Grand Round

Mycobacterium paratuberculosis cervical lymphadenitis, followed five years later by terminal ileitis similar to Crohn's disease

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Mycobacterium paratuberculosis was first described by John and Frothingham in 1895¹ as the cause of a chronic inflammatory disease of the intestine in a German cow. The organism was called John's bacillus, and the illness (in which millions of acid fast mycobacteria were visible in the diseased tissues), John's disease. In 1901 Thomas Dalziel, a surgeon at the Western Infirmary in Glasgow, operated on a colleague with chronic inflammation of the intestine. He was aware of the description of John's disease and of the subsequent bacteriological research in the field. He collected other cases and published his observations in the *BMJ* in 1913.² He wrote that the "histological characters" of the disease he had described in humans were so similar to those of John's disease that the diseases may be the same. Dalziel's dilemma was that he could not see acid fast mycobacteria in the diseased intestine in humans.³

M paratuberculosis belongs to the *M avium-intracellulare* group.⁴ The term paratuberculosis suggests close similarity to *M tuberculosis*, but in truth it is very different. Unlike *M tuberculosis*, it can survive in the environment and is highly resistant in vivo to most standard antituberculous drugs. *M paratuberculosis* cannot be reliably detected by culture in the laboratory; different subtypes of the organism with different preferred hosts⁵⁻⁶ range from very slow growing to unculturable.

M paratuberculosis can cause chronic inflammation of the intestine in many species of animals including primates.⁷⁻⁹ The disease in animals shows a broad range of histopathological types, from a pluribacillary (lepromatous) form with millions of typical acid fast bacilli visible in the tissues, to a paucimicrobial (tuberculoid) form in which *M paratuberculosis* cannot be seen in the tissues and cannot be detected by culture but in which there is a florid chronic granulomatous inflammatory response.¹⁰ Intermediate paucibacillary forms of the disease, in which only a few acid fast mycobacteria may be visible, also occur.

M paratuberculosis has only rarely been cultured in the laboratory from humans with chronic inflammation of the intestine of the Crohn's disease type.¹¹⁻¹⁵ When it has been grown, the organism has first appeared in the culture in an unrecognisable non-bacillary form, taking months or years of incubation to alter its phenotype to that of a mature bacillus with an established lipid rich cell wall able to be identified by conventional means. Detection and characterisation of *M paratuberculosis* has been advanced by the advent of DNA based methods that use as their target a 1451bp DNA insertion element IS900, of which there are about 16 copies stably integrated into the *M paratuberculosis* genome.¹⁶⁻¹⁷

The clinical case

In September 1988 a healthy boy aged 7 years 10 months developed enlarged lymph nodes on the right side of his neck. He was asymptomatic and had a negative result on the Mantoux test, and chest radiography was normal. He had not had a BCG vaccination. He was referred to the surgical service at Addenbrooke's Hospital, where the enlarged lymph nodes were removed. The histological picture suggested a mycobacterial infection. Samples of the diseased lymph nodes were incubated on Lowenstein-Jensen slopes with duplicates including pyruvate, but not mycobactin, for 12 weeks at room temperature, 30°C and 37°C. For two months he was treated with rifampicin 450 mg and isoniazid 150 mg once daily and pyrazinamide 250 mg three times daily, but when the cultures were negative drug treatment was stopped. The remaining lymph nodes on the right side of the neck progressively enlarged and were removed by block dissection in November 1989. Mycobacterial culture again proved negative. He remained well until March 1993 when he developed an arthritis affecting both knees, and an anaemia refractory to iron. By the end of 1993, he had daily abdominal pain, anorexia, 2-3 loose bowel motions a day, weight loss, and lethargy.

His weight was 37 kg (on the 30th centile) and his height 1.53 m. He looked pale and ill and had angular stomatitis. Firm lymph nodes were still palpable on the right side of the neck. There was no clubbing. In the

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Fig 1 Narrowing and distortion of 10 cm segment of terminal ileum with classic "cobblestoning" of the mucosa (arrowed). The appearances are the same as those seen in Crohn's disease

abdomen, an enlarged, tender loop of bowel was easily felt in the right iliac fossa. Radiologically, the small gut showed narrowing and distortion of a 10 cm segment of terminal ileum and prominent "cobblestoning" of the mucosa (fig 1). A white cell scan at one hour showed intense uptake of the label over the affected intestine (fig 2 (left)). These findings were considered typical of Crohn's disease.

