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Expression of NRAMP1 and iNOS in *Mycobacterium avium* subsp. *paratuberculosis* naturally infected cattle

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Abstract

Paratuberculosis (PTB) is a chronic disease caused by *M. avium* subsp. *paratuberculosis* (MAP) that affects several animal species, and some studies have suggested that there may be a relationship between Crohn's disease and PTB. Significant aspects of PTB pathogenesis are not yet completely understood, such as the role of macrophages. Natural resistance-associated macrophage protein 1 (NRAMP1) and the inducible nitric oxide synthase (iNOS) molecules have shown nonspecific effects against several intracellular pathogens residing within macrophages. However, these molecules have been scarcely studied during natural infection with MAP. In this work, changes in NRAMP1 and iNOS expression were surveyed by immunohistochemistry in tissue samples from MAP-infected cattle and healthy controls. Our findings show strong specific immunolabeling against both NRAMP1 and iNOS molecules, throughout granulomatous PTB-compatible lesions in ileum and ileocaecal

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lymph nodes from paratuberculous cattle compared with uninfected controls, suggesting a relationship between the expression of these molecules and the pathogenesis of PTB disease.

Keywords: Johne's disease; Cattle; Paratuberculosis; MAP; NRAMP1; Slc11a1 and iNOS

Résumé

La Paratuberculosis (PTB) est une maladie chronique causée par *M. avium subsp. paratuberculosis* (MAP) laquelle peut affecter plusieurs espèces animales, certaines études suggèrent une relation avec la maladie de Crohn. La pathogénie PTB n'est pas totalement comprise, certains aspects restant inconnus comme par exemple le rôle joué par les macrophages. La protéine 1 du macrophage associée à la résistance naturelle (NRAMP1) et l'oxyde nitrique synthase inducible (iNOS) ont démontré avoir des effets non spécifiques contre plusieurs pathogènes intracellulaires résidant dans les macrophages. Cependant peu d'études ont été réalisées pendant l'infection naturelle du MAP. Au cours de ce travail, en utilisant l'immunohistochimie, des changements dans l'expression de NRAMP1 et de l'iNOS dans des échantillons tissulaires de MAP de bétails infectés et de contrôles sains ont été mis en évidence. Ce travail démontre la présence d'un immunomarquage spécifique contre les deux molécules (NRAMP1 et iNOS), dans toutes les lésions granulomateuses compatibles avec la PTB, dans les ganglions lymphatiques de l'iléon et de l'ileocaecal du bétail ayant la paratuberculose en comparaison avec les contrôles non infectés, suggérant une relation entre l'expression de ces molécules et la pathogénie de la PTB.

Mots clés : La maladie de Johne ; Bétail ; Paratuberculosis ; CARTE ; NRAMP1 ; Slc11a1 et iNOS

1. Introduction

Paratuberculosis (PTB), also known as Johne's disease, is a chronic ailment caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP) in various animal species and is characterized by clinical signs such as diarrhea, weight loss, decreased milk production, and finally, death. PTB may affect cattle, sheep, goats, deer and muflons, as well as non-ruminant species, including rabbits, pigs and equines [6,7,9,12,21]. MAP infection was recently identified in one patient with HIV [23], and it has been implicated as a possible cause of Crohn's disease, a human intestinal disease of unknown etiology [13,20]. In cattle, advanced PTB produces granulomatous enteritis, characterized by epithelioid cells, giant cells and areas of necrosis; however, many aspects of PTB pathogenesis have not been elucidated. Animals become infected during the first months of life by consumption of contaminated milk or feed; but, in many cases, the disease remains latent without clinical manifestation [4]. The mechanisms responsible for this heterogeneous response are not well understood. It has been suggested that immunological and nutritional conditions, as well as ingested bacterial load may be important for PTB development. The microorganism penetrates the intestinal mucosa through the M cells, is engulfed by resident macrophage, proliferates slowly, and induces the development of early cell-mediated immunity (CMI) characterized by epithelioid and giant cells [19,24]. After the intestinal infection, bacilli may spread to the regional lymph nodes forming new granulomatous foci. Following the