Available online at www.sciencedirect.com
ScienceDirect
Veterinary Immunology and Immunopathology 126 (2008) 142–148
Veterinary immunology and immunopathology
www.elsevier.com/locate/vetimm

Short communication

Osteopontin immunoreactivity in the ileum and ileocecal lymph node of dairy cows naturally infected with *Mycobacterium avium* subsp. *paratuberculosis*

E.L. Karcher [a], C.S. Johnson [b], D.C. Beitz [a], J.R. Stabel [c,*]

[a] *Department of Animal Science, Iowa State University, Ames, IA 50010, USA*
[b] *Department of Veterinary Pathology, Iowa State University, Ames, IA 50010, USA*
[c] *USDA-ARS, National Animal Disease Center, 2300 Dayton Avenue, Ames, IA 50010, USA*

Received 16 January 2008; received in revised form 29 April 2008; accepted 20 May 2008

Abstract

Osteopontin (Opn), a highly acidic glycoprotein, promotes cellular adhesion and recruitment and has been shown to be upregulated in the granulomas of mycobacterial infections. Johne's disease, caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP), is associated with granulomatous enteritis. The objective of this experiment was to identify Opn in the ileum and ileocecal lymph node (ICN) of dairy cows naturally infected with MAP and to compare the frequency and intensity of staining between noninfected healthy controls, subclinical and clinical cows. Sections from these three groups of animals were selected from a tissue archive. Immunohistochemical analysis was used to determine the location and expression of Opn. The frequency and intensity of staining was also reported. Confirmation of acid-fast bacilli in the tissue sections was achieved by the Ziehl-Neelsen method. Within the ileal tissue, macrophages, lymphocytes, and plasma cells stained positive for Opn. Clinical cows expressed Opn at a greater frequency in the lamina propria. Control and subclinical cows did not have areas of granulomatous inflammation but cells staining for Opn were equally intense for the three groups. The frequency of staining for Opn in the ICN was not affected by MAP infection. Results of this study confirm for the first time, the expression of Opn in the ileum and ICN of MAP-infected cattle. Published by Elsevier B.V.

Keywords: *Mycobacterium avium* subsp. *paratuberculosis*; Osteopontin; Granulomas

1. Introduction

Mycobacterium avium subsp. *paratuberculosis* (MAP), the causative agent of Johne's disease, is currently a major economic and welfare issue for dairy producers in the United States. Once infected, animals may remain in the subclinical, or asymptomatic, stage of the disease for several years. The formation of granulomas at the site of MAP infection is critical for the early control of the infection (Coussens, 2001). In subclinical cows, well-demarcated, small granulomas located in the distal ileum and ICN were predominantly composed of macrophages, multinucleated Langherans giant cells, and few lymphocytes (Gonzalez et al., 2005). Lesions from clinically infected cows show diffuse granulomatous enteritis and consist primarily of lymphocytes, plasma cells, giant cells, and macrophages (Gonzalez et al., 2005).

Osteopontin (Opn) is a highly acidic glycoprotein that is produced by activated macrophages (Atkins et al., 1998), activated T-cells (Ashkar et al., 2000), and

* Corresponding author. Tel.: +1 515 663 7304; fax: +1 515 663 7458.
E-mail address: judy.stabel@ars.usda.gov (J.R. Stabel).

0165-2427/$ – see front matter. Published by Elsevier B.V.
doi:10.1016/j.vetimm.2008.05.030

dendritic cells (Kawamura et al., 2005). The role of Opn in mycobacterial infections is of interest based upon its reported ability to promote the production of Th1 cytokines and to enhance host resistance against mycobacteria (Nau et al., 1999; Weber et al., 2002). Stimulating macrophages with Opn resulted in the production of the proinflammatory cytokines, IL-12 and TNF-α (Weber et al., 2002). The Th1 cytokines TNF-α and IFN-γ are essential for the production of the protective granulomas in MAP-infected cows (Roach et al., 2002).

