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INFECTIOUS DISEASE

Inflammatory Lesion Patterns in Target Organs of Visna/Maedi in Sheep and their Significance in the Pathogenesis and Diagnosis of the Infection

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Summary

Ovine visna/maedi (VM) infection is characterized by the development of chronic inflammatory lesions in different organs, mainly in the lung, mammary gland and central nervous system (CNS), with either histiocytic or lymphocytic pattern predominance being described in the CNS. To help to understand the role of host immune response in the development of these patterns, 50 naturally-infected sheep and eight non-infected sheep from intensive milk-producing flocks were studied. The histological lesion patterns in the three main target organs in each sheep were characterized. Lesion severity was determined, including minimal lesions. A histiocytic pattern was observed in 23 sheep (46%), a lymphocytic inflammatory pattern in 19 sheep (38%) and a mixed inflammatory pattern in eight sheep (16%). Forty animals showed moderate or severe lesions (80%), while 10 had minimal lesions (20%). Moderate or severe lesions affected only one target organ in 20 sheep (50%), two organs in 14 sheep (35%) and all three target organs in six sheep (15%). Infection was confirmed by immunohistochemistry (IHC) using an antibody specific for p28 of VM virus/caprine arthritis and encephalitis virus and by polymerase chain reaction (PCR) in all sheep. Minimal inflammatory lesions associated with positive IHC and PCR were observed. The results suggest that the development of a predominant inflammatory pattern in different organs within the same animal may be related to the host immune response. Minimal and focal lesions, not considered previously, should be taken into account when formulating a differential diagnosis in affected sheep.

Keywords: immunopathology; lesion pattern; sheep; visna/maedi

Introduction

Ovine visna/maedi (VM) is a widespread disease caused by a retrovirus of the genus lentivirus and is related to caprine arthritis encephalitis virus (CAEV) and human immunodeficiency virus (Thormar, 2005). The disease has a significant economic impact, which is especially noted in flocks in northwest Spain where an intensive indoor farming system is widespread and seroprevalence reaches up to 96.8% (Sotelo, 1998; Peterhans *et al.*, 2004; Leginagoikoa *et al.*, 2006). VM is characterized by chronic inflammation of the lung, mammary gland and central nervous system (CNS) and rarely by arthritis. Microscopically, the changes in affected organs are interstitial pneumonia, mastitis and non-suppurative necrotizing encephalomyelitis, choroiditis and demyelination of the CNS (Cutlip *et al.*, 1979; Luján *et al.*, 1991; Benavides *et al.*, 2009; Minguijón *et al.*, 2015).

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The importance of cell-mediated immunity with regard to the severity of the lesions has been described for the CNS lesions (Torsteinsdóttir *et al.*, 1992; Polledo *et al.*, 2012a) and an individual immune response against VM virus (VMV) has been suggested to play a major role in the pathogenesis of the disease (Torsteinsdóttir *et al.*, 1992, 2007; Blacklaws, 2012; Polledo *et al.*, 2012a, b). In previous studies carried out on sheep with spontaneously arising neurological forms of VM, two main patterns of lesion were described with regard to the predominant inflammatory cells in the CNS infiltrates. The lymphocytic pattern is characterized by a predominance of T lymphocytes, particularly $CD8^+$ T cells, while the histiocytic pattern involves infiltration of macrophages and B lymphocytes (Polledo *et al.*, 2012b). Similar inflammatory patterns have been described in the lung and mammary gland (Gayo *et al.*, 2017).

In previous studies, moderate to severe VM lesions occurred in 35.5% of mammary glands and 32.3% of lungs in randomly selected sheep. These percentages increased to 58.1% and 54%, respectively, in seropositive sheep (Luján *et al.*, 1991). In the latter study, 25.6% of VM-seropositive sheep had no lesions in any organ, 20.2% had lesions in the mammary gland only, 16.2% had lesions in the lung only and 37.8% had lesions in both organs (Luján *et al.*, 1991). In subsequent studies an increase in these percentages was observed when mild lesions were also considered, with percentages of affected mammary glands rising to 46.7% (Benavides *et al.*, 2013). Minimal infiltrates of cells were not included in these investigations and were considered as normal, but the presence of virus in those lesions was not evaluated.

The aim of the present study was to determine whether the same inflammatory pattern is present in all of the target organs in individual infected sheep.

Materials and Methods

Animals

Fifty-eight Spanish Assaf sheep (1–3 years of age) from different intensive milk-producing flocks located in the northwest of Spain were selected from animals submitted to the Pathology Diagnostic Service, School of Veterinary Medicine, León, Spain, for routine necropsy examination. Fifty sheep were naturally infected and came from six VM-seropositive flocks subjected to VM control as described elsewhere (Polledo *et al.*, 2013). The seroprevalence in these flocks ranged between 62% and 97%. The remaining eight animals were selected as negative controls from three VMV-free flocks and showed no clinical signs. Sheep from affected flocks were culled because of loss of milk production and/or weight or because of the presence of neurological (e.g. hindlimb weakness, ataxia, hypermetria or paralysis usually leading to recumbency) or respiratory signs (e.g. dyspnoea). Animals in this study did not have gross or microscopical lesions compatible with other pathologies such as bacterial, fungal or parasitic pneumonias or mastitis.

Sampling, Histopathology and Immunohistochemistry

Tissue samples were collected systematically from the diaphragmatic and apical lung lobes, glandular parenchyma and udder cisterns and CNS from nine levels of the brain and nine levels of the spinal cord (Polledo *et al.*, 2012a). Mammary samples from six sheep and spinal cord samples from four sheep were not included in the histopathological studies as the tissues were not in good condition. Samples were fixed in 10% neutral buffered formalin for 48 h at room temperature. After fixation, samples were dehydrated through graded alcohols and embedded in paraffin wax. Sections (4 μm) were stained with haematoxylin and eosin (HE) and examined using light microscopy.

Additional sections (4 μm) were used for immunohistochemistry (IHC). The following primary antibodies were used: polyclonal anti-CD3 for T cells (Dako, Glostrup, Denmark); monoclonal anti-CD79 for B cells (Dako); monoclonal anti-CD68 for macrophages (Dako); and monoclonal anti-p28 of VMV/CAEV (VMRD, Pullman, Washington, USA) for viral detection. Subsequently, the sections were incubated for 30 min using the EnVision + system (EnVision + System Labelled Polymer-HRP anti-mouse or anti-rabbit; Dako) and labelling was 'visualized' using 3, 3′ diaminobenzidine as chromogen (Vector Laboratories, Burlingame, California, USA) for anti-CD3, CD79 and CD68 antibodies. An avidin–biotin–peroxidase complex (ABC) technique (Vectastain Elite, ABC Kit; Vector Laboratories) was used for detection of p28 of VMV/CAEV (Preziuso *et al.*, 2003). The slides were counterstained with haematoxylin and mounted. The specificity of the technique was evaluated using positive (previously confirmed tissues) and negative controls (previously confirmed tissues and non-immune serum replacing primary antibody).

Three lesion patterns were considered based on the characteristics of the inflammatory infiltrate. Histiocytic and lymphocytic patterns were considered as previously described (Polledo *et al.*, 2012b). Lymphocytic lesions were characterized by a predominance of $CD3^+$ cells within the inflammatory infiltrate, while in histiocytic lesions $CD68^+$ cells were the most abundant, together with some scattered $CD3^+$ and