He was treated daily with rifabutin 300 mg and clarithromycin 500 mg and was advised to have UHT ("long life") milk only. Eight days after the start of treatment lymph nodes on the left side of the neck and residual nodes on the right became enlarged and firm, with surrounding oedema. At the same time he had a transient episode of subacute intestinal obstruction, which subsided on conservative management. With continued rifabutin and clarithromycin treatment the nodes gradually subsided. By mid-1994 after six months' treatment he was virtually asymptomatic, and he was completely so by February 1995, by which time his weight had increased to 49 kg and his height to 1.60 m. White cell scanning then showed a complete resolution of the previously inflamed gut (fig 2 (right)). However, he was beginning to get obstructive symptoms. A barium enema showed no active disease, but there was a tight stricture of the terminal ileum with upstream dilatation. A limited resection was therefore carried out, with removal of the terminal ileum and adjacent ascending colon for a pale fibrotic stricture followed by end to end anastomosis. The histology of the resected stricture will be described. There was no other visible evidence of inflammatory disease throughout the gut.

Treatment with rifabutin and clarithromycin and restriction to UHT milk was continued. The drugs were stopped at the end of October 1996 after a total of 32 months' treatment. By January 1997 his weight was 67 kg (80th centile) and his height was 1.70 m. He was asymptomatic and a well developed adolescent on physical examination.

Fig 2 Left: White cell scan before treatment showing uptake of labelled cells by inflamed ileum characteristic of Crohn's disease. Right: White cell scan after one year of chemotherapy using combination of rifabutin and clarithromycin, showing apparent complete resolution

Fig 3 Microscopical appearance (top, $\times 400$; bottom, $\times 250$) of enlarged cervical lymph nodes showing typical Langerhans' giant cells with epithelioid cells, together with a non-caseating granuloma

Histopathology

Sections of the cervical lymph nodes initially removed showed areas of typical caseous necrosis with a only a few acid fast bacilli visible on Ziehl-Neelsen staining. Elsewhere there were marked chronic inflammatory changes with typical Langerhans' giant cells, non-caseating granulomata and an epithelioid cell infiltrate with focal microcalcifications (fig 3). The macroscopical appearances at operation were of a white fibrous stricture 5 mm in diameter and about 20 mm long in the terminal ileum (fig 4 (top)), with encroachment of fat over the serosal surface. There was no evidence of chronic inflammatory disease elsewhere in the gut. Microscopically the mucosa over the ileal stricture was intact with little gland distortion and villous atrophy, but the submucosa and gut wall showed extensive transmural fibrosis. There was no evidence of active inflammatory disease or granulomata (fig 4 (bottom)). The mesenteric lymph nodes showed reactive hyperplasia but were otherwise normal.

Identification of *M paratuberculosis* by IS900 polymerase chain reaction

Two of the paraffin embedded cervical lymph nodes removed in 1988 (fig 5) were subjected to DNA extraction using standard protocols and simultaneous process controls. IS900 polymerase chain reaction for *M paratuberculosis* was then performed on the DNA extract in triplicate using the primers p90 (5'-GAAGGGTGTTCGGGGCCGTCGCTTAGG-3') and

Fig 4 Top: Ileal stricture that developed after one year of treatment with rifabutin and clarithromycin. Bottom: Microscopic appearance ($\times 100$) of ileal stricture showing extensive intramural fibrosis

p91 (5'-GGCGTTGAGGTCGATCG CCCACGTGAC-3').¹⁸ Amplification products were run on agarose gel electrophoresis and identified by hybridisation at high stringency using a ^{32}P labelled 229 bp internal probe followed by autoradiography, as previously described.¹⁸ The DNA extract of the cervical lymph nodes was strongly positive in triplicate for *M paratuberculosis* in the presence of negative process controls and correctly reporting positive and negative internal polymerase chain reaction controls (fig 6). The strongly positive signal from the IS900 polymerase chain reaction that was obtained on the crude DNA extract from the cervical lymph nodes without hybridisation capture of target DNA¹⁸ probably reflects a relatively high microbial abundance of *M paratuberculosis* in the tissues at an early stage of the infection.

