MYCOBACTERIUM AVIUM SUBSP *PARATUBERCULOSIS* AND CROHN'S DISEASE

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RESUMO

Mycobacterium avium subsp *paratuberculosis* (MAP) é o agente etilógico da doença de Johne, uma doença inflamatória intestinal que afecta o gado, bem como outras espécies de mamíferos. Existem evidências de que, em humanos, MAP também despoleta a doença de Crohn, embora esta seja ainda uma hipótese controversa. Neste artigo será apresentado um sumário das características da doença de Johne e da doença de Crohn, e discutido o papel de MAP na doença de Crohn, com base em algumas evidências apresentadas na literatura.

PALAVRAS-CHAVE: Doença de Crohn; doença de Johne; *Mycobacterium avium* subsp *paratuberculosis*; citoquinas.

ABSTRACT

Mycobacterium avium subsp *paratuberculosis* (MAP) is the etiologic agent of Johne's disease, an inflammatory bowel disease occurring in cattle as well as in a number of mammal species. In humans, there are evidences that point to a role of MAP as an agent of Crohn's disease, although this is still a matter of controversy. In this review, a summary of the characteristics of Johne's disease and Crohn's disease will be presented, and the involvement of MAP in Crohn's disease will be discussed, based on reported evidences.

KEY-WORDS: Crohn's disease; Johne's disease; *Mycobacterium avium* subsp *paratuberculosis*; cytokines.

1. **INTRODUCTION**

The genus *Mycobacterium* comprise over 50 bacterial species. These bacteria possess a complex hydrophobic cell wall, which render them quite resistant to environmental stress. The majority of *Mycobacterium* species are environmental and non-pathogenic, closely related to the soil bacteria *Streptomyces* and *Actinomyces* (Cosma *et al.*, 2003). However, a few species are highly successful pathogens. *M. tuberculosis* is the most epidemic pathogen, with two millions infected worldwide and an estimated one third of the world population colonized. Other mycobacterial species also present a known pathogenic threat for humans and other animals, namely *M. leprae, M. ulcerans, M. bovis, M. avium* (Cosma *et al.*, 2003). The pathogenic species are intracellular pathogens, residing inside macrophages and dendritic cells. Those cells act as a growth niche.

M. avium subsp. *paratuberculosis* (MAP) is the etiologic agent of Johne's disease, an inflammatory bowel disease occurring in cattle, sheep, rabbits, deer, as well as in some primates (Glawischnig *et al.*, 2006; Judge *et al.*, 2005; McClure *et al.*, 1987). There is controversy about MAP being a cause of Crohn's disease in humans, although an increasing amount of scientific evidence suggests that this organism can be responsible for at least some cases of Crohn's disease (Ren *et al.*, 2008; Clancy *et al.*, 2007; Scanu *et al.*, 2007; Uzoigwe *et al.*, 2007; Naser *et al.*, 2004; Bull *et al.*, 2003b).

2. **JOHNE'S DISEASE**

Johne's disease has been primarily studied in domestic ruminants and is associated with an elevated economic impact. Cattle usually get primary infection as calves, by in utero transmission, or after birth, by ingestion of fecal contaminated material, collostrum or milk containing MAP. There is evidence that contamination may also result from contact with wild animals (Florou *et al.*, 2008; Corn *et al.*, 2005). Some calves become infected, although others show resistance to infection. A few infected animals develop clinical disease after a period of several years. In pre-clinical period, shedding of MAP is intermittent, while clinically affected cows may shed 10^6-10^8 CFU/g of fecal material. Clinical signs include weight loss, diarrhoea, poor milk production and increased morbidity. MAP organisms enter through the intestinal tract, and initial lesions consist of small granulomas formed in the intestinal wall and draining lymph nodes. With time, these granulomas become larger and may evolve into a diffuse granulomatous inflammation, with acid fast bacilli found inside macrophages and multinucleated giant cells. These lesions result in a thickness of the intestinal wall, resulting in decreased absorption, and fluid secretion into the gut lumen. Lesions are largely restricted to the ileum, or the ileocecal valve region of the small intestine(Wu *et al.*, 2007; Coussens, 2004; Sigurethardottir *et al.*, 2004; Stabel, 2000).

3. **INFLAMMATORY BOWEL DISEASE AND CROHN'S DISEASE**

Inflammatory bowel disease (IBD) describes a group of intestinal inflammatory disorders of unknown etiology. It may affect man and women equally, and also children. It usually presents in individuals at age 13-30, or 50-70. IBD comprises two major groups: ulcerative colitis (UC) and Crohn's disease (CD) (Chacon *et al.*, 2004). UC is characterized by a diffuse mucosal inflammation that may involve the rectum and spread to colonic tissue or even ileal tissue. The inflammatory lesions are continuous, without intact areas between them. CD is a transmural inflammatory disease that may involve any part of the gastrointestinal tract, from the mouth to the anus, although most of the patients develop terminal ileum CD. Symptoms vary with the affected area, however, the main gastrointestinal symptoms include diarrhea (that may be bloody), weight loss, constipation and vomiting. CD can also cause symptoms unrelated to

the intestinal tract, namely dermatologic, rheumatologic, ocular, hepatobiliary, and urologic manifestations (Chacon et al., 2004).

