Biochemical and Biophysical Research Communications xxx (2018) 1–7

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

PPAR-gamma pathways attenuate pulmonary granuloma formation in a carbon nanotube induced murine model of sarcoidosis

Matthew McPeek [a], Anagha Malur [a], Debra A. Tokarz [b], Gina Murray [c], Barbara P. Barna [a], Mary Jane Thomassen [a, *]

[a] Department of Internal Medicine, Pulmonary, Critical Care & Sleep Medicine, Brody School of Medicine, East Carolina University, Greenville, NC, 27834, USA
[b] Department of Population Health and Pathobiology, College of Veterinary Medicine, North Carolina State University, Raleigh, NC, 27695, USA
[c] Department of Pathology, Brody School of Medicine, East Carolina University, Greenville, NC, 27834, USA

ARTICLE INFO

Article history:
Received 6 June 2018
Accepted 12 June 2018
Available online xxx

Keywords:
Sarcoidosis
Granuloma
Carbon nanotube
Inflammation
Lipid transporters
Alveolar macrophage

ABSTRACT

Peroxisome proliferator activated receptor gamma (PPARγ), a ligand activated nuclear transcription factor, is constitutively expressed in alveolar macrophages of healthy individuals. PPARγ deficiencies have been noted in several lung diseases including the alveolar macrophages of pulmonary sarcoidosis patients. We have previously described a murine model of multiwall carbon nanotubes (MWCNT) induced pulmonary granulomatous inflammation which bears striking similarities to pulmonary sarcoidosis, including the deficiency of alveolar macrophage PPARγ. Further studies demonstrate alveolar macrophage PPARγ deficiency exacerbates MWCNT-induced pulmonary granulomas. Based on these observations we hypothesized that activation of PPARγ via administration of the PPARγ-specific ligand rosiglitazone would limit MWCNT-induced granuloma formation and promote PPARγ-dependent pathways. Results presented here show that rosiglitazone significantly limits the frequency and severity of MWCNT-induced pulmonary granulomas. Furthermore, rosiglitazone attenuates alveolar macrophage NF-κB activity and downregulates the expression of the pro-inflammatory mediators, CCL2 and osteopontin. PPARγ activation via rosiglitazone also prevents the MWCNT-induced deficiency of PPARγ-regulated ATP-binding cassette lipid transporter-G1 (ABCG1) expression. ABCG1 is crucial to pulmonary lipid homeostasis. ABCG1 deficiency results in lipid accumulation which promotes pro-inflammatory macrophage activation. Our results indicate that restoration of homeostatic ABCG1 levels by rosiglitazone correlates with both reduced pulmonary lipid accumulation, and decreased alveolar macrophage activation. These data confirm and further support our previous observations that PPARγ pathways are critical in regulating MWCNT-induced pulmonary granulomatous inflammation.

1. Introduction

Granulomatous lung diseases represent a significant health burden worldwide. Sarcoidosis is an inflammatory condition characterized by the presence of non-necrotizing granulomas which effects the lungs and mediastinal lymph nodes in 90% of clinical cases [1]. The etiology of sarcoidosis has not been determined, however current understanding suggest the presence of a poorly soluble antigen leading to an exuberant host immune response in genetically susceptible individuals [2]. Epidemiological studies have found a correlation between pulmonary sarcoidosis and exposure to wood-burning stoves, fireplaces and certain occupations such as firefighters [3–5]. An increased incidence of sarcoid-like granulomatous lesions have also been reported in first responders present at the September 2001 World Trade Center Disaster [6]. These environments have been shown to contain particulate matter of respirable sizes, including carbon nanotubes. Carbon nanotubes (CNT) are produced as byproducts of combustion or manufactured for a variety of commercial applications. Evaluation of the respiratory toxicology of these materials has found the

ABBREVIATIONS: ABCA1, ATP-binding cassette lipid transporter-A1; ABCG1, ATP-binding cassette lipid transporter-G1; BAL, Bronchoalveolar lavage; CD36, Cluster of differentiation-36; NF-κB, Nuclear Factor-Kappa-B; PPARγ, Peroxisome proliferator activated receptor-gamma.

* Corresponding author. Brody School of Medicine, East Carolina University, 600 Moye Blvd/3E-149, Greenville, NC, 27834, USA.
E-mail address: thomassenm@ecu.edu (M.J. Thomassen).

https://doi.org/10.1016/j.bbrc.2018.06.061
0006-291X/

potential to induce pulmonary granulomatous lesions in exposed animals [7,8]. These observations prompted our laboratory to investigate the use of multiwall carbon nanotubes (MWCNT) to generate a murine model of pulmonary granulomatous inflammation to study pulmonary sarcoidosis [9].

Previous methods utilized to induce pulmonary granulomas included the administration of pathogens or introduction of antigen bound sepharose beads into the tail vein of sensitized animals [10–12]. While these models have advanced our understanding of pulmonary granuloma formation they have notable drawbacks including the presence of active pathogens, eliciting granuloma in the pulmonary capillaries instead of the airways and a relatively short resolution time. In our studies, evaluation of mice 60 days following MWCNT instillation found the persistence of granulomatous lesions throughout the lung [9]. Further studies demonstrated that the MWCNT model closely mimics sarcoidosis pathophysiology, including elevated expression of inflammatory mediators and reduced expression and activity of alveolar macrophage peroxisome proliferator-activated receptor gamma (PPARγ) [9,13,14].

