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Mycobacterium avium subspecies paratuberculosis in the causation of Crohn's disease

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Mycobacterium avium subspecies paratuberculosis (MAP), originally called Johne's bacillus was first described from Germany in 1895 as the cause of a chronic inflammatory disease of the intestine in a cow. As the 20th century progressed, clinical and sub-clinical MAP infection in farm animals in Western Europe appeared to become more prevalent. Among the early reviews available are the excellent ones prepared by Doyle[1] from the Veterinary Laboratory, Weybridge, UK, and Riemann and Abbas[2] from the University of California, Davis. In general, the response on farms to the appearance of clinical Johne's disease was to cull infected animals. This practice over the course of the 20th century may have exerted a selection pressure on MAP favouring the emergence of strains which can infect animals for years without necessarily causing clinical disease. In the latter part of the 20th century the incidence of clinical disease due to MAP in some areas of Western Europe and North America appeared to decrease. The problem which confronts these regions now is subclinical MAP infection of domestic livestock throughout Western Europe and North America and the emergence of wildlife reservoirs including those in rabbits and their predators[3]. In the United States and Canada the herd prevalence of MAP infection is reported in the range 21% - 54%[4-8]. In Western Europe the herd prevalence lies in the same range, although a recent serological study of bulk-tanked milk from 900 dairy herds in Denmark reported that 70% of herds tested positive for MAP infection[9]. What is beyond doubt is that MAP is widespread in our domestic animals.

Subclinically infected dairy cows secrete MAP in their milk. It is one of the ways the organism passes from infected parent to offspring when the calf may be most susceptible. MAP is more robust than *M. bovis* or *M. tuberculosis* and the destruction of all viable MAP by exposure to current pasteurisation con ditions of 72℃ for 15 seconds is not assured. In on-going research in the Department of Food Science, University of Belfast, N. Ireland, small slow-growing, mycobactin-dependent, IS900 PCR positive colonies of MAP have been cultured from about 3% of retail units of pasteurised cows' milk, so far tested. In the U.K. what is also beyond doubt is that the human population is being exposed to MAP in retail milk supplies. These organisms accumulate particularly in the ileocolonic regions of the intest ine where they may remain for years and not cause clinical disease. This situation is similar in principal to the widespread exposure of human popul ations in Europe, North America and elsewhere, to M. bovis before the intro duction of milk pasteurisation and the tuberculin testing of dairy herds introduced in the middle third of the 20th century. With MAP, only those individuals with a particular inherited or acquired susceptibility may go on eventually to develop clinical disease.

Infected animals excrete MAP onto pastures. Wildlife reservoirs contribute to environmental contamination. The problems now being caused by MAP, differ from those previously caused by *M. bovis*, in that MAP can survive for long periods in the environment. Rains falling on contaminated land will wash MAP into ground and river waters. Although much research needs to be carried out in this area, it is probable that MAP in the environment is taken up into organisms such as amoebae in which they can survive. This may allow them to replicate, to increase their resistance to biocides and potentially acquire a phenotype which is more pathogenic for humans. Water abstracted from rivers and lakes contaminated with MAP may convey these organisms to human populations. MAP arriving at domestic outlets in high dilution may accumulate in biofilms lining household water systems. Either in the food chain therefore, or in water supplies, it is inevitable that humans sharing the same geographic areas with animals which are extensively infected, will be exposed to these pathogens.

The question as to whether MAP may also cause disease in humans has its origins in a proposition first published in 1913[10]. We have recently prepared a detailed analysis of this complex issue which is in general poorly understood, even by

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medical specialists in the field of chronic inflammatory diseases of the intestine[11]. It is now known that MAP can cause chronic inflammation of the intestine in a very broad range of animals including large and small ruminants, monogastrics such as dogs and pigs, and so far at least four types of sub-human primates. MAP shows a well defined tissue tropism and will end up causing chronic inflammation of the intestine even if administered experimentally by subcutaneous, intravenous or intraperitoneal routes. The histopathological features of MAP disease in animals ranges from one in which millions of typical ZN-positive MAP are visible microscopically in the inflamed intestine to the other extreme where no MAP can be seen at all, but there is chronic granulomatous inflammation. This is just what leprosy does in humans. One of the properties of MAP which has retarded our understanding of the problems it is causing is that it may be very difficult to culture in the laboratory. Patient work in many laboratories has, however, shown that MAP can be grown in conventional culture from about 5% of people with Crohn's disease, but not from normal people. Cultures have had to be incubated for months or years before any growth identifiable by conventional means, becomes visible.

