Frequently asked questions on the links between mycobacteria and Crohn's disease

K. Hruska and I. Pavlik
Veterinary Research Institute, Brno, Czech Republic

Keynote lecture delivered at the International Conference on Gastro-Intestinal Microbial Ecology, Kosice, Slovakia, 8 - 11 November 2010

A role for mycobacteria as food pathogens or immunomodulators is not yet generally accepted, although the links between them and autoinflammatory and autoimmune diseases are well documented. Their cell wall components activate inflammatory pathways and have a strong immunomodulatory potential. Links between Crohn's disease and paratuberculosis, a mycobacterial infection of ruminants, were proposed already 97 years ago by Dalziel (1913). The clinical picture of both diseases is similar and the incidence of both is also increasing in parallel worldwide.

Mycobacterium paratuberculosis (MAP, official name *Mycobacterium avium* subsp. *paratuberculosis*) cells are a unique source of bacterial triggers, frequently ingested and inhaled by adults, babies and formula-fed newborns. The distribution of milk and meat contaminated with *M. paratuberculosis* is not prohibited or controlled. Hence, the number of cells acting as bacterial triggers for proinflammatory cytokines, or altering intestinal permeability is not under control, although this number can be very high. Moreover, components from other mycobacterial species which act in a similar manner, and which contaminate tap or bottled water, can also affect the development of adaptive immunity and immunological memory. The onset of the clinical signs of Crohn's disease or autoimmune disease may appear only later under the influence of a booster effect of *M. paratuberculosis* or other ingested or inhaled bacteria from food, water or the environment, or other pathogens, which multiply during infectious disease. A significant association of formula feeding with Crohn's disease was reported as far back as 1998. The "protective effect" of breast feeding against Crohn's disease, type 1 diabetes, atopic allergies, multiple sclerosis, rheumatoid arthritis, psoriasis and other autoimmune diseases is now well known, and can conversely be interpreted to mean that formula feeding has harmful effects. The presence of dead mycobacterial cells evidently represents a crucial difference between mother's milk and formula in relation to the development of immunity postnatally. The missing environmental factors in the etiology of Crohn's disease and autoimmune diseases, mentioned by many authors, could be bacterial, namely mycobacterial triggers. These triggers can contaminate baby food anywhere in the world even at a distance of thousands of kilometres from the paratuberculosis infected herd and in countries without a dairy industry. Threshold level, genetic disposition, physical handicaps and general condition can influence disease onset. Huge numbers of mycobacterial cells in milk, meat and water, mycobacteria in aerosols, biofilms and showerheads, their long survival in soil, manure and in surface water and in swimming pools, and multiplication in zooplankton, all established facts, should be borne in mind when evaluating mycobacteria as food and environmental risk elements. Changes in the cell wall, problems with culture, heat and chlorine resistance of mycobacteria can also play an important role. Bacterial triggers, originating from mycobacteria, in particular from *M. paratuberculosis*, can be an important piece of the Crohn's disease puzzle.

We would like to focus attention on some important and well known but not fully respected data and to highlight findings, which have not yet been seriously and in a responsible manner linked with Crohn's disease. Our efforts are directed at recognizing and decreasing the risk of mycobacteria, namely in baby food. Mycobacterial species, although considered non-pathogenic in terms of Koch´s postulates, can participate in the pathogenesis of

autoinflammatory or autoimmune diseases by means of their components similarly to known allergens or xenobiotics. From the results of many experiments it is evident that components of mycobacterial cell walls can participate in pathogenesis not only as an integral part of an intact living cell but also as products of cell destruction or as synthetic molecules.

Inflammatory bowel diseases have been recorded as early as the 18th century by Mogliani. Crohn's disease was described at the beginning of the last century by (Leśniowski 1903) and named after Crohn, who published a report on fourteen cases in 1932 (Crohn et al. 1932). Crohn's disease is well recognised as a lifelong health problem of people of different ages. The incidence of the disease has been increasing over the last decades. The disease was formerly connected mainly with urban populations in well developed countries, however in recent times it has become global and differences between urban and rural populations are less pronounced. The onset of disease in childhood is now more and more common and geographical incidence extends to regions where the disease was formerly rare or unknown. The etiology and pathogenesis of Crohn's disease is not yet fully elucidated. What is well described and generally accepted is a genetic factor, namely NOD2 mutations, which influence the effect of muramyldipeptide MDP on intestinal cells. However, changes in NOD2 are not present in a majority of Crohn's disease cases and therefore some environmental factor with a role in etiology is suspected. Environmental bacterial factors likely participate in inflammatory pathways, but standard mechanisms of infection have not been observed. However, some alteration of innate immunity is highly probable. The quality of life is dramatically decreased in patients and the costs of treating some 600 000 Americans, 700 000 European citizens and perhaps millions of sufferers in other parts of the world are enormous. The number of Crohn's disease patients in recent decades has been steadily increasing, in parallel with an enormous increase in paratubeculosis in ruminants, predominantly in cattle.