PPARγ, a ligand-activated nuclear transcription factor, has been shown to limit pro-inflammatory macrophage activation [15]. PPARγ regulates gene expression by selectively binding PPARγ-response elements, promoting target gene expression, or through the inhibition of other transcription factors such as nuclear factor-κB (NF-κB) [16,17]. PPARγ, constitutively active in alveolar macrophages of healthy individuals, is deficient in multiple lung diseases including alveolar macrophages of pulmonary sarcoidosis patients [18–20]. The importance of alveolar macrophage PPARγ to pulmonary homeostasis is demonstrated by macrophage-specific PPARγ knockout mice. These animals exhibit increased Th-1 pro-inflammatory cytokine expression and dysregulated pulmonary lipid catabolism [21,22].

PPARγ maintains pulmonary lipid homeostasis through the expression of alveolar macrophage ATP-binding cassette lipid transporter-G1 (ABCG1) [22,23]. Following uptake by scavenger receptors, such as the PPARγ-regulated cluster of differentiation-36 (CD36), lipids are catabolized and effluxed to extracellular acceptors by ABCG1, and the complimentary lipid transporter ABCA1 [24]. Deficiency of ABCG1 or ABCA1 results in pulmonary lipid accumulation and elevated inflammatory mediators [25–28]. The inability to properly efflux cholesterol leads to increased sensitivity to extracellular inflammatory signaling in ABCA1/ABCG1 deficient macrophages [29]. Our recent studies in alveolar macrophages from sarcoidosis patients and MWCNT-instilled mice observed decreased expression of both ABCG1 and ABCA1 [30]. The deficiency of these lipid transporters correlates with increased alveolar macrophage lipid accumulation in MWCNT-instilled animals [30]. These observations suggest that downregulation of ABCG1 and ABCA1 may contribute to MWCNT-induced inflammation. We hypothesized that increase of the PPARγ-ABCG1 pathway would limit alveolar macrophage activation and pulmonary granuloma formation. To address this hypothesis we utilized the PPARγ-specific agonist rosiglitazone to activate PPARγ pathways. Results shown here indicate that rosiglitazone attenuates MWCNT-induced granulomatous inflammation.

2. Materials and methods

2.1. Animal care and treatment

Animal studies were conducted in accordance with the National Institutes of Health guide for the care and use of Laboratory animals and with approval from the East Carolina University Institutional Animal Care and Use Committee. C57BL/6 J wild type mice were purchased from The Jackson Laboratory (Bar Harbor, ME). An equal number of age and sex matched controls were randomly assigned into experimental groups. Rosiglitazone laden diets were produced by Teklad Diets (Madison, MI), incorporating rosiglitazone (Cayman Chemical. Ann Arbor, MI) into standard rodent chow (Prolab RMH 300, LabDiet; St. Louis, MO), delivering 2, 6 or 12 mg/kg/day (TD.160572, TD.160573 and TD.160574 respectively). Diets were administered daily, three days prior to instillation of multiwall carbon nanotubes (MWCNT) until necropsy at the indicated time point.

2.2. MWCNT model

Pulmonary granulomas were generated by oropharyngeal aspiration of 100 μg MWCNT (Cat.900–1501, Lot: GS1801) purchased from SES Research, Houston Texas as previously described [9]. Animals were euthanized with tribromoethanol and bronchoalveolar lavage (BAL) was performed for the collection of BAL cells as previously described [21]. Differential counts were performed on cytospins stained with modified Wright's stain. Histological analysis was performed on un-lavaged lungs inflated with formalin as previously described [9].

2.3. RNA purification and analysis

Total RNA was collected from BAL cell pellets with the miRNeasy Micro Kit and protocol (Qiagen. Valencia, CA). Primers were obtained from Qiagen for ATP binding cassette (ABC) transporter-A1 (Abca1, PPM03952F), Abcg1 (PPM03895A), chemokine (C-C motif) ligand-2 (Ccl2, PPM03151G), Glyceraldehyde-3-phosphate-dehydrogenase (Gapdh, PPM02946E), peroxisome proliferator activated receptor-gamma (PPARγ, PPM05108B) and osteopontin (Spp1, PPM03648C). Relative gene expression of complimentary DNA synthesized with the RT2 First Strand Kit was evaluated on an ABI Prism 7300 system (Applied Biosystems. Foster City, CA) in comparison to Gapdh using the $2^{-\Delta\Delta CT}$ method [31].

2.4. Immunohistochemistry

BAL cytospins of freshly isolated BAL cells were fixed with 4% paraformaldehyde, permeabilized with 0.3% Triton-X-100 and stained for NF-κB 1:200 (catalog# 6956 S. Cell Signaling. Danvers, MA) overnight at 4 °C followed by Alexa-conjugated goat anti mouse IgG 1:1000 (Invitrogen. Carlsbad, CA). Slides were counterstained with propidium iodide (Vector Laboratories. Burlingame, CA) to facilitate nuclear localization. Images were acquired on a LSM 700 confocal microscope (Zeiss. Oberkochen, Germany). Quantification of NF-κB nuclear localization was quantified using ZEN Blue software (Zeiss). Differential analysis of BAL cells revealed that alveolar macrophages constituted the majority of cells (93.4%–99.7%; $n \geq 6$) at either 10 or 20 days post treatment with no significant differences among BAL samples from control or treated groups.

2.5. Analysis of bronchoalveolar lavage fluid

Total cholesterol was measured using the Amplex Red Cholesterol Assay kit (Thermo Fisher Scientific. Waltham, MA) according to manufacturer's instructions in the presence of cholesterol esterase. Cholesterol was normalized to protein content of BAL Fluid measured by BCA assay (Thermo Fisher Scientific).

2.6. Quantitative analysis of tissue

Digital images of whole lung cross sections stained with

Please cite this article in press as: M. McPeek, et al., PPAR-gamma pathways attenuate pulmonary granuloma formation in a carbon nanotube induced murine model of sarcoidosis, Biochemical and Biophysical Research Communications (2018), https://doi.org/10.1016/j.bbrc.2018.06.061