MAP is very similar to other organisms of the M. avium complex (MAC) to which we are all exposed, so that immunological tests for MAP infection in humans using crude extracts of laboratory cultured organisms usually report no difference be tween Crohn's disease and normal people. Recent studies have however shown that if specific targets on MAP are carefully selected, highly significant differences in immune recognition can be demonstrated between Crohn's disease and normal people. An important example of this has come from recent research at UCLA which showed that the blood of 9 out of 10 people with Crohn's disease contained IgA which recognised a mycobacterial protein richly expressed on MAP called HupB[12]. HupB is identical to the laminin receptor used by *M. leprae* to enter Schwann cells round nerves causing the neural inflammation so characteristic of leprosy[13]. Neural inflammation is long known to be a specific feature in the inflamed gut in Crohn's disease[14] and antibodies to the chronic inflammatory disease associated autoantigen pANCA, cross-react with HupB.

In 1990, after spending 20 months carefully optimising sample processing and experimental procedures, we began a study which revealed the presence of MAP DNA in about two thirds of people with Crohn's disease using IS900 PCR[15]. It was also present in the intestine of 12% of normal people, which is just what would be expected to occur in a totally exposed population. Since then there have been 18 peer reviewed publications using a variety of experimental methods, 9 of which reported the presence of MAP in CD gut some or most of the time and 9 found MAP hardly ever or not at all[11]. Similar inconsistencies have occurred in the results of DNA tests applied to other chronic inflammatory diseases such as TB. Apart from some obvious methodological errors, the reasons for the uncertainty are the low abundance of MAP in Crohn's disease intestine and its extraordinarily tough protease-resistant phenotype. Recent research by Dr Saleh Naser and colleagues from the University of Central Florida [cited in 11], using improved liquid cultures and IS900 PCR on their centrifugal pellets, has demonstrated MAP in 86% of surgically resected Crohn's disease gut.The same authors have also demonstrated MAP in the centrifugal pellets of breast milk from each of 2 mothers with Crohn's disease who had recently given birth, but not in the breast milk of 5 normal women. Work in our own lab by Jun Cheng and Tim Bull, using much improved methods, is currently reporting Chinese MAP in 69% of Chinese surgical path blocks from Crohn's disease patients in China, and in 14% of path blocks of normal intestine from Chinese people. In our view these studies clearly demonstrate the presence of this chronic enteric pathogen in a substantial majority proportion of humans with chronic inflammation of the intestine of the Crohn's disease type. Although the incidence of Crohn 's disease in China is currently much lower than in Western Europe or North Ame rica, these recent studies in humans strongly suggest that Chinese people are exposed to these pathogens and that action may need to be considered at this early stage to limit the more extensive development of disease in humans.

It has long been known that infections due to non-tuberculous mycobacteria in immunocompetant people, particularly those caused by MAC, are usually resistant to standard anti-tuberculous drugs. These organisms can prevent the drugs penetrating the microbial cell and can rapidly develop mutations which confer drug resistance. Lasting resolution of MAP infections in animals using standard anti-mycobacterial treatment has never been convincingly demonstrated, and much the same outcomes have resulted from a similar treatment approach in humans with Crohn's disease[11]. MAP are however more susceptible to some newer drugs which are man-made chemical modifications of natural streptomyces antibiotics such as rifabutin and clarithromycin. These agents also have the advantage of being concentrated within macrophages where MAP in infected animals and in humans almost certainly resides. Our own studies from 1992[16], supported by the work of Dr. Tom Borody in Sydney, Dr. Ira Shafran at the University of Central Florida, and by recent work from the North of England[17] have shown that a substantial proportion of people with active Crohn's disease will go into remission with healing of the

intestine which is sometimes lasting, when treated with combinations of these drugs. A randomised controlled trial of this treatment was initiated in Australia in September 1999.

A question which is frequently asked is how can so few MAP cause so much chronic inflammatory disease? To answer this we must allow our thinking to escape from the immobilising presumption that it must be like TB, in which a major factor in the disease process is a direct immunological response to cell wall components. MAP in animals with the paucimicrobial form of the disease and in humans, does not have a classical mycobacterial cell wall. MAP colonising immunoregulatory cells like macrophages almost certainly causes an immune dysregulation. Together with defects in the integrity of the overlying mucosa, much of the disease itself is caused by an exaggerated immunological response to leakage into the gut wall of bacteria and food residues normally confined to the lumen. Clinical improvement can be achieved by suppressing or modulating the immune system, by reducing the allergic com ponent and altering the enteric flora with elemental diets, and by treatment with antibiotics such as ciprofloxacin and metronidazole. Without killing the underlying causative pathogens however, the benefit which follows such treatments is rarely lasting.

