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INFECTIOUS DISEASE

Histopathological Characterization of Cutaneous Delayed-type Hypersensitivity and Correlations with Intestinal Pathology and Systemic Immune Responses in Sheep with Paratuberculosis

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Summary

Cell-mediated immunity has been exploited historically in the diagnosis of mycobacterial diseases through elicitation of a delayed-type hypersensitivity (DTH) reaction following intradermal injection of an antigen. Here we describe the histopathological features of the cutaneous DTH reaction and its association with intestinal pathology and systemic immune responses in sheep with *Mycobacterium avium* subspecies *paratuberculosis* (MAP) infection. A mixed mononuclear cellular infiltrate dominated the DTH reaction and was present in perivascular and periadnexal patterns. Multiple multinucleate giant cells were present in the cellular infiltrate in one sheep while plasma cells were an obvious feature in six others. Sheep with paucibacillary intestinal lesions had the greatest degrees of cutaneous induration, more severe cellular infiltration in DTH lesions and high systemic interferon (IFN)-γ production. In contrast, sheep with multibacillary intestinal lesions, and particularly those with dissemination of MAP to extra-intestinal tissues, had minimal cutaneous induration, nil to mild cellular infiltration in DTH lesions and high serum anti-MAP antibody levels. Systemic IFN-γ production generally was augmented following skin sensitization. In general, the gross and histopathological features of the cutaneous DTH response matched the stage of paratuberculosis reflected by intestinal pathology and systemic measures of humoral and cellular immunity.

Keywords: cell-mediated immunity; delayed-type hypersensitivity; paratuberculosis; sheep

Introduction

Johne's disease (JD) or paratuberculosis is an economically important disease of ruminants caused by the pathogen *Mycobacterium avium* subspecies *paratuberculosis* (MAP). Economic losses stem from severe granulomatous enteropathy, which has an insidious clinical presentation, beginning with gradual weight loss and leading to death. In addition to direct production losses, there are costs of control and prevention (Clarke, 1997; Ott *et al.*, 1999; Bush *et al.*, 2006; Raizman *et al.*, 2009). Animals with clinical signs represent only a small proportion of the infected animals within a flock or herd. Those with subclinical disease can remain undetected for years, shed MAP in their faeces and so spread the disease.

Mycobacteria typically induce granulomatous lesions that are expressions of cell-mediated immunity (CMI) or immunopathology. Clinical manifestations depend on pathogen factors, such as tissue tropism (the distal small intestine in the case of MAP), and an array of host factors surrounding the immune response (Whittington *et al.*, 2012). Reflective of the stage of disease and immunity in individuals, a spectrum of histopathological lesions are observed in sheep with paratuberculosis (Perez *et al.*, 1996) and indeed in other species with other mycobacterial

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infections (Whittington *et al.*, 2012). Based on the type of gut pathology and the pathogen load, two disease phenotypes, paucibacillary and multibacillary, have been described in JD (Clarke and Little, 1996). Lesions vary from small foci of well-defined granulomata confined to the ileal Peyer's patches (type 1 disease), extension of foci into the overlying mucosa (type 2 disease) and thence into other gut layers away from the Peyer's patches (type 3 disease). Type 3 lesions are subdivided according to the degree of cellular infiltrate, cell type and the number of acid-fast bacilli (AFB) present. Subtype 3a lesions are an extension from type 2 into the lamina propria of the villi, with similar inflammatory patterns observed, and may involve the jejunum and draining lymph nodes. AFB may be seen in small numbers in types 1 and 2 and variable numbers from low to high in type 3a lesions. Subtypes 3b and 3c are characterized by diffuse enteritis causing marked gut wall thickening and villus shortening and fusion, and may be a continuation from subtype 3a. In subtype 3b there is a mosaic-like infiltrate of epithelioid macrophages and abundant AFB, while a lymphocytic infiltrate, small numbers of well-defined granulomata and few to no AFB characterize subtype 3c lesions. Although weight loss may be associated with type 3a (early paucibacillary) lesions (McGregor *et al.*, 2015), it is the subtype 3b (multibacillary) and 3c (paucibacillary) lesions that have been generally associated with clinical disease (Clarke and Little, 1996; Perez *et al.*, 1996; Dennis *et al.*, 2008).

Historically, the cutaneous delayed-type hypersensitivity (DTH) reaction, a test of CMI, has been applied in the diagnosis of mycobacterial diseases, including tuberculosis and leprosy in people, and bovine tuberculosis. The skin test for tuberculosis, first discovered by Robert Koch in 1882 (Black, 1999), is measured as a change in skin fold thickness or induration 48–72 h following intradermal injection of mycobacterial antigen, compared against diagnostic criteria to determine a positive or negative result. While the cutaneous DTH reaction to mycobacterial antigen has been well described in other species such as man and mice (Baumgarten and Wilhelm, 1969; Poulter and Lefford, 1977; Samuel *et al.*, 1985; Narayanan *et al.*, 1986; Beck *et al.*, 1988; Kaplan *et al.*, 1988; Black, 1999; Vukmanovic-Stejic *et al.*, 2006; Haholu *et al.*, 2008), it has not been described in MAP-affected sheep beyond the association of gut pathology with measurement of induration. High induration was associated with paucibacillary gut lesions, while no change in skin thickness or low levels of induration were associated with multibacillary gut lesions (Gilmour and Brotherston, 1966; Gilmour *et al.*, 1978; Perez *et al.*, 1999). The application of the cutaneous DTH reaction as a diagnostic tool for JD in ruminants is therefore limited because it is likely that many severely infected animals would not be detected.

Studies of paratuberculosis have reported two distinct immunological profiles, in that sheep with paucibacillary lesions show a T helper (Th)1-dominant immunological profile with strong CMI and lower pathogen numbers, compared with sheep with multibacillary lesions that show a Th2-dominant immunological profile, characterized by high serum antibody (Ab) levels and high pathogen load (Clarke, 1997; Gillan *et al.*, 2010). These contrasting profiles may overlap and represent a continuum, signifying a gradual decline in the functional capacity of the immune system, but the reason for this progression is unknown (Begg *et al.*, 2011). The links between these profiles, and whether pathology at the central site of infection in the intestine is mirrored in the peripheral DTH responses, have not been described. Therefore, the aim of this study was to describe the histopathological organization of the DTH reaction in skin of sheep with paratuberculosis and to explore its correlation with the degree of intestinal pathology and the systemic immune response.

Materials and Methods

All animal experiments carried out for this study were approved by the University of Sydney Animal Ethics Committee (approval number N00/8-2007/3/4649).

Animals

Merino sheep aged over 4 years were sourced from a farm near Orange in New South Wales, Australia, which had endemic JD. Blood samples taken from 149 sheep, which had not been vaccinated against paratuberculosis, were tested using a commercial serum Ab enzyme-linked immunosorbent assay (ELISA) kit (Institut Pourquier, Montpellier, France) and results were expressed as a sample to positive ratio (S/P) according to the formula: $S/P = 100 \times (OD_{450}$ value of the sample $- OD_{450}$ value of the negative control$)/(OD_{450}$ value of the positive control $- OD_{450}$ value of the negative control). A subpopulation of seropositive sheep with % S/P >70 was identified. Three groups of six sheep each were chosen at random to represent a spectrum of serum Ab concentrations: high (% S/P >149), medium (% S/P 100–149) and low (% S/P 70–90). These 18 sheep were transported to the University of Sydney farm at Camden and housed in pens for 2 weeks prior to further testing. They were fed a