Comment

M paratuberculosis infections in animals

Among animals, Johne's disease, or *M paratuberculosis* infection, occurs in domestic ruminants, especially cattle, sheep, and goats. Studies at the San Diego zoo and elsewhere, however, have shown that a broad range of animals, including subhuman primates, can be affected.⁸

Recently, *M paratuberculosis* has been identified in wild rabbits in Tayside, Scotland.¹⁹ In subclinically infected cows *M paratuberculosis* is secreted abundantly in the milk, so that infection is acquired early in the life of newborn calves, when they are particularly susceptible.²⁰ The gut is the main target organ; *M paratuberculosis* is shed in the faeces, but it can travel throughout the

Fig 5 Enlarged paraffin embedded cervical lymph nodes removed in September 1988 from which DNA extract was prepared

animal in macrophages. A long latent interval usually occurs before clinical disease emerges. The signs of the disease in cattle are weight loss and diarrhoea; diarrhoea is not common in sheep. The pathology of the pluribacillary "lepromatous" type of disease is a chronic enteritis with diffuse thickening of the gut wall especially the terminal ileum, but extension to involve the colon is common. Mesenteric lymph nodes are enlarged. The mucosa of the gut is reddened with crevicing and marked oedema but not usually ulceration. Microscopically, there are millions of small acid fast *M paratuberculosis* living in macrophages (fig 7(a)).

We have shown that in about one quarter of cases there is a different form of Johne's disease in sheep.¹⁰ Although the clinical signs and gross pathology are similar, microscopically there is a pronounced chronic granulomatous enteritis with heavy lymphocytic infiltration, typical Langerhans'-type giant cells and scattered granulomata. A few nests of acid fast bacteria can be found in about half of these cases of "tuberculoid" Johne's disease (fig 7 (b)). In the others, no bacillary form *M paratuberculosis* is visible in the tissues at all (fig 7 (c) and (d)).

Transmission of *M paratuberculosis* to humans

In the early part of this century, when milk supplies were at times heavily contaminated with *M tuberculosis* and *M bovis*,²¹ tuberculous cervical lymphadenitis was common. The organism entered through not only the

Fig 6 Results of IS900 polymerase chain reaction with amplification products analysed by agarose gel electrophoresis and autoradiography. Lanes 1, 2, and 3 are simultaneous process controls correctly reporting negative; lanes 4, 5, and 6 are the DNA extract from the enlarged nodes showing strongly positive triplicate reactions for *Mycobacterium paratuberculosis*; and on the right are negative and positive (10fg Mptb target DNA) internal polymerase chain reaction controls

Fig 7 Photomicrographs of sections of intestinal wall, illustrating range of histological appearances and abundance of visible *M paratuberculosis* organisms in infected sheep with different forms of Johne's disease: (a) Ziehl-Neelsen stain ($\times 1600$) showing classic pluribacillary Johne's disease in which large numbers of small, acid fast *M paratuberculosis* organisms are seen within macrophages, but with little chronic granulomatous response; (b) Ziehl-Neelsen stain ($\times 1600$) showing paucibacillary form of Johne's disease with scant *M paratuberculosis* visible in tissues and prominent lymphocytic infiltration; (c) Ziehl-Neelsen stain ($\times 800$) of paucimicrobial form of Johne's disease diagnosed by IS900 polymerase chain reaction, in which no bacillary form *M paratuberculosis* can be seen; (d) haematoxylin and eosin stain ($\times 800$) of paucimicrobial Johne's disease in a sheep showing typical Langerhans' giant cells and pronounced chronic granulomatous inflammation.

oropharyngeal lymphoid tissues but also the gut, causing tuberculous ileitis and disease in other parts of the body. The problem was overcome by tuberculin testing of dairy herds and the mandatory introduction of milk pasteurisation, using conditions that ensured the destruction of these well recognised pathogens.

