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Short communication

Immunohistochemical detection of interleukin-8 in inflamed porcine tissues

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ABSTRACT

The objective of this study was to identify the specific localization of interleukin-8 (IL-8) in cells *in situ* in a variety of inflammatory processes in different tissues from pigs. Our hypothesis was that IL-8 primarily is a neutrophil related cytokine present in all extravascular neutrophils while expression also occurs in other cell types in response to an inflammatory stimulus. Using IL-8 immunohistochemistry we discovered that neutrophils were the predominant IL-8 positive cell population while epithelial cell types and endothelium of postcapillary venules could be positive when situated in close vicinity of an inflammatory lesion. Furthermore, endothelial cells of newly formed vessels in granulation tissue were positive in some specimens. Some sub-populations of inflammatory neutrophils were, however, IL-8 negative which could reflect some phase of neutrophil swarming.

1. Introduction

The CXC chemokine, (IL-8), has received massive attention in recent years as it is believed to drive the pathogenesis of a number of acute and chronic inflammatory conditions characterized by an unbalanced immune response, as well as some cancer types (Qazi et al., 2011; Waugh and Wilson, 2008). This is achieved through its controlling function on the activation and magnitude of the innate immune response and a direct and indirect regulatory function on the adaptive immune response (Harada et al., 1994; Muller et al., 2009). An increased local or systemic concentration of IL-8 correlates with disease severity and works as a diagnostic and prognostic marker in several diseases (Bergin et al., 2013; Pearl et al., 2013; Shahzad et al., 2010). Compounds blocking the effects of IL-8 have been used successfully to impair neutrophil chemotaxis and improve the clinical outcome in various inflammatory animal disease models and in human disease (Citro et al., 2012; White et al., 1998). Studies of IL-8 in man, has revealed a cytokine with highly diverse origin and function. It is an essential chemoattractant, primer and activator of neutrophil granulocytes (Harada et al., 1994) that, like several other cell types, possess the capacity of IL-8 synthesis following a proinflammatory stimulus. Through its two high-affinity G-protein-coupled receptors, CXCR1 and 2, (Zeilhofer and Schorr, 2000) it induces responses in neutrophils, endothelial cells, subsets of T-cells and several other cell types (Mantovani et al., 2011; Waugh and Wilson, 2008). In pigs, a homologue of IL-8 with strong chemotactic activity towards neutrophils was

Abbreviations: HE, haematoxylin and eosin; IHC, immunohistochemistry; IL-8, interleukin 8; LPS, lipopolysaccharide; PAM, pulmonary alveolar macrophages; SIRS, systemic inflammatory response syndrome.

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identified in 1991, secreted by isolated LPS-stimulated porcine pulmonary alveolar macrophages (PAM) (Goodman et al., 1991). Increased mRNA expression after the proinflammatory stimulus was also documented (Goodman et al., 1992). IL-8 expression has been determined in other isolated cell types such as neutrophils (protein) (Gauthier et al., 2013; Zelnickova et al., 2008), monocytes (protein, mRNA) (Ondrackova et al., 2013; Pedersen et al., 2002; Zelnickova et al., 2008), venous endothelial cells (mRNA) (Zakkar et al., 2011), and intestinal epithelial cells (protein, mRNA) (Mc et al., 2010; Zhou et al., 2012) and is induced and acts via the porcine CXCR1 and 2 homologues (Ledger et al., 2004; Luo et al., 2011). Upregulated IL-8 gene expression in porcine organs is documented in several studies and this increase is for example seen in the intestine during a systemic inflammatory response syndrome (SIRS) (Mc et al., 2010), injured lung tissue (Soerensen et al., 2012), and in the liver of pigs with acute sepsis or lung infection with *Actinobacillus pleuropneumoniae* (Skovgaard et al., 2010; van Malenstein et al., 2010). The increasing use of pigs as animal models necessitates a continuous study of pathophysiology in this species. Knowledge of the precise cellular *in vivo* localization of the IL-8 protein in the balanced inflammatory response – and in diseases characterized by an imbalance in the release of anti – and proinflammatory mediators – is sparse in pigs (Christiansen et al., 2013; Nadworny et al., 2010; Soerensen et al., 2012). Our aim is to reveal, by immunohistochemistry, the cellular origin of IL-8 production *in situ* by testing various infectious and inflammatory lesions in different tissues in pigs. We hypothesize that IL-8 primarily is a neutrophil related cytokine present in all extravascular neutrophils and that expression in other cell types, as a response to an inflammatory stimulus, also occurs.

