International Immunopharmacology 27 (2015) 76–84

Contents lists available at ScienceDirect

International Immunopharmacology

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

Effect of elevated carbon dioxide on bronchial epithelial innate immune receptor response to organic dust from swine confinement barns

D. Schneberger [b], D. Cloonan [b], J.M. DeVasure [b], K.L. Bailey [a,b], D.J. Romberger [a,b], T.A. Wyatt [a,b,c,*]

[a] Research Service, Veterans Administration Nebraska Western Iowa Health Care System, Omaha, NE 68105, United States
[b] Pulmonary, Critical Care, Sleep & Allergy Division, Department of Internal Medicine, University of Nebraska Medical Center, 985910 The Nebraska Medical Center, Omaha, NE 68198-5910, United States
[c] Department of Environmental, Agricultural and Occupational Health, College of Public Health, University of Nebraska Medical Center, 985910 The Nebraska Medical Center, Omaha, NE 68198-5910, United States

ARTICLE INFO

Article history:
Received 17 September 2014
Received in revised form 27 March 2015
Accepted 14 April 2015
Available online 25 April 2015

Keywords:
Hypercapnia
TLR
LPS
Barn dust
Carbon dioxide
Lung
Bronchial epithelium

ABSTRACT

Hypercapnia is known to have immunoregulatory effects within the lung. Cell culture systems demonstrate this in both macrophages and alveolar cell lines, suggesting that the alveoli are affected by changes in CO_2 levels. We hypothesized that hypercapnia would also modulate human bronchial epithelial cell immune responses. Innate immune responses to Pam3CSK4 (TLR2 ligand), LPS (TLR4 ligand) and a complex innate immune stimulus, an extract from the organic dust of swine confinement barns (barn dust extract or BDE), were tested in a human bronchial epithelial cell line, BEAS-2B. Both TLR ligands showed a decrease in IL-6 and IL-8 production, and an increase in MCP-1 in response to elevated CO_2 indicating an enhancement in cytokine production to hypercapnia. This change was not reflected in expression levels of TLR receptor RNA which remained unchanged in response to elevated CO_2. Interestingly, barn dust showed an increase in IL-6, IL-8 and MCP-1 response at 9% CO_2, suggesting that elevated CO_2 exerts different effects on different stimuli. Our results show that airway epithelial cell immune responses to barn dust respond differently to hypercapnic conditions than individual TLR ligands.

© 2015 Elsevier B.V. All rights reserved.

1. Introduction

Workers in concentrated animal feeding operations (CAFOs) face a number of health problems over the course of their careers, many of these associated with the lungs due to organic dust exposure. These problems can include chronic bronchitis, COPD, and asthma [22,34]. Many of these conditions involve airflow obstruction in the lung, which may result in elevated CO_2 within the lung, such as observed in COPD patients [4]. Studies conducted with organic dusts show that innate immune responses to microbial ligands, such as endotoxin and peptidoglycan, found within these dusts are a major cause of many of these symptoms [13,23,35].

CAFOs also contain elevated levels of gasses, including CO_2, ammonia, and hydrogen sulfide [11,43]. The CO_2 levels found in these facilities may be up to 0.7% higher than ambient air [12]. Shorter common CO_2 exposures such as smoking may result in CO_2 exposures ranging up to 12.5% [31]. The combination may lead to significant changes in CO_2 levels deep within the lungs. Many cell culture studies to date study higher CO_2 test levels than are found occupationally or environmentally [1,16,32,45,47]. Examining how varying CO_2 levels can induce immune changes in the lung is vital to determining whether exposure to environmental CO_2 affects innate immunity.

In addition to CAFO workers, hypercapnia also occurs clinically, such as in acute respiratory distress syndrome (ARDS). Work in ARDS studies suggests that permissive hypercapnia in ventilated patients may have beneficial effects in reducing lung inflammation [24]. Some link these effects not to reduced mechanical stretch of the lungs, but to elevated CO_2 [10]. In contrast, others show increased inflammation due to hypercapnia [30], and animal studies have yielded conflicting results, with some showing reductions in cytokines, while others show increased inflammation in other cell systems [1,36,42].

