EDITORIAL

Crohn's disease caused by *Mycobacterium avium* subspecies *paratuberculosis:* a public health tragedy whose resolution is long overdue

Crohn's disease is a systemic disorder whose principal clinicopathological manifestation is chronic inflammation of the intestine. Any part of the gastrointestinal tract from mouth to anus may be involved in the chronic granulomatous process, but the terminal ileum and colon are the regions most frequently affected. The disease commonly presents with abdominal pain, loss of energy and loss of weight, night sweats, mouth ulcers and joint pains. About 60% of patients have diarrhoea which contains mucous, pus and blood. The tissues around the anus and perineum may be chronically inflamed discharging pus from multiple sinuses. In children, growth and sexual maturation are retarded or arrested. Treatment is limited to the suppression or modulation of the inflammatory process. Surgery is required if the disease gets out of control, or if specific complications develop. These take the form of obstruction due to stricturing, abdominal abscesses, perforation of the gut, or fistulous connections leading to discharge of intestinal content from other organs such as the bladder or vagina. About 40% of patients with colonic Crohn's disease will end up with an abdominal bag collecting intestinal effluent from an ileostomy or colostomy.

Crohn's is a disease of developed societies in temperate regions of the globe, with efficient intensive farming. Although earlier cases are recorded, Crohn's disease emerged in Britain in the late 1940s, slightly ahead of the rest of Western Europe. It also emerged in North America and equivalent latitudes of the southern hemisphere. Thereafter the incidence of the disease in the UK has continued to climb, in recent years particularly in children [1]. We do not have accurate population data on the actual incidence, prevalence and geographic distribution of Crohn's disease in the UK because the Department of Health has so far failed to make the disease reportable. However, if we take the figures for incidence at 8.3/100 000/year and point prevalence (on 1 Jan. 1995) at 144.8/100000 which resulted from a survey of 135 723 GP clinical records in the North of England [2], and apply them to the whole population there would be 86 880 people with Crohn's disease in Britain. Likewise, we do not have an accurate figure for the direct health-care costs (DHC), but if we take data from other countries, a conservative estimate of mean DHC for the UK would be about £3000 per person per year ongoing, so the total DHC may be at least £270 million per year.

There is a very strong probability that most Crohn's disease is caused by infection with *Mycobacterium avium* subsp. *paratuberculosis* (MAP) [3]. This organism, originally called Johne's bacillus, was first identified in 1895 as the cause of chronic inflammation of the intestine in a German cow. Johne's disease (JD) appeared to increase in domestic livestock in Western Europe and North America up to the middle of the 20th century. The response on farms to a clinically sick cow with diarrhoea and low milk yield was to kill the animal. This understandable practice over the course of a century may have exerted a selection pressure on MAP towards the emergence of strains able to live in the gut of animals for years without causing clinical disease. The problem we face now is widespread subclinical as well as clinical MAP infection in cattle, sheep and goats, with herd prevalences in Western Europe and North America reported in the range 7-55% [4]. Infected dairy cows secrete MAP in their milk. MAP is tougher than M. *bovis* and can survive pasteurisation conditions of 72°C for 15 or 25 s [5]. Research in the UK initiated by the Ministry of Agriculture, Fisheries and Food (MAFF) and continued by the Food Standards Agency, has reported the presence of live MAP cultured in the laboratory from 1.7% of units of pasteurised milk on retail sale [6]. As MAP is very slow growing and difficult to isolate in conventional culture, the true proportion of units of retail pasteurised milk in Britain which contain live MAP may be higher. Furthermore, MAP shed on to pastures can survive for prolonged periods. There are wildlife reservoirs in rabbits and hares and their predators such as foxes, weasels and carrion birds [7]. MAP has also been reported in insects on infected farms [8]. Cattle consume rabbit droppings which act as a source of re-infection [9]. Environmental MAP is probably taken up by protozoa [3], and in an MRC-funded project in collaboration with Professor Roger Pickup at the Centre for Ecology and Hydrology, Lake Windermere, we are currently investigating the possibility that these pathogens may be conveyed to human populations in water supplies. What is certain, however,

is that in Britain, and by implication maybe elsewhere, human populations living in the same regions as endemically infected animals are exposed to MAP