The risk that something similar is happening with *M paratuberculosis* is substantial, although the organism is much less virulent for humans than is *M tuberculosis*.²² Subclinical infection of dairy cows with *M paratuberculosis* is widespread in Britain and elsewhere in Europe,²³ and such animals shed the organism in their milk.²⁰⁻²² Conditions in the laboratory simulating commercial pasteurisation, which demonstrably kill *M bovis* do not always kill the more environmentally robust *M paratuberculosis*.²⁴⁻²⁵ Outcomes analysis of the treatment of humans with chronic enteritis of the Crohn's disease type with a combination of drugs predicted to be active against non-bacillary forms of *M paratuberculosis* in vivo suggests that the use of such a drug combination can cause substantial remission in many patients with active Crohn's disease.²⁶

The young boy in this case was probably infected by milk contaminated with non-tuberculous mycobacteria including *M paratuberculosis*. The organisms would have entered his cervical lymph nodes and his terminal ileum, as also occurs in animals.²⁷ After a latent interval of about five years, first an arthritis and then a chronic

enteritis emerged. This was similar clinically, radiologically, and on white cell scanning, to Crohn's disease. Shortly after starting to take rifabutin and clarithromycin he had a transient flare of the disease, with widespread enlargement of the cervical lymph nodes, and an episode of subacute intestinal obstruction, similar to disease exacerbations reported in some cases of leprosy and tuberculosis after treatment is started.²⁸⁻²⁹ In this case, the gut healed with prolonged treatment, leaving a scar that needed excision.

Discussion

Angus Dalgleish: As *M tuberculosis* is associated with caseation and *M paratuberculosis* is not, could both have been involved?

CF: The involvement of *M tuberculosis* is unlikely as it was not cultured from the lymph nodes, and the boy had a negative result on the Mantoux test. Other organisms such as *M scrofulaceum* can cause caseation in cervical lymph nodes. The information we have on this boy suggests that this was a mixed, non-tuberculous mycobacterial infection, which on this occasion also included *M paratuberculosis* in considerable abundance. We do not of course see caseation in the intestinal lesions of Crohn's disease.

George Griffin: Why do we not all get Crohn's disease?

JH-T: Probably for the same reasons that not everybody got tuberculosis earlier this century. In addition, *M paratuberculosis* has a much lower pathogenicity for humans than *M tuberculosis*. Several susceptibility factors are required for chronic enteritis and clinical disease due to *M paratuberculosis* to develop. These include firstly, an inherited susceptibility,³⁰ well known in Crohn's disease clinically, especially among Jewish people³¹ but possibly also in Celtic races. An understanding of the molecular genetics of susceptibility is just beginning to emerge.³²⁻³⁴ Secondly, intercurrent microbial infection (particularly gastroenteritis) is well known to be associated with the emergence of Crohn's disease. Lastly, psychological stress factors,³⁵ which are known to be able to trigger the emergence of Johne's disease in animals, may be associated with both the development of Crohn's disease and its exacerbation.

Devinder Kumar: If *M paratuberculosis* is a ubiquitous environmental organism how do you explain the geographical distribution of Crohn's disease?

JH-T: The environmental distribution of *M paratuberculosis* is not known. Efficient farming methods in industrialised societies, however, may have created localised conditions favouring the amplification of *M paratuberculosis* in our domestic livestock and their controlled habitats over the course of the century. Persistence of the pathogen would be favoured in temperate regions with a high water table.³⁶ Such conditions may also have increased the mutational frequency of *M paratuberculosis* and resulted in the emergence of a strain with increased pathogenicity for humans.

Robert Boyd: There is clearly a need for a randomised controlled trial of treatment with rifabutin and clarithromycin in patients with Crohn's disease.

NB: This certainly seems to be the case. A trial in children would seem to be particularly appropriate because they present relatively early on during the course of the disease, before they have developed the irreversible tissue damage and the range of complications that often occurs in adults.

George Griffin is professor of medicine, Angus Dalgleish is professor of oncology, Robert Boyd is principal, and Devinder Kumar is consultant surgeon at St George's Hospital Medical School, London.

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Conflict of interest: JH-T has prosecuted patents on IS900 and would share with coworkers in a proportion of revenues generated by the successful commercial exploitation and use of this technology.

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