Data available from the Czech Republic are based on the well managed system of information collected by the Institute of Health Information and Statistics of the Czech Republic from the whole country since its existence in 1995. The index of all Crohn's disease patients between 1995 and 2009 has increased nearly 5 times and in the young population nearly seven times. The increase in Crohn's disease incidence is very likely associated with the opening of the Czech Republic to the Western European market in 1990. While no or negligible paratuberculosis cases in cattle were observed in the Czech Republic up until 1990, the contamination of meat, milk and baby food with *M. paratuberculosis* in the Czech Republic has been steadily rising during the last 20 years as a consequence of the increasing incidence of paratuberculosis in domestic herds and the massively increased import of cattle and dairy foods. Unfortunately, paratuberculosis is not a notifiable disease and exact data of incidence are not available. Nevertheless, the estimated herd incidence in the Czech Republic is around 30%, while in some other countries in Europe it is reported to be as high as 70% to 90%.

Another well documented link between paratuberculosis and Crohn's disease has been reported from Iceland (Pedley et al. 2004). Prior to 1930 paratuberculosis in Iceland was virtually unknown. Then in 1933, twenty sheep were imported from Germany and distributed to 14 farms (Fridriksdottir et al. 2000). Although apparently healthy, some of the sheep were subclinically infected with *M. paratuberculosis*. They transmitted MAP to the Icelandic sheep population though they never developed disease themselves. By 1938 clinical paratuberculosis appeared in Icelandic sheep on five of the original farms. By about 1945, clinical paratuberculosis was observed in the cattle on the same farms. The infectious organism isolated from these cattle was later confirmed as the sheep strain of *M. paratuberculosis* (Whittington et al. 2001). Slowly the infection spread so that by the late

1950s the disease had reached epidemic proportions with about 30% of sheep farms affected and huge annual losses. The mean incidence of Crohn's disease (number of cases/100 000 per year) in the human population was 0.4 from 1950-59, 0.45 from 1960-69, 0.9 from 1970-79, 3.1 from 1980-89 and 5.6 cases per 100 000 from 1990-94, inclusive. Young people were particularly affected (Bjornsson 1989; Bjornsson et al. 1998; Bjornsson and Johannsson 2000).

The bacterial triggers, mostly muramylpeptides, need not originate only from *M. paratuberculosis*. Different species of mycobacteria are common in biofilms in communal piping systems and in showerheads. The use of water from municipal sources is increasing and babies are increasingly being showered and brought to swimming pools in the last decades. Moreover, cattle farming technologies produce more liquid manure and contaminate river water with *M. paratuberculosis*. The presence of this organism in aerosols over rivers and their links with the incidence of Crohn's disease has been described. In summary, muramyl peptides originating from different mycobacteria increasingly come into contact with people of all age groups and will be ingested or inhaled until it is considered a risk and until it is under some control. As a result, people are challenged with live or dead mycobacterial cells, which contaminate milk, dairy and beef products, potable and surface water and water droplets in aerosols.

Bacterial cell wall components have a high immunomodulatory potential. Mycobacteria have been used in the complete Freund adjuvants for more than 50 years (in: Coulombe et al. 2009). Muramyldipeptides were discovered as the minimal structures responsible for the improved reaction to antigens in 1974 (Ellouz et al. 1974; Traub et al. 2006; Coulombe et al. 2009). This ability has been proved in synthetic molecules and recently Coulombe et al. (2009) reported that *N*-glycolyl MDP has a greater NOD2-stimulating activity than *N*-acetyl MDP. The former is produced by degradation of mycobacterial peptidoglycans.

