Differential Inflammasome Activation in M1 and M2a Pulmonary Macrophages

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Abstract

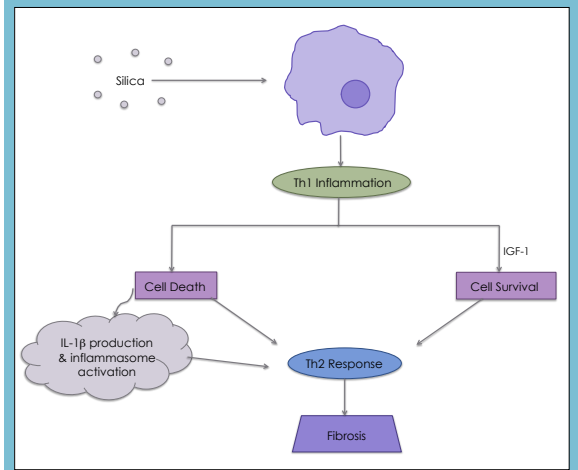
Macrophages are a critical cell type in protection of various environments and modulation of immune responses. Many different phenotypes and subpopulations of macrophages have been described and shifts in subpopulation proportions have been show to be important in the development of numerous diseases. Previously differences have been found in antigen presenting cell (APC) activity in distinctive interstitial macrophage populations following silica (Si) exposure. Prolonged Si exposure can lead to silicosis, which is defined as chronic inflammation and pulmonary fibrosis. Although this is a human condition, the same disease occurs after silica exposure in mouse models. In our mouse model silica instillation initially results in Th1-associated inflammation driven by activation of the multi-protein complex called the inflammasome and subsequent production of IL-1β by pulmonary macrophages. We propose that the level of inflammasome activation is distinct among separate subsets of pulmonary macrophages, and that there is a relationship between the level of inflammasome activation and the role of Th2 immunity in pulmonary fibrosis. Following Si exposure, Th2-associated macrophages show increases in pro-IL-1β production and IL-1β secretion when compared to untreated and Th1-associated controls. Additionally differences in caspase-1 activation between autofluorescent positive and autofluorescent negative populations of interstitial macrophages 24 and 72 hours following Si treatment have been found. These data suggest that the various macrophages subsets do have different levels of inflammasome activation and that the Th2-associated subpopulation has the highest inflammasome activation and IL-1β production.

Introduction

Pulmonary macrophages are a key cell type in developing immune responses and preserving the integrity of the lung. Pulmonary macrophages are a heterogeneous population consisting of two major groups, the alveolar macrophages and the interstitial macrophages. Within each of these two groups there is a diverse array of subpopulations resulting from differences in activation pathways, surface protein expression, and protein secretion. Macrophage phenotypes are considered plastic and can transform from one subpopulation to another depending on the environment. Exposure of pulmonary macrophages to a variety of deleterious agents shifts the normal array of subpopulations promoting one type of immune response over another. These shifts have been shown to be critical in the pathophysiology of many diseases. Altering of the number of cells occupying a certain subpopulation can determine the subsequent adaptive immune response and therefore the long-term result from the initial injury. Two of the well-described subsets involved in macrophage biology are the classically activated (M1) macrophage and the alternatively activated (M2a) macrophage. M1 macrophages are activated by two signals, interferon (IFN)-γ, and tumor necrosis factor (TNF)-α, while M2a macrophages are activated by the Th2-associated factors interleukin (IL)-4 or IL-13. In addition to differences in activation pathways, M1 macrophages are said to be classically inflammatory and Th1 associated while M2a macrophages are Th2 associated and classically a regulatory and recover phenotype. (Migliaccio & Holian, 2010)

Silicosis is defined as chronic inflammation and pulmonary fibrosis caused by inhalation of silica dust (Cassel et.al., 2008). The progression of the disease has been extensively studied and involves a shift in the immune response from Th1-associated inflammation to a Th2 fibrotic response. In the Balb/c silicosis model changes in interstitial macrophage subpopulations have previously been found (Migliaccio, 2005). Initially following silica inhalation there is Th1 associated inflammation driven by activation of the multi-protein complex called the inflammasome and subsequent production of Il-1β (Cassel et.al., 2008k Franchi et.al., 2009). We propose that the level of inflammasome activation is distinct among separate subsets of pulmonary macrophages, and that there is a relationship between the level of inflammasome activation and the role of Th2 immunity in pulmonary fibrosis.

