J. Comp. Path. 2014, Vol. ■, 1–9

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INFECTIOUS DISEASE

Immunohistochemical Investigation of Extracellular Matrix Components in the Lymphoid Organs of Healthy Pigs and Pigs with Systemic Disease Caused by Circovirus Type 2

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Summary

The extracellular matrix (ECM) provides a scaffold for cell growth, impacts on cellular behaviour and plays an important role in pathological conditions. Several components of the ECM of lymphoid tissues have been shown to be crucial in the maturation, differentiation and migration of lymphocytes and other immune cells and, therefore, in the development of immune responses. Little is known of the composition and function of the ECM in porcine lymphoid tissues. The present study characterizes immunohistochemically the expression of several ECM-related molecules (i.e. hyaluronan [HA] and its receptor CD44, tenascin-C [TN-C] and versican) in primary and secondary lymphoid organs of healthy pigs and animals affected by porcine circovirus type 2-systemic disease (PCV2-SD). These ECM molecules displayed a highly defined expression pattern in healthy animals, suggesting that they may have a role in the compartmentalization of immune cells within lymphoid tissues. HA was abundant in the medulla of the thymus and follicles of secondary organs; CD44 and TN-C were present in the thymic medulla and parafollicular areas of secondary lymphoid organs; however, there was minimal expression of versican in healthy tissues. In PCV2-SD-affected animals, HA and CD44 showed a similar but more diffuse distribution. TN-C was increased in the T-cell-dependent areas and in tonsillar crypts, and versican was more abundantly expressed, with expression restricted to vascular structures and trabeculae and also surrounding tonsillar crypts. The altered expression in PCV2-SD-affected pigs was most probably related to a higher content of connective tissue secondary to tissue destruction and remodelling attempts as part of the disease process.

Keywords: extracellular matrix; lymphoid organs; PCV2-systemic disease; pig

Introduction

The primary (i.e. thymus and bone marrow) and secondary (i.e. lymph nodes, tonsil and spleen) lymphoid organs play a major role in the development of the immune response. Primary lymphoid organs participate in the generation of lymphocytes from immature progenitor cells, while secondary or peripheral lymphoid organs maintain mature naïve lymphocytes and initiate adaptive immune responses against foreign antigens.

The extracellular matrix (ECM) is a complex network of macromolecules that, in addition to having a structural role in the maintenance of tissue integrity, participates in a variety of cellular functions and provides a source of cytokines and growth factors. In lymphoid tissues, the ECM is involved in the regulation of several aspects of immune cell behaviour, including lymphocyte migration, differentiation and activation. Increasing evidence suggests that the

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0021-9975/$ - see front matter

http://dx.doi.org/10.1016/j.jcpa.2014.03.005

Please cite this article in press as: Docampo MJ, et al., Immunohistochemical Investigation of Extracellular Matrix Components in the Lymphoid Organs of Healthy Pigs and Pigs with Systemic..., Journal of Comparative Pathology (2014), http://dx.doi.org/10.1016/j.jcpa.2014.03.005

ECM is also actively involved in pathological conditions, such as infection and inflammation (Taylor and Gallo, 2006; Sorokin, 2010).