Hypotheses on the etiology of Crohn's disease reflect the exceptional character of the condition. The pathological intestinal lesions and pathogenesis are identical with a similar chronic disease in cattle and other ruminants – paratuberculosis. Both diseases have a long latent period; live *M. paratuberculosis* cells or cultivable spheroplasts, e.g., cell wall-deficient *M. paratuberculosis* cells can be cultivated from blood and can be present in milk. The link between mycobacteria and Crohn's disease was mentioned for the first time as early as 1913 by Dalziel and has been supported by many authors since then. Critics of this theory base their objections on Koch's postulates, the lack of of culturable *M. paratuberculosis* in intestinal lesions from all Crohn's disease patients and the only partial therapeutic effect of antimycobacterials. However, in view of Crohn's disease as an autoinflammatory disease or a condition leading from altered innate immunity these objections are not conclusive. Crohn's disease is obviously a multifactorial disease with genetic, environmental, probably bacteriological, and immunological factors.

Many other hypotheses have been proposed. Psychrotropic bacteria and chilled food, extreme hygiene, adhesive E. coli, measles or other infections and vaccinations are some of the suggested theories. Undoubtedly none of these can be accepted exclusively, but all can play their role in individual cases and can be in agreement with the assumption that Crohn's disease is triggered in genetically or immunologically affected people by bacterial structures called pathogen-associated molecular patterns. Their interaction with the pattern recognition receptors can result in activation of anti-bacterial host defence reactions. It seems to be highly probable that food and environmental mycobacteria, considered to be non-pathogenic or potential pathogenic and being subject to no regular inspection and control could have a dominant role as the missing environmental factor in the pathogenesis of Crohn's disease.

The culture of *M. paratuberculosis* is extremely difficult and some authors believe that only 10% of life cells are produce colony forming units. This phenomenon has likely led to the underestimation of the seriousness *M. paratuberculosis* contamination. However, the question regarding triggering cells is not whether they are alive or dead but the issue is rather whether food and water are contaminated in a negligible concentration or are contaminated over the (yet to be adopted) DNA limits.

There is no doubt that *M. paratuberculosis* is present in dairy and beef products because no regulatory measures aimed at eliminating mycobacteria from the food chain and from the environment have yet been implemented worldwide. Quantitative determination of *M. paratuberculosis* DNA in real-time has been available only for the last few years and comprehensive data are not yet available. However, a higher ingestion of muramyldipeptides means a higher risk for consumers.

Some preliminary data on the highest numbers of *M. paratuberculosis* cells per gram are as follows:

milk for baby formula (per gram)	1,61E4
bulk tank milk (per ml)	1,59E2
milk from individual cow (per ml)	2,72E2
bovine masseter muscle (per gram)	1,37E4
bovine diaphragm pillar muscle	5,79E4
salami (per gram)	2,43E4
watering place water (per ml)	1,36E3
water plant reservoir (sediment, per gram)	1,00E2
faeces (per gram)	2,30E8
liquid manure (per gram)	6,80E4
dunghill lagoon (per gram)	3,18E3

This means a possible consumption of 1,5 million cells from 150 g beefsteak or more than 8 million cells from one package of formula milk. One 550 g package is consumed during less than 7 days by a newborn baby, and during 4 days by a baby 3 months old.

The contamination of infant formula milk with bacterial triggers poses a possible risk because muramylpeptides can activate immunomodulatory pathways during early development and likely influence T-cells and their participation in immunity mechanisms.

Links between breast/formula feeding and different (autoimmune and autoinflammatory) diseases have been published; conversely, the protective effect of breastfeeding against Crohn's disease, type 1 diabetes, atopic allergies, multiple sclerosis, rheumatoid arthritis, psoriasis and other autoimmune diseases has also been reported.

Breast feeding or limited access to dairy products from the western market can be connected with the lower incidence of Crohn's disease:

- in the Gipsy population of Hungary
- in Maori population of New Zealand
- in Palestinians in Israel
- in Indians in Canada
- in people with higher social position
- in some ethnic groups living in underdeveloped countries in comparison with immigrants from the same ethnic group in developed countries

