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Can early host responses to mycobacterial infection predict eventual disease outcomes?

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ABSTRACT

Diagnostic tests used for Johne's disease in sheep either have poor sensitivity and specificity or only detect disease in later stages of infection. Predicting which of the infected sheep are likely to become infectious later in life is currently not feasible and continues to be a major hindrance in disease control. We conducted this longitudinal study to investigate if a suite of diagnostic tests conducted in *Mycobacterium avium* subspecies *paratuberculosis* (MAP) exposed lambs at 4 months post infection can accurately predict their clinical status at 12 months post infection. We tracked cellular and humoral responses and quantity of MAP shedding for up to 12 months post challenge in 20 controls and 37 exposed sheep. Infection was defined at necropsy by tissue culture and disease spectrum by lesion type. Data were analysed using univariable and multivariable logistic regression models and a subset of variables from the earliest period post inoculation (4 months) was selected for predicting disease outcomes later on (12 months). Sensitivity and specificity of tests and their combinations in series and parallel were determined. Early elevation in faecal MAP DNA quantity and a lower interferon gamma (IFNγ) response were significantly associated with sheep becoming infectious as well as progressing to severe disease. Conversely, early low faecal MAP DNA and higher interleukin-10 responses were significantly associated with an exposed animal developing protective immunity. Combination of early elevated faecal MAP DNA or lower IFNγ response had the highest sensitivity (75%) and specificity (81%) for identifying sheep that would become infectious. Collectively, these results highlight the potential for combined test interpretation to aid in the early prediction of sheep susceptibility to MAP infection.

1. Introduction

Disease outcomes following exposure to virulent mycobacteria are not uniform; not all exposed individuals become infected and, amongst those that do, factors such as the rate of disease progression and disease pathology are variable (American Thoracic Society, 2000). This may partly be due to the nature of the pathogen as virulent mycobacteria are notoriously slow-growing organisms and can switch between dormant and active phases (Magombedze and Mulder, 2012). The host response also plays a pivotal role in orchestrating the progress of mycobacterial disease (Kunnath-Velayudhan and Gennaro, 2011).

Mycobacterium avium subsp. *paratuberculosis* (MAP) causes Johne's disease in ruminants, a chronic debilitating disease resulting in chronic diarrhoea and eventually death. In ruminants the usual route of exposure to MAP is oral, via ingestion of contaminated milk or faecal matter. Once MAP enters the intestinal wall, initial contact is with phagocytic cells such as macrophages. At this stage, these cells

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of the innate immune system may be able to destroy the pathogen. Alternatively, MAP may actively evade intracellular killing mechanisms and take residence within these cells (Weiss et al., 2002). As a result, a more complex host response is required. Antigen presenting cells such as the macrophages and dendritic cells are able to indicate the presence of infection by presenting pathogen-derived antigens on their cell surface. These cells also release a variety of signals in the form of cytokines and chemokines (Weiss and Souza, 2008). The initial response predominantly involves the antigen-specific release of interferon gamma (IFNγ) by T lymphocytes and as disease progresses this response is replaced by an antibody response. In sheep, this classical response is as common as a simultaneous IFNγ and antibody response (Begg et al., 2011). IFNγ activates bystander macrophages and facilitates intracellular killing of MAP. While the IFNγ response is important in the cell-mediated control of intracellular pathogens like MAP it is not always a predictor of disease outcome (Jungersen et al., 2012). To counteract the host's immune response, MAP can also actively induce certain cytokines to suppress and evade immune cells; interleukin (IL)-10 and tumour growth factor (TGF)β are two such cytokines (Weiss and Souza, 2008). IL-10 can also reduce the ability of macrophages to kill intracellular MAP (Weiss et al., 2005). The presence of specific antibodies produced by the humoral arm of the adaptive immune system is widely used as an indicator of disease although the exact mechanism by which it acts against an intracellular organism is not clear. As disease progresses (with the expression of clinical disease) in some animals there is a general suppression of the immune system and this is thought to be due to an increase in the secretion of immunosuppressive cytokines such as IL-10. However, we have previously shown that an early IL-10 response also occurs after exposure to MAP (de Silva et al., 2011).

Infected sheep can shed huge amounts of MAP in their faeces – as high as 10^8 mycobacterial bacilli per gram of faeces (Whittington et al., 2000) – and thus contaminate pasture and act as major source of infection for susceptible animals. Therefore, control of Johne's disease is usually based on culling these highly infectious animals or by using management practices to avoid or minimise their contact with susceptible animals. Although vaccines can be used as a preventative measure, they are not fully protective in sheep as some continue to shed MAP after vaccination (Reddacliff et al., 2006; Eppleston et al., 2011).

For any of the control measures to be fully effective, there is need of a sensitive and specific diagnostic test, or a suite of tests, to identify infectious animals – not when they are already shedding huge amounts of bacilli – but at a younger age when they are still in early stages of infection. Achieving this goal would enable removal or separation of such animals from a flock prior to their being able to spread the disease. Such a test should also prevent removal of animals that, although infected, are unlikely to become highly infectious but instead are likely to clear infection (Dennis et al., 2011).

Most of the currently available diagnostic tests for MAP infection have poor sensitivity or specificity in younger age and only detect animals in later stages of infection, when they have already made substantial contributions to contamination of the pasture and have already infected many susceptible animals. Sensitive and specific diagnosis of disease in early age or our ability to identify infected animals prior their becoming highly infectious would be a major step forward in controlling the disease. We conducted this longitudinal study to investigate if there are any such diagnostic indicators that singly and in combination can predict the eventual clinical outcome of sheep. In this study, we monitored several potential indicators of infection and immune responses in experimentally challenged sheep from time of exposure to up to 1 year post inoculation (p.i.) and evaluated if indicators from an early age can predict pathological and clinical status of animals 1 year post-infection.

Identifying indicators of protection against paratuberculosis has benefits in addition to the possible prediction of disease outcome (Berry et al., 2010; Kunnath-Velayudhan and Gennaro, 2011). Understanding the initial changes in immune parameters would inform the design of novel vaccines that drive a similar immune signature leading to the production of a more efficient vaccine.

2. Materials and methods

2.1. Animals

The use of animals (Merino sheep) in this study was approved by the University of Sydney Animal Ethics Committee.

Merino lambs were obtained from the University of Sydney farms in NSW, Australia where the parent flocks were shown to be free from MAP infection by repeated testing using faecal culture (by the radiometric BACTEC method) and antibody ELISA (Institut Pourquier). The lambs were brought to and held within a control farm free from MAP infection for two weeks to acclimatise to the new environment. Negative infection status of the lambs was verified by antibody ELISA, IFNγ ELISA and faecal culture prior to experimental inoculation (Begg et al., 2010). All animals were managed similarly under conventional Australian sheep farming conditions in open paddocks; control animals were kept on pasture isolated from MAP-exposed animals. Fifty-seven lambs (3–4 months of age) were drafted into control and exposed groups using systematic sampling and either left unexposed ($n=20$) or orally exposed to MAP S strain ($n=37$). MAP exposed groups received a total of 6.72×10^9 of a clonal isolate or 7.52×10^9 of a gut homogenate from a sheep with clinical Johne's disease (de Silva et al., 2010). For this study, data from animals exposed to the clonal isolate and gut homogenate were considered together as 'MAP exposed' animals.

2.2. Sampling

Blood/serum and faecal samples were collected at 4, 8 and 12 months p.i. and tissue samples were collected at 12 months p.i. when the animals were sacrificed.