Although Opn is constitutively expressed, it is upregulated in inflamed tissues, such as granulomas (Nau et al., 1997; O'Regan et al., 1999). Macrophages interact with Opn through the CD44 receptor and engagement induces chemotaxis and chemoattractant activity (Weber et al., 2002). This is important for the formation of granulomas, as the interaction facilitates the movement of macrophages and lymphocytes from peripheral blood to the tissue.

The objective of this study was to identify Opn in the ileum and ileocecal lymph node (ICN) of dairy cows naturally infected with MAP and to compare differences in immunoreactivity between the different infection groups. This is the first study to report Opn localization in the intestinal tract of MAP-infected cows and differences in Opn expression at the site of infection.

2. Materials and methods

2.1. Samples

This study was designed as a retrospective study in which sections were selected from an archive of tissue samples from healthy control dairy cows and cows with subclinical and clinical MAP infection. While in the herd, animals were routinely evaluated on a quarterly basis to determine infection status, which was monitored by culturing the feces for MAP using standard culture methods (Stabel, 1997). By definition, clinical animals were shedding more than 100 CFU/tube of feces and presented with weight loss and intermittent diarrhea. The noninfected control cows were characterized by repeated negative fecal cultures performed quarterly over a 3- to 5-year period. In addition, the animals were negative on serologic assays (i.e., production of antibody specific for MAP and IFN-γ) performed during that period. Standard procedure in our laboratory mandates a complete necropsy of animals on-site after death, including tissue collection for histological and immunohistochemical examination. Ileum and ICN are two tissues routinely collected. The number of ileal sections included samples from 4 controls, 4 subclinical, and 3 clinical cows. Sections of ICN were obtained from 5 control, 5 subclinical, and 5 clinical cows. When available, sections of ileal and ICN tissues were utilized from the same animals. Samples of tissues from all cows were fixed in neutral-buffered 10% zinc-formalin, processed routinely, and embedded in paraffin.

2.2. Immunohistochemistry

Sections were cut at 4 μm and fixed on ProbeOn™ Plus microscope slides (Fisher Scientific; Pittsburgh, PA). Sections then were deparaffinized in xylene and rehydrated by an ethanol series (100%, 95%, 70%, distilled water). An antigen retrieval step was performed by boiling slides in citrate buffer (10 μM citric acid, pH 6; Mallinckrodt, Hazelwood, MO) plus 0.05% Tween 20 (Sigma, St. Louis, MO) for 20 min and then incubated at RT for an additional 20 min. Slides were washed in 0.1% saponin (Sigma, St. Louis, MO)-PBS solution for 5 min. Endogenous peroxidases were quenched for 30 min by placing the slides in a 3% hydrogen peroxide (Fisher Scientific, Pittsburgh, PA) solution prepared with 0.1% saponin-PBS. Slides were washed for 5 min in 0.1% saponin-PBS before blocking for 30 min in 10% normal goat serum (KPL, Gaithersburg, MD). Rabbit anti-bovine Opn (generously supplied by Dr. Gary Killian; Pennsylvania State University, Almquist Research Centre, University Park, PA) was added to each slide (1:25, diluted in tris/PBS/BSA) and allowed to incubate overnight at 4 °C in a humidified chamber. A slide with normal rabbit serum served as a no primary antibody control for each tissue type. The following day, slides were washed in 0.1% saponin-PBS for 5 min and incubated at RT for 30 min with biotinylated goat anti-rabbit IgG (KPL, Gaithersburg, MD). Following a 5 min wash, slides were treated with streptavidin-horseradish peroxidase (KPL, Gaithersburg, MD) for 30 min. Slides were washed in 0.1% saponin-PBS and incubated in DAB-nickel substrate solution (Vector Laboratories, Burlingame, CA) for 20 min. Washed (3 × 5 min) slides were stained with Harris Hematoxylin (Newcomer Supplies, Middleton, WI). Slides were then washed (3 × 5 min) in water, treated with blueing water (ammonium hydroxide) for 1 min, and then washed an additional 3× (1 min/wash). Slides were then dehydrated by an ethanol series (water, 95%, 100%) and xylene, coverslipped with Permount (Fisher Scientific, Pittsburgh, PA), and evaluated for the presence of Opn by light microscopy at 40× magnification.