Some cases or outbreaks demonstrating evident links between *M. paratuberculosis* and Crohn's disease have been reported (Pedley et al. 2004). Differing prevalences of Crohn's disease have been reported from within Cardiff. This city is divided by the river Taff, which is contaminated with *M. paratuberculosis* from pastures. Residents, living on the river side with prevailing winds suffer more from Crohn's disease. The reason for this is very likely the contaminated aerosol, which is able to concentrate mycobacterial cells 14 000 fold. Heavily grazed pastures have been associated with conspicuous clusters of Crohn's disease. In the village of Blockley, a rural community of about 2000 people in Gloucestershire, England 12 people developed Crohn's disease between 1960 and 1983. The village, which had its own water supply from local springs, lay in a hollow surrounded by upland pastures grazed by cattle in which clinical paratuberculosis was evident. Seven cases of Crohn's disease amongst 285 graduates of the Mankato West High School class of 1980 were reported. All seven had a common hobby, swimming in local ponds and lakes. There are also data which implicate domestic hot water systems. Two case control epidemiological studies carried out independently in the United Kingdom, each unexpectedly identified the availability of fixed hot water supplies in the early childhood home as a significant risk factor for the subsequent development of Crohn's disease, but not for ulcerative colitis (Gent et al. 1994; Duggan et al. 1998). This constitutes a very interesting complement to the hygienic theory of the origin of Crohn's disease.

The information in this presentation should not be considered as alarming rumours, but as a public health problem which cannot be ignored. It is not possible to expect the eradication of paratuberculosis from cattle herds in the next 20 or 30 years or the elimination of mycobacteria from municipal water distribution systems. As a start it would be useful to learn and remember a few not altogether new or original definitions:

- mycobacteria are immunomodulators and potential food pathogens or allergens
- bacteria need not be alive to be harmful
- breast feeding is a priority
- bottle feeding is vital if breast feeding is not possible; however, the number of mycobacteria in the formula should be under strict control

REFERENCES

Bjornsson S (1989): Inflammatory bowel disease in Iceland during a 30-year period, 1950-1979. Scand.J.Gastroenterol.Suppl 170**,** 47-49.

Bjornsson S, Johannsson JH (2000): Inflammatory bowel disease in Iceland, 1990-1994: a prospective, nationwide, epidemiological study. Eur.J.Gastroenterol.Hepatol. 12**,** 31-38.

Bjornsson S, Johannsson JH, Oddsson E (1998): Inflammatory bowel disease in Iceland, 1980-89. A retrospective nationwide epidemiologic study. Scand.J.Gastroenterol. 33**,** 71-77.

Coulombe F, Divangahi M, Veyrier F, de LL, Gleason JL, Yang Y, Kelliher MA, Pandey AK, Sassetti CM, Reed MB, Behr MA (2009): Increased NOD2-mediated recognition of N-glycolyl muramyl dipeptide. J Exp.Med 206**,** 1709-1716.

Crohn BB, Ginzburg L, and Oppenheimer GD. (1932): Regional ileitis; a pathologic and clinical entity. Journal of the American Medical Association 99, 1323-1329.

Dalziel TK (1913): Chronic interstitial enteritis. British Medical Journal 2, 1068-1070

Duggan AE, Usmani I, Neal KR, Logan RFA (1998): Appendicectomy, childhood hygiene, Helicobacter pylori status, and risk of inflammatory bowel disease: a case control study. Gut 43**,** 494-498.

Ellouz F, Adam A, Ciorbaru R, Lederer E (1974): Minimal structural requirements for adjuvant activity of bacterial peptidoglycan derivatives. Biochemical and Biophysical Research Communications 59**,** 1317-1325.

Fridriksdottir V, Gunnarsson E, Sigurdarson S, Gudmundsdottir KB (2000): Paratuberculosis in Iceland: epidemiology and control measures, past and present. Vet.Microbiol. 77**,** 263-267.

Gent AE, Hellier MD, Grace RH, Swarbrick ET, Coggon D (1994): Inflammatory Bowel-Disease and Domestic Hygiene in Infancy. Lancet 343**,** 766-767.

Leśniowski A (1903): Przyczynek do chirurgii kiszek. Medycyna (Warszawa) 31, 460-464

Pedley S, Bartram J, Rees G, Dufour A, Cotruvo JAE (2004): Pathogenic Mycobacteria in Water - A Guide to Public Health Consequences, Monitoring and Management. Emerging Issues in Water and Infectious Disease Series, World Health Organization titles with IWA Publishing 1-222.

Traub S, von Aulock S, Hartung T, Hermann C (2006): MDP and other muropeptides - direct and synergistic effects on the immune system. Journal of Endotoxin Research 12**,** 69-85.

Whittington RJ, Taragel CA, Ottaway S, Marsh I, Seaman J, Fridriksdottir V (2001): Molecular epidemiological confirmation and circumstances of occurrence of sheep (S) strains of Mycobacterium avium subsp. paratuberculosis in cases of paratuberculosis in cattle in Australia and sheep and cattle in Iceland. Vet.Microbiol. 79**,** 311-322.