The Effects of Woodsmoke on Murine Alveolar Machrophages

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Abstract

In many rural areas and undeveloped countries, the main source of pollution and particulates is often biomass smoke such as woodsmoke (WS). Much is known about the specific health effects of urban particulate matter (PM), however not much is known about the effects of woodsmoke PM. Many epidemiological studies have shown a correlation between WS exposure and respiratory infections, especially in children and individuals with preexisting conditions. It has also been previously demonstrated in our mouse model that post exposure to WS there is decreased bacterial clearance in the lungs. This study will test the hypothesis that the decreased bacterial clearance is due to decreased alveolar macrophage (AM) function. To test this hypothesis AMs were isolated from WS exposed mice 2 and 24 hours post exposure and from mice 24-post instillation with 125µg of ambient particulate matter. AMs were utilized in an antigen presenting cell (APC) assay. Supernatants collected from the APC assay were analyzed for T cell cytokine levels. T cells were collected for analysis by flow cytometry. T cell activation showed a significant decrease ($p<.05$) in the WS-exposed mice over the control two hours post exposure. In addition, there was a decrease in interferon-gamma levels (IFN-gamma). Analysis of AMs 24 hours post exposure to WS showed a small decrease in phagocytic activity. In mice instilled with $PM_{2.5}$ there was a significant increase in tumor necrosis factor-alpha (TNF-alpha). WS instilled mice showed the lowest levels of TNF-alpha and IFN-gamma. The results seem to indicate some degree of suppression of macrophage function after exposure to WS or instillation with WS particulates. Furthermore, the promptness of the response seems indicative of a direct effect of WS on the alveolar macrophage.

Introduction

Much of the ambient air pollution present in urban areas is caused by combustion of fossil fuels. In contrast, the primary source of pollution in rural areas is often biomass smoke such as woodsmoke. Much is known about the specific health effects of urban particulate matter, however not much is known about the effects of woodsmoke particulate matter. Exposures to biomass smoke ranges from acute, in the case of seasonal woodland fires, to chronic, in the use of biomass fuel for household cooking. Biomass burning is thought to comprise 10% of the total energy consumption worldwide, making any adverse effects of concern to those with daily or occupational exposure (Naeher et. al., 2007). Many epidemiological studies have shown a correlation between woodsmoke exposure and respiratory infections, especially in children and individuals with preexisting conditions (Naeher et. al., 2007). The particulates that are produced by the burning of wood are of particular interest when discussing the health effects of exposure because woodsmoke contains a significant amount of $PM_{2.5}$. $PM_{2.5}$ is particulate matter less than 2.5 µm in diameter and penetrates deep into the alveolar spaces of the lungs, in contrast to the coarse fraction of PM, which does not penetrate as deeply. The first line of defense for fighting off infections in the lung is the alveolar macrophage. The macrophage is a specialized immune cell that is responsible for responding to pathogens and regulating a localized immune response to the pathogen (Mosser, 2003). It has been shown previously that the macrophage population in the lung is responsible for clearing and responding to particulates such as silica and asbestos (Holian et.al., 1997). The macrophage population in the lung has also been shown to have decreased pathogen clearing ability after exposure to particulates (Sun, Metzger, 2007;Yin et.al.,2006; Zhou, Kobzik, 2006). This study will test the hypothesis that decreased bacterial clearance post woodsmoke exposure is due to down-regulated alveolar macrophage function.

Materials and Methods

Intranasal Instillations

- Mice were anaesthetized with ketamine and instilled intranasally with 25µl of 5 mg/ml $PM_{2.5}$, diesel particulates (DEP), or woodsmoke particulates (WS) in PBS
- Particles were sonicated before use

Woodsmoke Exposure

- Locally-derived wood was burned in a non-EPA certified woodstove (50g bundles added every 5-10 minutes) and vented through aluminum tubing to an exposure chamber
- PM levels were monitored during the 2-hour exposure inside the chamber using a TSI DustTrak

Lung Lavages

- Lungs were lavaged with 3-5 ml of sterile PBS, 1 ml at a time
- First lavage fluid was frozen for further analysis