The present analysis of the MAP problem suggests that particularly in Western Europe and North America, we are challenged by a public health issue of substantial proportions for which a range of remedial measures are needed. These measures include conditions of pasteurisation of retail milk which do ensure the destruction of all viable MAP. We need to use the improved culture systems available together with modern molecular methods to ensure our domestic livestock are free of sub-clinical infection. We need to test water supplies to make sure they are clean. In high incidence areas, we need to make Crohn's disease reportable so we have accurate data to monitor the effect of these measu res on the overall problem. We need to make a rapid increase in the volume and intensity of research in the field, to sequence the MAP genome and to develop preventative vaccines for animals and therapeutic vaccines for humans.

REFERENCES

1 Doyle TM. Johne's Disease. *Vet Rec*, 1956;68:869-886
2 Reimann HP, Abbas B. Diagnosis and control of bovine paratuberculosis (Johne's Disease). *Adv Vet Sci & Comp Med*, 1983;27:481-506
3 Beard PM, Henderson D, Daniels MJ, Pirie A, Buxton D, Greig A, Hutchings MR, McKendrick I, Rhind S, Stevenson K, Sharp JM. Evidence of paratuberculosis in fox (Vulpes vulpes) and stoat (Mustela erminea). *Vet Rec*, 1999;145:612-613
4 Merkal RS, Whipple DL, Sacks JM, Snyder GR. Prevalence of Mycobacterium par atuberculosis in ileocecal lymph nodes of cattle culled in the United States. *JAVMA*, 1987;190:676-680
5 NcNab WB, Meek AH, Duncan JR, Martin SW, Van Dreumel AA. An epidemiological study of paratuberculosis in dairy cattle in Ontario: study design and prevalence estimates. *Can J Vet Res*, 1991;55:246-251
6 Collins MT, Sockett DC, Goodger WJ, Conrad TA, Thomas CB, Carr DJ. Herd preva lence and geographic distribution of, and risk factors for, bovine paratuberculosis in Wisconsin. *J Am Vet Med Assn*, 1994;204:636-641
7 Wells SJ, Ott SL, Garber LP, Bulaga LL. Johne's disease on U. S. dairy opera tions: results from the NAHMS Dairy 96 Study. In: Chiodini RJ, Hines ME, Collins MT, eds. Proceedings of the Fifth International Colloquium on Paratuberc ulosis Rehoboth, MA, USA: Int. *Assn for Paratuberculosis*, 1996:140-142
8 Johnson Ifearulundu Y, Kaneene JB. Distribution and environmental riskf actors for paratuberculosis in dairy cattle herds in Michigan. *Am J Vet Res*, 1999;60:589-596
9 Nielsen SS, Thamsborg SM, Houe H, Bitsch V.Bulk tank milk ELISA antibodies for estimating the prevalence of paratuberculosis in Danish dairy herds. *Prev Vet Med*, 2000;44:1-7
10 Dalziel TK. Chronic interstitial enteritis. *BMJ*, 1912;ii:1068-1070
11 Hermon Taylor J, Bull TJ, Sheridan JM, Cheng J, Stellakis ML, Sumar N . The Causation of Crohn's Disease by Mycobacterium avium subsp. paratuberculos is. *Can J Gastroenterol*, 2000:in press
12 Cohavy O, Harth G, Horwitz M, Eggena M, Landers C, Sutton C, Targan SR, Braun J. Identification of a novel mycobacterial histone H1 homologue (HupB) as an antigenic target of pANCA monoclonal antibody and serum immunoglobulin A from patients with Crohn's disease. *Inf Immun*, 1990;67:6510-6517
13 Shimoji U, Ng V, Matsumura K, Fischetti VA, Rambukkana A. A 21 kDa surface protein of Mycobacterium leprae binds peripheral nerve laminin 2 and mediates Schwann cell invasion. *Proc Natl Acad Sci*, 1999;96:9857-9862
14 Geboes K, Rutgeerts P, Ectors N, Mebis J, Penninckx F, Vantrappen G,Desmet VJ. Major histocompatibility class II expression on the small intestinal nervous system in Crohn's disease. *Gastroenterology*, 1992;103:439-447
15 Sanderson JD, Moss MT, Tizard MLV, Hermon Taylor J. Mycobacterium paratuberculosis DNA in Crohn's disease tissue. *Gut*, 1992; 33:890-896
16 Gui GPH, Thomas PRS, Tizard MLV. Two year outcomes analysis of Crohn's disease treated with rifabutin and macrolide antibiotics. *J Antimicrob Chemo*, 1997;39:393-400
17 Douglass A, Cann PA, Bramble MG. An open pilot study of antimicrobial therapy in patients with unresponsive Crohn's disease. *Gut*, 2000;46:A11

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