MAP is a member of the *M.* avium complex (MAC). MAC are widely distributed in the environment and in healthy animals and people, and do not usually cause disease unless the host is debilitated or immunocompromised. By contrast, MAP is a pathogen and a specific primary cause of chronic inflammation of the intestine in many different animals including large and small ruminants, monogastrics such as dogs and pigs, and at least four types of subhuman primates [10]. MAP exhibits a considerable tissue tropism and, in animals, will result in chronic inflammation of the intestine even if given experimentally by subcutaneous or intravenous routes. The natural clinical disease in animals demonstrates a broad range of histopathological features from pluribacillary disease with millions of Ziehl-Neelson (ZN)-positive organisms visible microscopically within macrophages, to paucimicrobial disease with no visible organisms and chronic granulomatous inflammation, much like leprosy in man [3]. In animals, genetic differences may substantially influence susceptibility to the emergence of clinical disease. With the opportunity to amplify in crowded domestic livestock for over a century, MAP has almost certainly undergone an adaptive diversification. At least 28 genotypes of MAP have so far been identified with distinct genetic and phenotypic differences between bovine and ovine strains [11]. Comparison of the total genome sequence of *M.* avium 104 with that of a bovine isolate of MAP confirms the very close genetic relationship between MAP and other MAC. The genome of MAP is 0.2-0.3 Mb larger than that of M. avium 104 and this may be linked to its pathogenic phenotype. The extra DNA includes 14-18 copies of IS*900* which may influence gene regulation in MAP [12]. Furthermore, MAP has a 10974-bp insertion at IS*900* locus 6 containing genes which include a MAP catalase, a polyketide synthase, a cytochrome B and associated transcriptional regulators. Catalase activity is important for intracellular survival [13]. Polyketide synthases are involved in the biosynthesis of membrane polyunsaturated fatty acids [14] and in the production of polyketide toxin associated with mycobacterial virulence [15]. MAP also contains a single 6496-bp low G+C mol % cassette designated `GS' which contains genes for the biosynthesis, derivatisation and transport of fucose to the microbial cell surface [16]. Glycopeptidolipid putatively capped with derivatised fucose may contribute to the extraordinarily physical robustness of MAP, its ability to adopt a ZN-negative phenotype and minimise immune recognition.

The acquisition of a resident population of MAP in the intestine of people exposed to these potential pathogens, may be cumulative. As in animals, the terminal ileum and colon are preferentially involved and the organisms may be harboured for years without the development of clinical disease. As in animals, clinical disease may eventually emerge in individuals with an inherited or acquired susceptibility, and may be triggered by physical or psychological stress. Research into the molecular basis for such inherited susceptibility has recently identified mutations in the carboxy-terminal portion of the *NOD2* gene encoded on human chromosome 16, affecting 15-20% of people with Crohn's disease [17-19]. NOD2 is a receptor for bacterial lipopolysaccharides expressed on macrophages and it will be interesting to see how the expression of mutant NOD2 by macrophages may alter their relationship with MAP Other mutations such as that in the nuclear protein STATI dimer which binds to interferon (IFN)-y-activating sequences may also affect susceptibility to mycobacterial disease [20], and further definition of the molecular nature of the inherited susceptibility to Crohn's disease can be expected.

MAP has been grown in conventional culture from the inflamed intestine of people with Crohn's disease, but only in about 5% of cases. In the laboratory it slowly reverts to its ZN-positive phenotype after months or years of incubation. The early results of PCR studies on DNA extracts of Crohn's disease and uninflamed intestine were often conflicting, because of the use of a wide variety of sometimes inadequate sample processing procedures, as well as primers and PCR conditions which would amplify other elements in the IS*900* family present in some MAC, such as IS*1626* and IS*1613* [21]. Recent research in our own laboratory (unpublished) and at the University of Central Florida, with improved liquid cultures and methods optimised to ensure lysis of MAP with access to its DNA, together with nested IS*900* PCR uniquely specific for MAP, shows unequivocally that MAP is present and can often be cultured from the inflamed intestine of the substantial majority of people with Crohn's disease [3, 22]. For MAP in this situation not to contribute to pathogenesis and merely to have a bystander role, it would be necessary to accept that despite its specific ability to cause chronic inflammation of the intestine in so many animals including primates, it is somehow harmless to man. That this is unlikely to be the case is suggested by four independent open studies including our own initial work, all of which show that a substantial proportion of people with active Crohn's disease will get better with healing of the intestine when treated with drug combinations including rifabutin and clarithromycin. Rifampicin and erythromycin will inhibit a wide range of gut organisms, but they are not active against MAP infections and do not heal Crohn's disease. Rifabutin and clarithromycin also kill commensal gut organisms, but they are more active against MAP and can heal Crohn's disease. A large multi-centre randomised placebo-controlled trial of rifabutin, clarithromycin and clofazimine treatment in Crohn's disease began in Australia in September 1999 and should report in 2003.

