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Short communication

Altered patterns of toll-like receptor gene expression in cull cows infected with *Mycobacterium avium* subsp. *paratuberculosis*

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ABSTRACT

Johne's disease caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP), is a chronic enteric disease of cattle. The mechanism how MAP can co-exist in the gastro-intestinal tract despite a massive infiltration of immune cells is not known. Toll-like receptors (TLRs) are known to play an important role in both innate and acquired immune responses but it is unclear what role different TLRs play in response to MAP. In this study, 38 cull cows from herds infected with MAP were classified into four groups, based on MAP culture from gut tissues and histopathological lesion scores. The expression of TLR1, 2 and 4 mRNA from MAP antigen-stimulated mesenteric lymph node (MLN) cultures and peripheral blood mononuclear cells (PBMCs) and in the MLN and ileum tissues of these animals was determined. MAP antigen-specific expression of TLR1 in MLN and PBMC was significantly lower in the MAP-infected groups than the non-infected control group, suggesting that in MAP-infected animals there is impairment in the up-regulation of TLR1 in response to MAP antigen. TLR4 expression in MLN tissues was significantly higher in the severely infected group than the control group suggesting up-regulation of endogenous TLR4 expression at a site of MAP infection in animals severely affected with Johne's disease. A preliminary screening of TLR1, 2 and 4 in the cull cows revealed the presence of polymorphisms in TLR1 and TLR2. In summary, one mechanism how MAP may subvert the immune system is that there is an apparent lack of recognition of MAP antigens as foreign by TLR1 in MAP-infected cows.

1. Introduction

Johne's disease caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP) is a chronic enteric disease of cattle and other ruminants and causes major economic losses in many countries (Whittington et al., 2011). MAP is transmitted, primarily, via the faecal-oral route, with infection occurring in young animals (Windsor and Whittington, 2010), although is followed by a long subclinical period ranging from 2 to 5 years (Cocito et al., 1994). At the advanced stage, the disease is manifested as a chronic granulomatous enteritis leading to reduced production, wasting, weight loss and eventually death (Harris and Barletta, 2001). In advanced disease, MAP is able to co-exist with a massive infiltration of immune cells in the gastro-intestinal tract, but the mechanism how MAP dampen or distort immune responses at the mucosal sites is not known.

Host recognition of pathogen associated molecular patterns displayed by MAP is one of important factors for development of an effective immune response against Johne's disease (Sohal et al., 2008). Toll-like receptors (TLRs) are a class of pattern recognition molecules with a unique function in the innate immune system and host

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defence in response to foreign antigens (Cristofaro and Opal, 2006). TLRs on the surface of macrophages and dendritic cells bind pathogen associated molecular patterns leading to induction of cytokines and reactive oxygen and nitrogen intermediates (Werling et al., 2006). Activation of TLRs has been shown to coordinate not only innate immune response but also adaptive immune response to microorganisms (Schnare et al., 2001; Takeda et al., 2003). TLR1, 2 and 4 have been associated with cellular recognition of mycobacteria by binding cell wall components including lipoproteins (Purdie et al., 2011). There is uncertainty in the precise role that each TLR plays in response to MAP infection. TLR2 and 4 were shown to have an involvement in recognition of MAP in mice with macrophages from TLR2 and TLR4 knockout mice producing less cytokines than wild type mice in response to MAP antigens (Ferwerda et al., 2007). A study on MAP-infected bovine monocytes suggested that MAP interaction with TLR2 leads to suppression of antimicrobial responses by initiating IL-10 transcription and inhibiting phagosome maturation (Weiss et al., 2008).

There have been several reports on the expression of TLR genes in sheep (Nalubamba et al., 2008; Plain et al., 2010; Taylor et al., 2008) but no study on TLR gene expression in response to MAP infection in cattle has been reported. Determining the profile of TLR gene expression in cattle with different severity of Johne's disease may contribute to understanding the role TLRs play during MAP infection. In addition, there is growing evidence that single nucleotide polymorphisms in TLR genes influence the course of infectious and inflammatory disease (Schroder and Schumann, 2005). Linkage between TLR1, 2 and 4 gene mutations and increased susceptibility to MAP infections has been reported in cattle (Ruiz-Larrañaga et al., 2011; Koets et al., 2010; Mucha et al., 2009) and sheep (Bhide et al., 2009). Mutations in TLR genes have been targeted as possible gene markers for susceptibility to MAP infection (Koets et al., 2010).

In this study, mRNA expression of TLR1, 2 and 4 were measured in immune cells from blood and mesenteric lymph nodes (MLNs) stimulated with MAP antigen and in gastro-intestinal tract tissues of cull cows which had varying severity of histopathological lesions associated with Johne's disease. The TLR1, 2 and 4 genes from the cull cows were analysed to identify mutations which may be associated with severity of disease.

2. Material and methods

2.1. Animals

The 38 cull cows were purchased from seven dairy herds in the lower North Island, New Zealand which had a history of Johne's disease. These dairy herds remained outdoors all year and were on a pasture-based diet. These animals were culled because of poor milk production, not in-calf or had a positive serological response to MAP. The cows which were negative for MAP serology were randomly selected from the cull cow populations. The breeds of the cows were Friesian, Jersey or Friesian/Jersey cross and had a mean age of 5.3 years. All procedures performed on the animals were approved by the Institutional Animal Ethics Committee (AgResearch Grasslands, Palmerston North, New Zealand).

2.2. Sample collection

Prior to slaughter of the cows, 40 ml of blood was collected into heparinised tubes (BD Vacutainer, Becton Dickinson, NJ, USA) for peripheral blood mononuclear cell (PBMC) culture. Five microlitres of blood was collected for serum separation and sera stored at −20 °C. Following slaughter, a sample of MLN (1 cm^3) draining the ileum was collected for cell culture studies. Samples of MLN, ileo-caecal lymph node, distal ileum (50 cm from the ileo-caecal valve) and ileo-caecal valve were collected for MAP culture and histopathological assessment.

2.3. MAP culture

Procedures for MAP cultures have been described previously (Shu et al., 2011). A cow was classified as MAP-infected if MAP was cultured from one or more tissue samples. Faecal samples were not collected for MAP culture as culture for MAP from tissues was considered more definitive.

2.4. Histology

Tissue samples for histology were fixed in 10% buffered-formalin and 4 μm sections were stained with Haematoxylin Eosin (HE) and Ziehl-Neelsen (ZN). Histopathological changes from sections of distal ileum and ileo-caecal valve were scored as described previously (Shu et al., 2011). Briefly, the scoring system was based on the condition of villi, cellular infiltrate in the lamina propria, the presence and severity of granulomatous lesions, and presence of acid fast bacilli (AFB), while sections of the mesenteric and ileo-caecal lymph nodes were scored on the presence and severity of granulomatous lesions, and presence of AFB. Each parameter was scored separately from 0 to 3, progressing from no pathology (0) to severe pathology (3). Scores of the four parameters (villi, cellular infiltration, granulomas and AFB) were pooled to give a score for an individual intestine sample and scores for the two intestinal samples were added together to provide a combined intestinal tissue score (maximum score of 24). Similarly, scores of the two parameters (granulomas and AFB) for the two lymph node samples were added together for a combined lymph node score (maximum score of 12) and a total lesion score was obtained by combining the intestinal and lymph node scores (maximum score of 36). The scoring of the sections was undertaken blindly by one person.

2.5. Serology

Serological responses to MAP were measured using the Pourquier ELISA (IDEXX Laboratories, USA) as described by the manufacturer.