Veterinary Immunology and Immunopathology 157 (2014) 20–30

Contents lists available at ScienceDirect

Veterinary Immunology and Immunopathology

journal homepage: www.elsevier.com/locate/vetimm

Organic barn dust extract exposure impairs porcine macrophage function *in vitro*: Implications for respiratory health

Susan M. Knetter[a], Christopher K. Tuggle[a], Michael J. Wannemuehler[b], Amanda E. Ramer-Tait[c,*]

[a] *Department of Animal Science, Iowa State University, Ames, IA 50011, USA*
[b] *Department of Veterinary Microbiology and Preventive Medicine, College of Veterinary Medicine, Iowa State University, Ames, IA 50011, USA*
[c] *Department of Food Science and Technology, University of Nebraska-Lincoln, Lincoln, NE 68583, USA*

ARTICLE INFO

Article history:
Received 6 April 2013
Received in revised form 28 October 2013
Accepted 30 October 2013

Keywords:
Organic dust extract
Pig
Macrophage
Airway inflammation
Respiratory disease

ABSTRACT

Respiratory diseases are responsible for a significant amount of animal morbidity and mortality in the swine industry, including the majority of nursery and grower/finisher deaths. Innate immunity, including the maintenance of lung macrophage health and function, is an important defense mechanism against respiratory pathogens and their associated losses. Chronic exposure of swine industry workers to airborne barn dust results in significant predisposition to airway diseases and impairment of alveolar macrophage (AMϕ) function. Because of their importance in maintaining normal respiratory function, this study was designed to evaluate the impact of barn dust on swine macrophages. As measures of macrophage function, we evaluated the activation of NF-κB, cytokine production, cell surface marker expression and the phagocytic and antibacterial capabilities of porcine macrophages after *in vitro* exposure to an organic swine barn dust extract (ODE). ODE treatment induced AMϕ secretion of both pro- and anti-inflammatory cytokines, suggesting a complex activation profile. Additionally, ODE induced expression of genes (TLR2, NOD2) involved in sensing Gram-positive bacteria, a major component of barn dust. ODE exposure also enhanced the expression of several cell surface markers of activation, including a receptor for the porcine reproductive and respiratory syndrome virus. Moreover, two key functions of AMϕ, phagocytosis and bacterial killing, were impaired after exposure to ODE. Treatment with ODE for the first 72 h of differentiation also inhibited the ability of monocyte-derived macrophages to translocate NF-κB to the nucleus following endotoxin stimulation. Taken together, these results demonstrate, for the first time, that organic dust extract exposure negatively affects pig macrophage activation and function, potentially enhancing host susceptibility to a variety of respiratory infections.

© 2013 Elsevier B.V. All rights reserved.

1. Introduction

In the swine industry, respiratory diseases account for the highest percentage of all nursery deaths, cause the majority of grower/finisher deaths (NAHMS, 2006) and contribute to costly production losses by decreasing feed intake and average daily gain (Jericho and Harries, 1975; van Reeth and Nauwynck, 2000). The etiology of respiratory disease is complex, and susceptibility to infection may be complicated by a variety of environmental factors, including exposure to swine barn dust. Indeed, conditions known to contain high levels of swine barn dust impair

* Corresponding author. Tel.: +1 402 472 7293.
E-mail address: aramer-tait2@unl.edu (A.E. Ramer-Tait).

0165-2427/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.vetimm.2013.10.019

human lung function, and swine confinement operation employees are significantly predisposed to airway diseases, including rhinitis, bronchitis and chronic obstructive pulmonary disease (Von Essen and Romberger, 2003).

Swine barn dust is composed of a myriad of components derived from feed, dander, fecal waste, microbial particles and other sources capable of stimulating immune responses. Alveolar macrophages (AMϕ) are among the first immune cells to respond to these inhaled particles (Poole and Romberger, 2012). Microbial constituents of organic dust are rich in highly conserved pathogen-associated molecular patterns (PAMPs) recognized by host pattern recognition receptors (PRRs) present on AMϕ and other antigen presenting cells. Signaling cascades initiated via these PRRs ultimately activate cellular inflammatory responses (Barton and Medzhitov, 2002; Poole and Romberger, 2012). The non-allergic inflammation elicited by inhaled dust is accompanied by local and systemic production of inflammatory cytokines, such as TNF-α, IL-1β and the chemoattractant CXCL8, resulting in pyrexia, enhanced mucus production and neutrophil influx into the airways (Larsson et al., 1997; Wang et al., 1998; Sahlander et al., 2012). Exposure to swine barn organic dust extract (ODE) *in vitro* impaired human and murine macrophage function (Poole et al., 2008) and altered human dendritic cell maturation (Poole et al., 2009a). *In vivo*, dust inhalation has been directly linked to increased airway inflammation and lung pathology in mice, humans and pigs (Donham et al., 1995; Urbain et al., 1999; Poole et al., 2009b).