2. Materials and methods

2.1. Tissues, HE- and Luna-staining and morphological characterization

Paraffin wax-embedded porcine tissue specimens obtained from different prior studies were included along with some specimens sampled from tissues condemned at meat inspection. Thus, both experimental and spontaneous lesions were represented. Included tissues comprised skin (7), bone (10), lung (15), lymph node (5), heart (3), kidney (8), liver (8), stomach (4), intestine (2), synovial membrane (9), and brain (2). Infectious etiologies were *Staphylococcus aureus* (15), *Escherichia coli* (2), *Pasteurella multocida*, *Porcine circovirus* 2, *Mycoplasma hyorhinis* and *Mycoplasma hyopneumoniae* (4), *Mycoplasma hyosynoviae* (2), *Erysipelothrix rhusiopathiae* (2), Influenza A virus (H1N1 and H1N2) (4), *Lawsonia intracellularis* (1), *Streptococcus suis* (2), parasitic (3), and mixed flora (5). Non-infectious etiologies included foreign body (4), sterile bacterial culture (2), unknown (16), and controls (11). Lesional ages were acute (26), subacute (3), chronic (33), and controls (11). For every tissue type with lesions, a non-lesional control specimen obtained from clinically normal pigs was included. The study comprised a total of 61 pigs, and the age of the pigs ranged from 1 to 6 months. All pigs were originally bred for the purpose of meat production. Fixation was performed in 4% neutral buffered formaldehyde or paraformaldehyde (four specimens) for 1–7 days followed by paraffin wax embedding.

Serial sections were made from each paraffin wax block, and one section was stained with haematoxylin and eosin (HE) while the other was stained using an IL-8 immunohistochemical (IHC) staining technique. Sections for IHC were mounted on adhesive glass slides (SuperFrost Ultra Plus®, Menzel-Gläser, Braunschweig, Germany or Starfrost®, Waldemar–Knittel, Braunschweig, Germany). Additional sections were made from a few selected blocks and were Luna stained to identify eosinophil granulocytes.

HE and Luna stains were performed following the standard protocol described by Stevens and Wilson (1996) and Luna (1968), respectively. The findings were summarized into a histopathological diagnosis for each of the lesions.

2.2. IL-8 immunohistochemistry and analysis

Detection of IL-8 in the lesions was determined by IL-8 IHC staining using the technique described by Christiansen et al. (2013) utilizing mouse anti-sheep IL-8 monoclonal antibody (MCA1660, AbD Serotec, Oxford, UK). Cross reactivity of this antibody to porcine IL-8 in flow cytometry and Western blot analysis was confirmed in a previous study (Pedersen et al., 2002). Antibody dilution varied between 1:500 and 1:1000 and the Lab Vision™ Ultravision™ LP Detection System HRP (Thermo Fischer Scientific, Runcorn, UK) was utilized for antibody detection. All IHC staining batches included, as a positive staining control, a chronic, abscess-forming hepatitis while the same tissue, stained with a nonsense antibody of similar isotype and concentration (X0943, Dako, Glostrup, Denmark), was included in the first three batches as a negative staining control.

Identification of structures and cells with positive or negative (only neutrophils investigated) IL-8 staining were done by comparing the IHC stained sections with the HE stained serial sections, or, in a few cases, by comparison with Luna stained sections. The staining pattern of positive cells was recorded.

3. Results and discussion

Seventy three sections were examined comprising 62 inflammatory lesions from 55 pigs and 11 tissues from six clinically normal pigs. The lesions encompassed both acute and chronic inflammatory processes present in various tissues. Furthermore, different etiologies such as bacteria, viruses and parasites were represented as well as foreign body reactions.

Cells positive by IL-8 IHC consisted mainly of extravascular neutrophilic granulocytes located inside or in close vicinity of the inflammatory focus (Fig. 1A.) in accordance with our hypothesis and earlier studies (Christiansen et al., 2013; Soerensen et al., 2012). Extravascular neutrophil staining in all, but a few lesions was homogenous, *i.e.* the vast majority of neutrophils were positive, regardless of the tissue or organ, inflammatory type, lesional age, or etiology. Most of the positive neutrophils contained