Hypercapnia cell culture studies with cells found in the alveolar space also yield conflicting results. Alveolar epithelial cells show increased inflammatory cytokine production [1]; whereas, a differentiated monocyte cell line shows reduced IL-6 production [50]. Hypercapnia appears to primarily affect cytokine production, as cell viability and cell cycle progression appear unaffected [47], though this is also unresolved [49]. One possible reason for these conflicting results could be that different cell types within the lung respond quite differently to the same hypercapnic conditions, so that while inflammation is reduced in the alveolar space, it may be increased elsewhere. Differences in exposure and reporting systems may also

* Corresponding author at: Pulmonary, Critical Care, Sleep & Allergy Division, Department of Internal Medicine, University of Nebraska Medical Center, 985910 The Nebraska Medical Center, Omaha, NE 68198-5910, United States. Tel.: +1 402 559 3817; fax: +1 402 559 4140.
E-mail address: twyatt@unmc.edu (T.A. Wyatt).

http://dx.doi.org/10.1016/j.intimp.2015.04.031
1567-5769/© 2015 Elsevier B.V. All rights reserved.

play a role. As airway epithelial cells remain unstudied, we tested the human bronchial epithelial BEAS-2B cell line for changes in inflammatory response to common toll-like receptor (TLR) ligands and organic barn dust extract using a range of CO_2 levels, ranging from standard cell culture conditions to levels shown to induce hypercapnic changes (5%–9%). We hypothesized that similar to alveolar epithelial cells, bronchial epithelial cells would show an increase in inflammatory cytokine production. TLR2 (synthetic triacylated lipoprotein; Pam3CSK4) and TLR4 (lipopolysaccharide; LPS) ligands were chosen given the role both TLRs have in barn dust immunogenicity [14,35] and because others show that hypercapnic effects alter NF-κB signaling, through which both of these receptors signal [45]. We further examined at what level above standard culture conditions (5% CO_2) any possible response to elevated CO_2 could be measured. We show that not only do bronchial epithelial cells produce less IL-6 and IL-8 but also more MCP-1 to TLR stimulation ligands under hypercapnic conditions. These changes are observed at as little as a 2% increase in CO_2 over standard 5% culture conditions. In contrast, exposure to barn dust under hypercapnic conditions resulted in an increase in IL-6, IL-8 and MCP-1, showing that response to different stimuli is affected in different ways by hypercapnia.

2. Materials and methods

2.1. Cell culture system

BEAS-2B, a human bronchial epithelial cell line, was purchased from American Type Culture Collection (ATCC, Manassas, VA), cultured and grown in Vitrogen-coated (Invitrogen, Carlsbad, CA) tissue culture flasks at 37 °C and 5% CO_2 in 1:1 LHC9:RPMI supplemented with penicillin and streptomycin (Gibco, Grand Island, NY). Monolayers were harvested by treatment with trypsin for 10 min at 37 °C. 100 μl trypsin inhibitor was added (Sigma, St. Louis, MO) to inactivate trypsin, resuspended in media and centrifuged to wash cells and replace media and counted. Normal human bronchial epithelial cells (NHBE) were similarly cultured using serum free bronchial epithelial basal media (Lonza, Walkersville, MD). Media was tested at normal and 9% CO_2 levels for changes in pH by RapidPoint 500 blood gas analyzer (Siemens, Tarrytown, NY). Monolayers were harvested by treatment with TrypLE Express (Gibco, Denmark) for 10 min at 37 °C. 100 μl trypsin inhibitor was added to inactivate trypsin, resuspended in media and centrifuged to wash cells and replace media and counted. 12-well tissue culture plates were coated for a minimum of 10 min with 1% Vitrogen before adding 0.3×10^6 cells in 800 μl to each (approximately 70% confluence). Cells were allowed to attach overnight then washed with PBS, pH 7.4 and fresh media added at the time of treatment. After treatment with TLR ligands or BDE, cells were cultured at 37 °C at 5%–9% CO_2 in 1% increments in a standard cell culture incubator (Model MCO-19AICUV-PA; Sanyo, Wood Dale, IL). The same incubator was used for all trials and calibrated using a fyrite analyzer (Bacharach, New Kensington, PA). A control using acidified (10 mM HCL, media pH 7.0) media at 5% CO_2 levels was also performed to control for the effects of CO_2 induced media acidosis.