To date, few studies have examined the impact of chronic barn dust exposure on the swine immune system, and none have attempted to directly test whether dust impairs porcine macrophage phenotype or function. We sought to define the functional alterations in cytokine production, cell surface marker expression and phagocytosis of pig AMϕ exposed to ODE obtained from swine barns. ODE exposure induced both pro- and anti-inflammatory cytokine production, enhanced surface expression of activation markers and enhanced the expression of genes involved in sensing Gram-positive bacteria. Both phagocytosis and bacterial killing were diminished following ODE treatment. Moreover, ODE exposure during the early differentiation of monocyte-derived macrophages (MDMs) reduced translocation of nuclear factor kappa B (NF-κB) to the nucleus following endotoxin stimulation. Together, these data demonstrate that swine barn ODE suppresses macrophage function. Considering that respiratory immunity must be optimal for ensuring disease resistance and efficient growth in today's modern swine production facilities, barn dust exposure may be an underappreciated underlying cause of porcine respiratory disease outbreaks.

2. Materials and methods

2.1. Organic dust extract (ODE)

ODE was a kind gift from J. A. Poole, University of Nebraska Medical Center; it was collected, prepared and analyzed for composition as previously described (Romberger et al., 2002; Poole et al., 2007, 2012). Briefly, settled dust was collected three feet above the floor from a swine confinement facility of 500–700 animals. Dust samples were solubilized, vortexed and centrifuged. The supernatant was filter-sterilized (0.22 μm) and frozen (−20 °C) until use. The collected dust was analyzed via gas chromatography-tandem mass spectrometry; results were consistent with previous reports (Poole et al., 2007). Analysis revealed high muramic acid (424.0 ± 17.7 pmol/mg), a component of peptidoglycan, high 3-hydroxy fatty acid (3109.8 ± 152.6 ng/mg), a component of endotoxin, and low ergosterol (9.3 ± 0.4 pmol/mg), a component of fungi. The aqueous dust extract was diluted to 12.5% (vol/vol) in sterile PBS for analysis of protein and endotoxin concentrations, and independent batches of ODE were prepared and tested. To confirm the absence of any live microorganisms, ODE was plated on either brain heart infusion (BHI) (Difco, Detroit, MI) agar or 5% sheep blood agar plates and incubated at 37 °C. Plates were confirmed negative for bacterial growth at 12, 24, 48 and 96 h. Additionally, no cytopathic effects were observed in cultures of AMϕ grown in the presence of ODE for 24 or 48 h, suggesting a lack of live virus in the ODE preparation. The range of diluted extract contained 2.91–3.88 mg/mL of total protein and 22.1–91.1 EU/mL of endotoxin as measured by the limulus amebocyte lysate assay (Sigma, St. Louis, MO). ODE concentrations of either 0.1% or 1% were employed to conservatively model low swine barn dust exposure conditions (Poole et al., 2008, 2009a).

2.2. Animals, lavage and macrophage culture

Ten crossbred pigs between 8 and 12 weeks of age of either sex were obtained from a porcine reproductive and respiratory syndrome virus (PRRSv), swine influenza virus and porcine circovirus 2 (PCV2) negative herd. Prior to eight weeks of age, pigs were vaccinated against porcine parvovirus, *Mycoplasma hyopneumoniae* and leptospirosis. Pigs were euthanized with an overdose of sodium pentobarbital given intravenously according to Iowa State University Laboratory Animal Resources experimental guidelines. The IACUC at Iowa State University approved all protocols involving animals. Lungs were removed and lavaged twice with cold PBS. Lavage fluid was centrifuged at 500 × *g* for 15 min; cell pellets were pooled and washed once in cold PBS. Erythrocytes were lysed in ACK lysis buffer (0.15 M NH_4Cl, 10 mM $KHCO_3$, 0.1 mM EDTA). Cells were washed again in cold PBS, resuspended in complete tissue culture media (CTCM; RPMI 1640, 5% heat-inactivated normal swine serum (Sigma–Aldrich, St. Louis, MO), 5 mM HEPES, 1 mM L-glutamine, 100 U/mL penicillin, 100 μg/mL streptomycin, and 15 μg/mL gentamicin sulfate), plated in 150× 15 mm tissue culture-treated dishes and allowed to adhere for 2 h at 37 °C with 5% CO_2. After 2 h, non-adherent cells were removed and discarded. Adherent cells were harvested by scraping and then washed and counted via trypan-blue exclusion. AMϕ were plated in duplicate wells within pig and treatment at a density of 5×10^5 per well in 24-well plates. Cells were cultured in the presence of 10 μg/mL endotoxin, ODE as a percentage of total culture volume as indicated (0.1% ODE or 1% ODE) or a medium only equivalent. No evidence of cell death was observed following incubation with any of the treatments.

Download English Version:

https://daneshyari.com/en/article/5796839

Download Persian Version:

https://daneshyari.com/article/5796839

Daneshyari.com