Reid, J., and R.J. Chiodini. Serologic reactivity against *Mycobacterium paratuberculosis* antigens in patients with sarcoidosis. *Sarcoidosis* 10:32–35 (1993).

18. Walmsley, R.S., J.P. Ibbotson, H. Chahal, and R.N. Allan. Antibodies against *Mycobacterium paratuberculosis* in Crohn's disease. *Q. J. Med*. 89:217–221 (1996).

19. Tanaka, K., M. Wilks, P.J. Coates, M.J.G. Farthing, J.A. Walker-Smith, and S. Tabaqchali. *Mycobacterium paratuberculosis* and Crohn's disease. *Gut* 32:43–45 (1991).

20. Rowbotham, D.S., P.D. Howdle, and L.K. Trejdosiewicz. Peripheral cell–mediated immune response to mycobacterial antigens in inflammatory bowel disease. *Clin. Exp. Immunol*. 102:456–461 (1995).

21. Ebert, E.C., B.D. Bhatt, S. Liu, and K.M. Das. Induction of suppressor cells by *Mycobacterium paratuberculosis* antigen in inflammatory bowel disease. *Clin. Exp. Immunol*. 83:320–325 (1991).

22. Hardin, L.E., and J.G. Thorne. Comparison of milk with serum ELISA for the detection of paratuberculosis in dairy cows. *J. Am. Vet. Med. Assoc*. 209:120–122 (1996).

23. Streeter, R.N., G.S. Hoffsis, S. Bech-Nielsen, W.P. Shulaw, and D.M. Rings. Isolation of *Mycobacterium paratuberculosis* from colostrum and milk of subclinically infected cows. *Am. J. Vet. Res*. 56:1322–1324 (1995).

24. Millar, D., J. Ford, J. Sanderson, S. Withey, M. Tizard, T. Doran, and H. Hermon-Taylor. IS900 PCR to detect *Mycobacterium paratuberculosis* in retail supplies of whole pasteurized cows' milk in England and Wales. *Appl. Environ. Microbiol*. 62:3446–3452 (1996).

25. Chiodini, R.J., and J. Hermon-Taylor. The thermal resistance of *Mycobacterium paratuberculosis* in raw milk under conditions simulating pasteurization. *J. Vet. Diagn. Invest*. 5:629–631 (1993).

26. Meylan, M., D.M. Rings, W.P. Shulaw, J.J. Kowalski, S. Bech-Nielsen, and G.S. Hoffsis. Survival of *Mycobacterium paratuberculosis* and preservation of immunoglobulin G in bovine colostrum under experimental conditions simulating pasteurization. *Am. J. Vet Res*. 57:1580–1585 (1996).

27. Grant, I.R., H.J.Ball, S.D. Neill, and M.T. Rowe. Inactivation of *Mycobacterium paratuberculosis* in cows' milk at pasteurization temperatures. *Appl. Environ. Microbiol*. 62:631–636 (1996).

28. Grant, I.R., H.J.Ball, and M.T. Rowe. Thermal inactivation of several *Mycobacterium* spp. in milk by pasteurization. *Lett. Appl. Microbiol*. 22:253–256 (1996).

return to top

FRI home page [FRI Briefings]

Last modified: 10 June 1998

University of Wisconsin–Madison