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Immunohistochemical characterization of Kisselev nodules (ectopic lymphoid follicles) in wild boar (*Sus scrofa* L.)

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Abstract

This paper describes the histopathological features and cellular distribution of T lymphocytes (CD3), B cells (CD79), follicular dendritic cells (FDC) and macrophages (alpha-1-antitrypsin, lysozyme) in lymphoid aggregates (Kisselev nodules) found in the lung, kidney and liver of wild boar (*Sus scrofa* L.). The distribution of immunoreactive cells, tested for antibodies, was similar to that found in the cortex of lymph nodes: lymphoid follicles with germinal centers mainly consisting of $CD79^+$ B cells with sparse interfollicular tissue ($CD3^+$ T lymphocytes). This finding and the association of these structures with helminthic infections suggests that local humoral immunity is central to the organism's response to parasitic challenge. The presence of follicular dendritic cells confirms the high degree of organization of these lymphoid-like structures. The role of other pathogenic factors and the induction of chronic inflammatory reaction in these ectopic lymphoid sites is also discussed.

Keywords: Parasitic diseases; Immunohistochemistry; Kisselev nodules; Ectopic lymphoid follicle; Wild boar; Haemopoietic tissue

1. Introduction

Lymph node-like structures (Kisselev nodules) have been described in various parasitic diseases: pigs with *Ascaris suum* or *strongyles* (Schwartz and Alicata, 1933; White, 1941; Taffs, 1967; Ferguson et al., 1968; Copeman and Gaafar, 1972; Batte et al., 1975; Nakagawa et al., 1983; Guarda et al., 1996); lambs and calves infected by *Cysticercus tenuicollis* and *Dictyocaulus viviparus*, respectively (Jarret and Sharp, 1963); and domestic and wild ruminants infected by *Schistosoma bovis* (Massoud, 1973), *Eleophora schneideri* (Madden et al., 1991) and *Eleophora elaphi* (Carrasco et al., 1995). These structures are usually found in the liver but they have also been observed in the lung (Schwartz and Alicata, 1933; Guarda et al., 1996), kidney (Carrasco et al., 1995), rete mirabile cerebri (Madden et al., 1991) and the hepatic intralobular vein (Carrasco et al., 1995). In all cases, they consist of germinal centers with sparse interfollicular lymphoid tissue similar to lymph nodes. These complex lymphoid aggregates have also been described in humans associated with: chronic interstitial pneumonia (Koss et al., 1987); chronic gastritis (Lee et al., 1990); hepatitis C (Murakami et al., 1999); Sjögren's syndrome (Prochorec-Sobieszek et al., 2004); and rheumatoid arthritis (Takemura et al., 2001). Since these structures share many features with secondary lymphoid tissue, they have been called ectopic lymphoid follicles, follicle-like structures or tertiary lymphoid tissue. The formation of such lymphoid centres at an extranodal site is considered an example of lymphoid neogenesis (Takemura et al., 2001). The functional role of these nodules is not understood completely, especially in veterinary pathology as the simultaneous occurrence of lymphoid aggregates and parasites in some cases has not been demonstrated. Up to present, only two studies on the immunohistochemical characterization of nodules (Carrasco et al., 1998; Perez et al., 2001) have been reported and neither of these looked at wild boar.

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The aim of this study was the immunohistochemical analysis of T and B lymphocytes, macrophages and follicular dendritic cells, and their distribution in ectopic lymphoid follicles in wild boar, in order to understand their function and the immune response of wild boar during parasitic infestations.

2. Materials and methods

2.1. Tissue samples and histopathology

Tissue samples (lung, kidney, liver and tracheo-bronchial lymph nodes and spleen) were collected from 47 wild boar, fixed in 10% buffered formalin and embedded in paraffin wax. Tissue sections (5 μm) were cut and stained with haematoxylin and eosin and then studied; images (40×) were generated by a microscope (Leica DMLS) connected to a camera (JVC Digital 1/2 in. CCD, TK-C1381; Japan) and stored on a computer for processing. A digital image software system (Leica Qwin; Leica Imaging System Ltd.; Cambridge, England) was used for image analysis. The area (mm^2) of lymphoid structures was calculated by outlining profiles and expressed as mean ± SD (standard deviation).