4. **IS MAP CAUSING CD?**

Incidence of CD is increasing in developed countries (Bernstein *et al.*, 1999; Loftus *et al.*, 1998; Munkholm *et al.*, 1992). Genetic and environmental factors seem to be involved in the etiology of CD, as well as immune disregulation. CD is probably a multifactorial disease, because although environmental factors (as infection or diet) and at least eight genetic loci have been implicated, none alone have been proven to cause the disease (Brant and Shugart, 2004; Chamberlin *et al.*, 2001).

Evidences exist that support an infectious etiology in CD: (i) a concordance rate of only 44% between monozygotic twins with inflammatory bowel disease (Tysk *et al.*, 1988); (ii) clustering of IBD cases among spouses and other members of affected families; (Comes *et al.*, 1994); and (iii) clustering of IBD among unmarried individuals having close contact (Aisenberg and Janowitz, 1993). Infected individuals with pre-disposing condition may develop CD, although infection may be one but not the only cause for the disease.

Although some reports suggest that the intestinal commensal flora as a whole may be involved in the development of CD (Girardin *et al.*, 2003), several microrganisms have been suggested to have a role in the disease etiology. Similarities between CD and Johne's disease point to a possible link to MAP infection. The fact that MAP can cause disease in several mammal species including primates, is an argument in favour of the link between MAP and Crohn's disease in humans. Indeed, MAP was detected by PCR and culture from intestinal tissue, blood and breast milk of CD patients (Naser *et al.*, 2004; Bull *et al.*, 2003a; Naser *et al.*, 2000). Serum antibodies against MAP antigens were also detected in CD patients (Naser *et al.*, 1999). Recently, a report showed that IL-10-deficient mice, which develop spontaneous colitis under conventional housing conditions, fail to do so when the mice are housed in a germfree environment, suggesting a microbial trigger. Mice with spontaneous colitis (under conventional housing conditions) presented significantly higher IgG2a specific antibody against MAP as compared to controls. Administration of MAP to IL-10-deficient mice housed in a germfree environment led to colitis development, increase in serum TNFα, IFNγ and chemokines, and also an increase in lymphocyte proliferation and IL-2 production (Singh *et al.*, 2007).

MAP in humans assumes an obligate intracellular spheroplast form, residing in macrophages and dendritic cells, as other mycobacteria (Jiao *et al.*, 2002; Markesich *et al.*, 1988). Production of pro-inflammatory cytokines by MAP infected cells of susceptible hosts may contribute to generate the inflammatory process in CD. There are reports with bovine and murine macrophages and dendritic cells that support this pro-inflammatory effect of MAP (Langelaar *et al.*, 2005; Zur Lage *et al.*, 2003). Studies performed in bovine peripheral blood monocyte-derived macrophages suggest a primary stimulation of pro-inflammatory cytokine production by MAP infection, followed by an increase in IL-10 production (Coussens *et al.*, 2004). IL-10 was found to decrease killing of MAP, and IL-10 expression is suggested to be a determinant of virulence for this organism (Weiss *et al.*, 2005). Induction of IL-10 seems to be dependent on the MAPK(p38) pathway (Souza *et al.*, 2006). In a study using bovine peripheral blood monocyte-derived dendritic cells, MAP was found to stimulate pro-inflammatory cytokine production (strong IL-12 induction), while MAP heat-shock protein (Hsp) 70 resulted in a more inhibitory type of cytokine gene expression, suggesting it may be a virulence factor for MAP (Langelaar *et al.*, 2005). There are still no reports on cytokine induction by MAP in human cells. Those studies could contribute to elucidate MAP involvement in Crohn's disease.

Mechanisms of MAP survival inside macrophages have also been addressed for bovine and murine macrophages. MAP was found to inhibit acidification of phagosomes and to decrease phagosome maturation (Weiss *et al.*, 2004; Hostetter *et al.*, 2003) in these cells. A recent report of *in vitro* infection of peripheral blood neutrophils from CD patients with MAP, showed that MAP mimicked *M. tuberculosis* both in high frequency of colocalization with an early endosomal marker and in survival rate inside the phagocytes, which suggests that MAP can be potentially pathogenic for humans (Rumsey *et al.*, 2006). Survival of MAP in human macrophages has not been addressed and is important to understand the pathogenesis of MAP to humans.

Further research on the interaction of MAP with human phagocytes is needed to evaluate MAP possible role as an etiologic agent of Crohn's disease.

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