Silicosis Model



Results

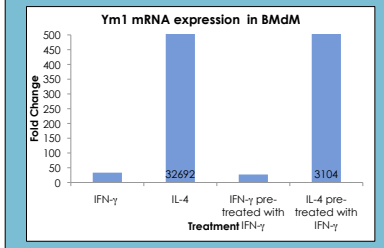
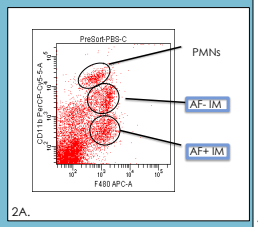


Figure 1: In vitro generation of M2a macrophages
BMdM were treated with cytokines for 24 hours to generate macrophage subsets. Following cytokine treatment subsets were assayed for mRNA production of the M2a differentiation marker Ym1. Bars represent the mean fold increase (n=3), compared to an untreated control, in Ym1 mRNA production in BMdM treated with IFN-γ and IL-4. BMdM were treated with cytokines for 24 hours before collection for mRNA analysis. The values displayed on the IL-4 treated and IL-4 pre-treated with IFN-γ bars are the actual fold increase values for those samples. IL-4 treated BMdM showed significant increases ($p<0.001$) in Ym1 expression when compared to untreated controls. The two bars on the far right of the graph represent BMdM that have been pre-treated with IFN-γ before treatment with either IL-4 or IFN-γ. With IFN-γ pre-treatment, the IL-4 treated cells still showed a large increase in Ym1 expression compared to untreated controls.



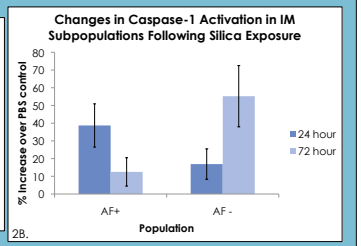
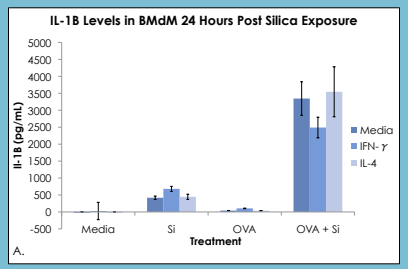
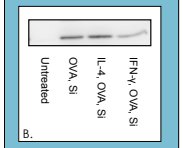


Figure 2: Caspase-1 activation in interstitial macrophage (IM) populations following silica exposure Balb/c mice were instilled transorally with Si or PBS. Twenty-four and 72 hours post instillation, lungs were collected and interstitial macrophages were isolated for analysis of caspase-1 activity by flow cytometry. Figure (B) demonstrates the gating. Figure (A) shows the mean ± SEM (n=7 for PBS n=8 for Si) percent increase in caspase-1 activation compared to a PBS control in interstitial macrophages 24 and 72 hours post Si instillation. The AF+ interstitial macrophage population shows larger increases in caspase-1 activity 24 hours after silica exposure while the AF- population shows smaller differences 24 hours after exposure. Seventy-two hours after exposure the trend shifts and the AF+ population shows smaller increases while the AF- population shows large increases in caspase-1 activity compared to the PBS control.





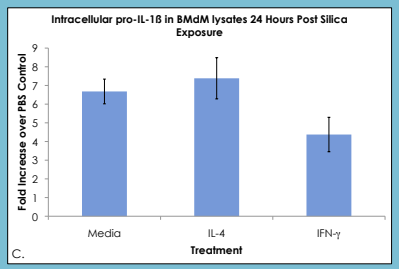


Figure 3: Silica induced pro-IL-1β production and IL-1β secretion in *in vitro* BMdM subsets
BMdM were cultured for 24 hours in the presence of cytokines to generate macrophage subsets. Subsequently BMdM were treated with silica for one hour followed by OVA. Supernatants from treated BMdM were collected after 24 hours and assayed for IL-1β secretion. Cells were also collected for lysates, which were assayed for pro-IL-1β levels by western blot. Figure (A) shows secreted IL-1β levels in BMdM supernatants as determined by ELISA. BMdM treated with IL-4 showed an increase in IL-1β secretion compared to IFN-γ and untreated controls. Figure (C) shows the fold increase in pro-IL-1β compared to an untreated control as determined by western blot (Figure B). The western blot data are consistent with the ELISA data showing that there is the highest fold increase in the IL-4 treated BMdM cultures. Bars in (A) and (C) represent the mean value ± SEM (n=2-6).

