MicroRNA-Mediated Calcineurin Signaling Activation Induces CCL2, CCL3, CCL5, IL8 and Chemotactic Activities in 4,4'-Methylene Diphenyl Diisocyanate Exposed Macrophages

Introduction

Occupational exposure to 4,4'-methylene diphenyl diisocyanate (MDI), the most widely used monomeric diisocyanate, is one of the leading causes of occupational asthma (OA). Pathophysiological mechanism(s) by which how MDI causes OA is still warranted to be elucidated. Alveolar macrophages are the most abundant immune cell type in the lung, and these cells serve as one of the first immune responders against inhaled pathogens, particles, stimuli, and chemical allergens such as dNCOs. Upon encountering outside stimuli, alveolar macrophages react by phagocytosis as well as producing and secreting different mediators such as cytokines, chemokines, and others, into the alveoli microenvironment to orchestrate the initiation of inflammatory/immune responses. Dysfunction of alveolar macrophages, including elevated production and secretion of pro-inflammatory cytokines and other immune mediators, has been shown to play an important role in asthma pathogenesis. In the clinical setting, the levels of many immune mediators produced by macrophages have been found elevated in the asthmatic airway. However, both the levels of these asthma-associated, macrophage-secreted inflammatory/immune mediators in MDI-OA patients' airways and how expression of these mediators change in response to MDI exposure in alveolar macrophages are largely undetermined. Previously, we demonstrated a microRNA (miR)-206-3p and miR-381-3p mediated PPP3CA/Calcineurin signaling induced *inducible nitric oxide synthase* (*iNOS*) transcription in macrophages and bronchoalveolar lavage cells (BALCs) after acute MDI exposure; however, whether this mechanism participates in regulation of other asthma-associated mediators secreted by macrophages/BALCs after MDI exposure is currently unknown. The first aim of this study was to identify candidate asthma-associated, macrophage-secreted mediators that can be regulated after MDI exposure. After identified the candidate mediators can be regulated by MDI-exposure, we investigated the roles of miR-mediated calcineurin signaling in regulation of these candidate mediators' expressions in relation to the exposure to MDI.

Methods Collection

1. Bronchoalveolar lavage cells (BALCs) RNA from MDI aerosol exposed mice
    - Stored RNA from previous studies using animals exposed to MDI aerosol.
    - BALB/c mice were exposed to 4580 ± 1497 μg/m^3 MDI aerosol or pure house air control (Ctl) for 1 h followed by bronchoalveolar lavage at 24 h post-exposure
2. Cell culture and cell differentiation
    - All cells were obtained from ATCC.
    - Enhanced differentiated THP-1 macrophages were prepared using media containing 100 nM phorbol 12-myristate 13-acetate (PMA) to induce differentiation for 3 days and then enhanced by refeeding fresh media after removing PMA containing media for additional 3 days.
    - Dendritic cell differentiation was prepared using undifferentiated THP-1 monocytes cultured in serum-free RPMI-1640 culture medium supplemented with 100 ng/ml rhGM-CSF, 10 ng/ml rhTNF-α, and 200 ng/ml ionomycin for 3 days.
    - Neutrophil differentiation was prepared using HL-60 cells cultured in complete RPMI-1640 media containing 1.5% DMSO for 7 days.

- Eosinophil differentiation was prepared using HL-60_C15 cells cultured in complete RPMI-1640 media containing 0.5 mM butyric acid for 7 days.

3. MDI-GSH conjugation
    - 10 mM GSH solution was prepared in 200 mM sodium phosphate buffer (pH= 7.4).
    - MDI-GSH conjugation was prepared by adding MDI/acetone directly into GSH solution. End-to-end mixing for 1 h at 25 °C.
4. Transient transfection and PPP3CA overexpression
    - Plasmid DNAs were transfected into THP-1 macrophages using Mirus TransIT-2020 transfection reagent according to manufacturer's instructions.
    - miR-inhibitors were transfected into THP-1 macrophages using Lipofectamine RNAiMAX transfection reagent according to manufacturer's instructions.
5. Mediator gene expression (gene and protein)
    - Total RNA was isolated using *mirVana*™ miR isolation kit according to manufacturer's instructions.
    - TaqMan gene expression assays were obtained from ThermoFisher Scientific.
    - Real-time PCR assays were performed on Applied Biosystems 7500 RT-PCR System.
    - Human CCL2, CCL3, and IL8 ELISA kits were obtained from ThermoFisher Scientific. Human CCL5 ELISA kit was obtained from R&D systems. ELISA were performed according to manufacturer's instructions.
6. Chemotaxis assays and quantification of migrated cells
    - 3 μm pore size Transwell™ assay insert was used for chemotaxis/cell migration.
    - Immune cells were added into Transwell™ assay inserts and allowed those cells responded to conditioned media obtained from THP-1 macrophages exposed to MDI-GSH or transfected with miR-inhibitors.
    - Migrated immune cells were quantified by using CyQUANT GR proliferation assay according to manufacturer's instructions.

Citations – Publications based on the data set

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Chen-Chung Lin, mjy2@cdc.gov

Brandon F. Law, bhl7@cdc.gov

Justin M. Hettick, ayf2@cdc.gov

Contact information
For further information, contact:

Allergy and Clinical Immunology Branch (ACIB),
Health Effects Laboratory Division (HELD),

National Institute for Occupational Safety and Health (NIOSH),
Morgantown, WV
304.285.6360