2.2. Immunostimulatory ligands

Ligands for two of the most commonly studied TLRs, Pam3CSK4 (TLR2) and LPS (TLR4), were administered to cells at either 10 ng/ml or 100 EU respectively and immediately exposed to cell culture CO_2 conditions as various levels. These doses were determined to be stimulatory, but not maximal (results not shown). Both ligands were diluted in LHC9:RPMI cell culture media.

BDE extracts were prepared from combined settled dust samples taken from two separate swine confinement facilities. Dust extracts were prepared as previously described [38]. Briefly, dust (1 g) was mixed with 10 ml HBSS without calcium. This mixture was incubated for 1 h at room temperature before 10 min centrifugation, with the media being decanted and sterile-filtered for use, for a final concentration of approximately 0.105 g/ml dust. Extracts were used at a concentration of 5% v/v of culture per well (40 μl) or about 0.005 g/ml dust.

2.3. Lactate dehydrogenase assay

Lactate dehydrogenase was measured in media samples to determine cell viability using the Lactate Dehydrogenase Activity Assay Kit (BioVision, Milpitas, CA) according to the instructions provided. Media samples from cultured cells were tested for 24 h exposure at all CO_2 levels and treatments ($n = 3$ per group). No significant changes in cell death were noted as a result of treatments.

2.4. ELISAs

Cell culture media was collected and tested using a sandwich ELISA. IL-6, IL-8, and MCP-1 were tested in duplicate and quantified as per manufacturer's instructions (R&D Systems, Minneapolis, MN). Plates were read using an Epoch microplate reader (BioTek, Winooski, VT). Values given are reported in pg/ml.

2.5. Chemokine array assay

Cell culture media from 24 h cell culture exposures were sampled equally to obtain a 1 ml sample pool for testing. Media was tested using a semi-quantitative protein microarray according to the manufacturer's instructions (Ray Biotech, Norcross, GA). Sample binding and secondary antibody binding steps were incubated on arrays overnight at 4 °C. Arrays were developed with fluorescent marker and exposed to film (GeneMate Blue Ultra; ISC BioExpress, Kaysville, UT) for approximately 3 s. Developed film was quantitated via densitometry using ImageJ software (http://rsbweb.nih.gov/ij/) and compared to each other by mathematically equalizing control spots (lower right duplicate) on different arrays to one another.

2.6. NF-κB translocation/binding

Cells were cultured onto 96-well plates and transfected using the Cignal Vector Reporter for NF-κB (SABiosciences; Valencia, CA) as per manufacturer's protocol using lipofectamine 2000 (Invitrogen; Grand Island, NY). Cells were treated with 5% BDE for 24 h and incubated at 37 °C at 5% or 9% CO_2. Cells were harvested using Promega Dual-Glo Luciferase Reagent (Promega; Madison, WI). Firefly and renilla luciferase activities were measured using a VICTOR 3V plate reader (Perkin Elmer; Waltham, MA).

2.7. Real-time RT-PCR

cDNA synthesis was done using the TaqMan reverse transcription kit (Applied Biosystems, Branchburg, NJ) with 100 ng of template RNA purified from cells using the Qiagen Minprep Kit (Qiagen, Valencia, CA). cDNA synthesis (RT-PCR) reactions contained the following reagents: 1× TaqMan RT buffer, 5.5 nM $MgCl_2$, 500 μM of each dNTP, 2.5 μM random hexamers, 0.4 U/μl RNase inhibitor, and 1.25 U/μl MultiScribe Reverse Transcriptase. Samples were incubated at 25 °C for 10 min, then 48 °C for 30 min, and 95 °C from 5 min in a thermocycler (MJ Mini; Bio-Rad, Hercules, CA). Real-time PCR reactions consisted of 1× TaqMan Master Mix along with human IL-6 and IL-8, and TLR1, 2, 4, 5, and 6 primers and probes (Applied Biosystems, Branchburg, NJ; Hs03929033_u1, Hs01567913_91,Hs00413978_m1, Hs00152932_m1, Hs00152939_m1, Hs00152825_m1, and H200271977_s1 respectively). PCR was completed in an ABI PRISM 7700 Sequence Detection

Download English Version:

https://daneshyari.com/en/article/2540662

Download Persian Version:

https://daneshyari.com/article/2540662

Daneshyari.com