Materials and Methods

Isolation of Pulmonary Macrophages
- Alveolar macrophages (AM) were isolated by whole lung lavage
- Interstitial macrophages (IM) were isolated from collagenase-treated lungs and further isolated using Percoll gradient centrifugation for analysis of mRNA and by flow cytometry.

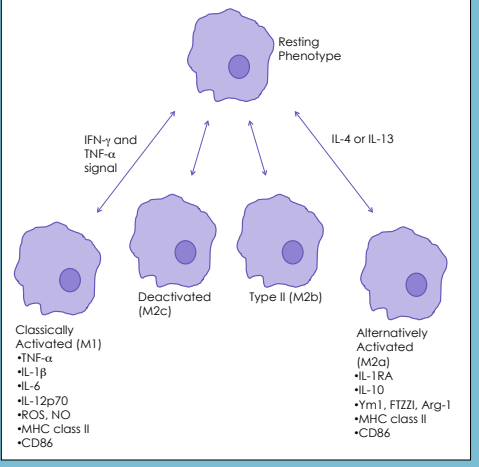
Silica Exposure
- Balb/c mice were anaesthetized with isoflurane
- Mice were then instilled transorally with 1 mg of crystalline silica in 25 μL phosphate buffered saline (PBS)

Flow Cytometry Analysis
- Leukocytes collected from gradient centrifugation were stained for flow cytometry analysis
- Macrophage populations were assessed by staining with anti-F4/80, anti-CD11b, and Caspalux fluorescently tagged peptide substrate for caspase-1 (Oncolmmunin, Inc.)
- Analysis of florescence was performed on a FACSAria system (BD)

***In vitro* Silica Exposure**
- Bone marrow cells were isolated from Balb/c mice and cultured for 10 days with macrophage colony stimulating factor (M-CSF)
- Cells were treated with 12.5ng/mL of interferon-γ, or 10ng/mL interleukin-4 for 24 hr at 37°C
- Following cytokine treatment cells were exposed to silica (200 μg/mL) for 1 hr at 37°C
- Cells were plated at a concentration of $1x10^5$ cells/well in a 96-well plate with 10mg/mL ovalbumin at 37°C for 24 hr after which supernatants were collected and assayed for interleukin-1β and lactate dehydrogenase levels (LDH)
- Treated bone marrow derived macrophages (BMdM) were also collected for mRNA analysis

Western Blotting
- Lysates from BMdM treated with cytokines, ovalbumin, and silica were separated with a 10% SDS-PAGE gel by electrophoresis
- Immunoblotting was accomplished using a rabbit anti-pro-IL-1β antibody

Differentiation of Macrophage Subsets



Summary

- Naïve and classic BMdM treated with Il-4 demonstrated significant levels of mRNA for Ym1, a Th2 associated marker
- IL-1β secretion and pro-IL-1β production are increased in IL-4 treated macrophages when compared to untreated and IFN-γ treated controls
- There is no difference in LDH release between IL-4 treated macrophages, untreated macrophages, and IFN-γ treated controls
- There is differential caspase-1 activation in distinctive subsets of interstitial macrophages 24 hours and 72 hours following silica instillation
- There are differing levels of inflammasome activation in macrophage subsets based on IL-1β production and caspase-1 activity; the highest IL-1β production happening in Th2 associated macrophage subsets

Further Research

- Further experiments will be aimed at determining the mechanism for increased production of IL-1β in Th2-associated subsets of macrophages
- *In vivo* experiments must also be performed to determine if the differences between BM subsets hold true for *in vivo* subsets
- The driving force for Th2 differentiation of pulmonary macrophages *in vivo* and subsequently its relationship with inflammasome activation will also need to be determined

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Acknowledgments

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