Amongst the many components of the ECM, hyaluronan (HA) plays an important role. HA forms a complex with other components, such as versican and tenascin-C (TN-C), and interacts with cells through specific membrane receptors, which include CD44 and others (Toole, 2004). HA is a glycosaminoglycan with no protein component and its classical role is in maintenance of the stability and structure of the ECM, since it controls water homeostasis, lubrication, structural integrity, the sequestration of free radicals and plasma protein distribution (Taylor and Gallo, 2006). Increased HA deposition is a consistent feature of tissue after injury and during inflammation, and HA deposition has been reported in a range of human disorders such as inflammatory bowel disease (Kessler *et al.*, 2008). Versican is a chondroitin sulphate proteoglycan that aggregates with HA and modulates cellular adhesion, proliferation, migration and apoptosis of several cell types, as well as ECM assembly (Wight, 2002; Theocharis, 2008). Versican is highly expressed in tissue compartments undergoing active cellular proliferation and migration, such as during embryonic morphogenesis and tissue remodelling, and in disorders such as cardiovascular disease, cancer and inflammation. TN-C is a large, hexameric glycoprotein with a highly regulated pattern of expression. It appears in a specific spatiotemporal fashion during embryogenesis and shows very little expression in healthy adult tissues, but is up-regulated in response to tissue injury or in tissue remodelling. Due to its complex domain structure, TN-C is able to interact with a variety of ECM molecules and cell surface receptors and may exert both adhesive and anti-adhesive properties (Jones and Jones, 2000; Midwood and Orend, 2009). All of these molecules have been implicated in lymphocyte adhesion, migration and differentiation (Clark *et al.*, 1997; Jiang *et al.*, 2011; Evanko *et al.*, 2012); however, little is known about the composition of the ECM in porcine lymphoid organs.

Porcine circovirus type 2 (PCV2)-systemic disease (PCV2-SD), formerly known as post-weaning multisystemic wasting syndrome (PMWS) (Segales, 2012), is a multifactorial disease for which PCV2 is considered the essential infectious agent (Segales *et al.*, 2005). The disease has a profound economic impact on pig production worldwide. The clinical signs are relatively non-specific and variable. The major histopathological lesions consist of generalized lymphocyte depletion, together with histiocytic infiltration of lymphoid organs, linked with the presence of PCV2 (Darwich *et al.*, 2004).

The aim of the present study was to describe the normal distribution of different components of the ECM, including HA and its receptor CD44, versican and TN-C, in normal porcine lymphoid organs and to study how the expression pattern of these molecules was altered in pigs suffering from PCV2-SD, a swine disease characterized by chronic inflammation of lymphoid tissues.

Materials and Methods

Tissue Samples

Formalin-fixed and paraffin wax-embedded samples of porcine lymphoid tissues, including thymus, superficial inguinal lymph node and tonsil, were obtained from the archives of the Servei de Diagnòstic de Patologia Veterinària at the Facultat de Veterinària of the Universitat Autònoma de Barcelona. Samples from healthy control pigs ($n = 5$) and PCV2-SD-affected pigs ($n = 5$) were studied. All pigs came from the same farms managed by the same producer and were of the same genetic background (i.e. crossbreds including mainly of the Landrace breed). The mean ages of the control and diseased animals were 14.0 ± 1.1 and 13.8 ± 1.4 weeks, respectively. No diseased thymic tissue was available for this study. PCV2-SD cases with moderate to severe lymphoid lesions (e.g. lymphocyte depletion and granulomatous inflammation), a moderate to high amount of PCV2 antigen/nucleic acid and clinical wasting were selected. A detailed description of the criteria for PCV2-SD diagnosis is reported in Grau-Roma *et al.* (2009). Sections (4 μm) were cut from wax blocks, mounted on silane-coated slides and stained with haematoxylin and eosin (HE).

Antibodies and Hyaluronan-binding Protein

Primary antibodies used in immunohistochemistry (IHC) include mouse anti-porcine CD44 monoclonal antibody (clone PORC24A; IgG2a isotype; VMRD, Pullman, Washington, USA), mouse anti-human TN-C monoclonal antibody recognizing all TN-C isoforms (clone 4F10TT; IgG1 subclass; IBL, Hamburg, Germany) and mouse anti-human large chondroitin sulphate monoclonal antibody (clone 2B1; IgG1 subclass; Seikagaku, Tokyo, Japan). Biotinylated hyaluronan-binding protein (bHABP) was obtained from Seikagaku. Polyclonal rabbit anti-human CD3 (Dako, Glostrup, Denmark; Chianini *et al.*, 2003) was kindly provided by Dr. M. Montoya (CReSA, UAB-IRTA). Secondary biotinylated goat anti-mouse and goat anti-rabbit immunoglobulins were from Dako. Mouse IgG1, mouse IgG2a and rabbit immunoglobulin fraction were from Dako.

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