2.2. Immunohistochemistry

The following antibodies were used on tissue sections containing ectopic lymphoid follicles: (1) monoclonal mouse anti-human CD79αcy (DakoCytomation, Glostrup, Denmark) used for the identification of B-lineage cells (Comans-Bitter et al., 1997), (2) polyclonal rabbit anti-human CD3 (DakoCytomation), highly specific for T cells (Campana et al., 1987), (3) monoclonal mouse anti-human follicular dendritic cell, clone CNA.42 (DakoCytomation), labelling a 120 kDa antigen expressed on follicular dendritic cells (Raymond et al., 1997), (4) polyclonal anti-human lysozyme (DakoCytomation) and polyclonal anti-human alpha-1-antitrypsin (Novocastra Laboratories Ltd., Newcastle upon Tyne, United Kingdom) which stain monocytes and macrophages (Isaacson et al., 1981; Mörsky, 1988), (5) polyclonal rabbit anti-human von Willebrand Factor (DakoCytomation) specific for endothelial cells lining the lumen of capillaries, arteries and veins (Ruggeri and Ware, 1993) (Table 1). The streptavidin–biotin–peroxidase method was used for CD79 and FDC (BIOSPA MR*HRP-610, Biospa, Milan, Italy) and the envision–horseradish peroxidase (HRP) method (Dakocytomation, Glostrup, Denmark) for CD3, lysozyme and alpha-1-antitrypsin (α1AT) macrophages and von Willebrand factor (vWF). Endogenous peroxidase activity was blocked by incubation with 3% hydrogen peroxide in methanol for 45 min and antigens unmasked in a microwave oven (850 W) using EDTA–Tris buffer, pH 9 (CD79αcy, CD3, FDC, vWF) or citric acid, pH 6 (lysozyme, α1AT). After blocking background staining, the samples were incubated with primary antibodies for 1 h at room temperature (RT) (CD3 and vWF), overnight at RT (CD79αcy and FDC) and at 4°C (lysozyme and α1AT). A biotinylated goat anti-rabbit and anti-mouse immunoglobulin G and a horseradish peroxidase complex were used for 10 min for the streptavidin–biotin–peroxidase method while for the Envision method, a peroxidase labelled polymer conjugated to goat anti-mouse and goat anti-rabbit immunoglobulins was used in Tris–HCl buffer for 30 min. Tissue sections were then incubated for 5 min in 3.3-diaminobenzidine (DAB) solution (D-5905, Sigma–Aldrich, St. Louis, MO, USA), counterstained with Papanicolau's haematoxylin (Merck, Darmstadt, Germany), dehydrated and mounted. Sections were incubated with blocking solution instead of primary antibodies and an irrelevant antibody directed against an unrelated antigen for the negative controls (polyclonal rabbit anti-papillomavirus – DakoCytomation, Glostrup, Denmark – when testing anti-human follicular dendritic cells; monoclonal mouse anti-human cytokeratin – DakoCytomation – for the other antibodies). Pig and wild boar lymph node tissue sections were used as positive controls. The assessment of immunolabelling was based on two methods: (1) a semi-quantitative analysis (Perez et al., 2001) based on the percentage of immunoreactive cells in lymphoid follicles and interfollicular tissue: tissue samples were graded ± when 0–15% of the cells were

Table 1
Details of the primary antibodies used in the study

Antibody (type)	Treatment	Blocking[a]	Dilution	Immunohistochemical method	Secondary antibodies[d]
CD79αcy (mAb)	Microwave[e]	NGS	1:1200	Streptavidin–biotin–HRP[b]	B
CD3 (pAb)	Microwave[e]	DM	1:100	Envision–HRP[c]	P
Follicular dendritic cells (mAb)	Microwave[e]	NGS	1:800	Streptavidin–biotin–HRP[b]	B
Lysozyme (pAb)	Microwave[f]	DM, 5% BSA	Ready-to-use	Envision–HRP[c]	P
Alpha-1-antitrypsin (pAb)	Microwave[f]	DM, 5% BSA	1:200	Envision–HRP[c]	P
von Willebrand factor (pAb)	Microwave[e]	5% BSA	1:1000	Envision–HRP[c]	P

[a] NGS, normal goat serum; DM, dry milk; BSA, bovine serum albumin.
[b] BIOSPA MR*HRP-610, Biospa, Milan, Italy.
[c] Dakocytomation, Glostrup, Denmark. HRP, horseradish peroxidase.
[d] B, biotinylated goat anti-rabbit and anti-mouse IgG and horseradish peroxidase complex; P, peroxidase labelled polymer conjugated to goat anti-mouse and goat anti-rabbit immunoglobulins.
[e] Incubation in EDTA–Tris buffer, pH 9 in a microwave oven (850 W).
[f] Incubation in citric acid, pH 6 in a microwave oven (850 W).