The intracellular phenotype of MAP in human patients with Crohn's disease **and in animals with the** paucimicrobial form of Johne's disease, is one that does not demonstrate a classical ZN-positive lipid-rich mycobacterial cell wall, and it is unlikely that pathogenic mechanisms are the same as in tuberculosis [3]. It is much more likely that parasitisation of immunoregulatory macrophages and other cells by MAP in its protease-resistant ZN-negative phenotype, is associated with a variable immune dysregulation. Together with a leaky mucosa, the chronic transmural inflammation itself results from a perturbed inflammatory response to penetration into the gut wall of food residues and bacteria present in the lumen. This explains why Crohn's disease can be improved by immunomodulation or by reducing the intensity of the allergic component, with accompanying changes in enteric flora, by treatment with elemental diets. It also explains the temporary clinical improvement which may be achieved by general antimicrobial agents. However, without eradicating the underlying causative organisms, such treatments do not usually achieve lasting resolution of the disease. Highly significant antibody recognition by Crohn's disease sera of MAP proteins such as the p35 and p36 antigens [23], and others such as the 14-kDa secreted protein from MAP and AhpC [24], strengthen the case for the involvement of this enteric pathogen. A high proportion of Crohn's disease sera also contain IgA which binds a protein expressed by MAP called HupB [25]. HupB is very closely related to the 21-kDa surface protein of *M. leprae* which mediates Schwann cell invasion [26]. HupB also shares homology with the polymorph leucocyte antigen pANCA, recognition of which by circulating autoantibody characterises a clinical subgroup of Crohn's disease [27]. Specific neuronal inflammation in the gut wall, associated with MHC Class II expression, is a well-known feature of Crohn's disease [28] and also occurs in sheep with naturally acquired and experimental Johne's disease [29]. Taken together these data are consistent with MAP sharing some of the neuropathic properties of *M. leprae* in man and animals, and an opportunity for the involvement of molecular mimicry in aspects of the disease process.

It is likely that in the causation of Crohn's disease by MAP transmitted to people in milk and by other means, we are confronted with a public health problem of tragic proportions. Given the scale of this problem, the knowledge that MAP was first proposed as a cause of chronic inflammation of the intestine in man as early as 1913 [30], and the consistent enteric pathogenicity of MAP in such a wide range of animals, the families of teenagers newly diagnosed with Crohn's disease as well as informed public opinion, might reasonably ask why it is taking so long for medicine and science to realise what is going on under our noses and respond? If this were tuberculosis, AIDS or vCJD there would be large scale co-ordinated research and remedial measures on a national and international scale, to contain and reverse the problem. There are many reasons for the delay. The early expectation that if Crohn's disease was caused by MAP it should be possible to isolate MAP in culture or visualise it by light microscopy. Because of the intracellular phenotype of MAP and reluctance, particularly of human strains, to grow on conventional media, it is not. The superficial expectation that if Crohn's disease was caused by MAP, immune responses to it must be like TB, but they are not. The expectation that if Crohn's disease were caused by a mycobacterium it should heal on ordinary anti-tuberculous drugs [31], but it does not. The expectation that if Crohn's disease were caused by MAP, farmers and animal health-care workers would always be developing the disease, whereas repeated exposure to the mature ZN-positive bacillary form of MAP in animal faeces almost certainly confers some protection [32, 33]. The expectation that standard laboratory reagents like SDS-protease K and 6 M guanidine thiocyanate which reliably release DNA from other bacteria and other mycobacteria in a sample, are effective for MAP, they are not [3]. The resulting PCR data on MAP in Crohn's diseased intestine reported in 18 fully refereed publications between 1994 and 1999 were wholly conflicting [3].

We need now to disperse the climate of doubt and misconception and address ourselves to a clear-sighted recognition of this issue. This should lead to the implementation of a range of co-ordinated and incremental remedial measures. Ensuring that our milk is safe and that our farm animals are as free from MAP infection as can reasonably be achieved by modified farm practises, is a good start. Given the presence of wildlife reservoirs and the complexities of the overall problem, an effective veterinary vaccine which does not interfere with the diagnosis of tuberculosis and makes the gut as well as the body of the animal a hostile place for MAP [3], is an achievable priority. Clinical MAP infections in animals and man are very difficult to eradicate. We urgently need improved pharmaceuticals that are active against MAP and a therapeutic vaccine for human use to assist in immune-mediated microbial clearance.

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