APC assay

- Alveolar macrophages (AMs) were aliquoted to a 96-well plate (1 x 10^5 cells per well) and incubated for three hours with ovalbumin (OVA; 10 mg/ml) at 37°C
- T cells isolated from a D011.10 mouse were co-cultured with AMs at a ratio of 4:1
- AMs and T cells were incubated at 37°C for 48 hours
- Supernatants and T cells were collected for further analysis

Flow Cytometry Analysis

- T cells collected from the APC assay were stained with anti-CD3 and anti-CD25 antibodies and incubated for 20 minutes
- Analysis of florescence was performed on a FACSAria system (BD)

Phagocytosis Assay

- AMs were cultured for 24 hours, incubated with florescent particles,, and then quenched with trypan blue (Vybrant Phagocytosis assay kit, Molecular Probes)

Literature Cited

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Results

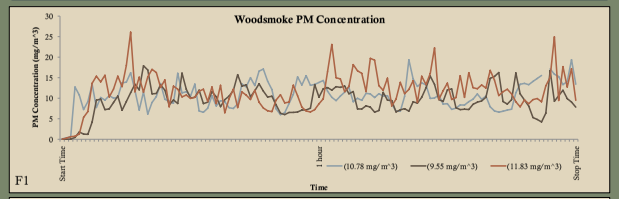
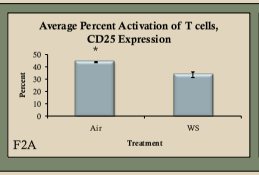


Figure 1: PM levels inside the mouse woodsmoke-exposure chamber. Each exposure is designated by a color and the average PM level shown in parentheses. Measurements were taken once a second and averaged over every minute. The peaks and valleys are representative of a typical indoor exposure to woodsmoke produced by a woodstove.



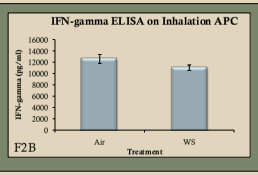


Figure 2: A: Flow analysis of T cell activation from a 2 hour post WS exposure APC assay. Figure shows the average percent activation for each group (n=7) ± the S.E.M. The woodsmoke treated mice showed significantly lower ($p<0.05$) T cell activation than the control mice. **B: IFN-gamma levels in 2 hour post WS exposure APC assay**. Figure shows the average IFN-gamma levels ± the S.E.M. The woodsmoke-exposed mice show a decreased level of IFN-gamma. This data indicates that there is some down regulated alveolar macrophage function 2 hours post exposure.

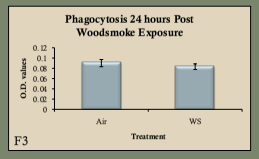


Figure 3: Analysis of AM phagocytic behavior 24 hours post woodsmoke exposure. Figure shows the average optical density values for each group (n=8) ± the S.E.M. There is a slight decrease in phagocytosis in the WS treated cells.

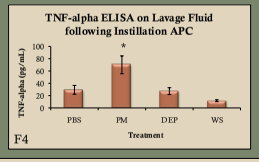


Figure 4: TNF-alpha levels in lavage fluid 24 post particle instillations. Figure shows average TNF-alpha levels for each group (n=6 for DEP & PBS; n=7 for PM & WS) ± the S.E.M. PM-instilled mice had a significantly higher TNF-alpha level than the other treatments. WS-instilled demonstrated the lowest levels.

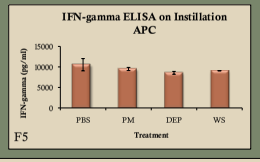


Figure 5: IFN-gamma levels in 24 hour post instillation APC. Figure shows the average IFN-gamma levels for each group (n=12 for WS, n=13 for PBS, n=14 for DEP, n=23 for PM) ± S.E.M. There is a slight decrease in IFN-gamma levels in the WS-instilled mice.

Summary

- There was a significant decrease in T cell activation in woodsmoke exposed mice over the control 2 hours post exposure
- Woodsmoke exposed mice also demonstrated a decreased level of IFN-gamma in the APC supernatants
- 24 hours post exposure to woodsmoke there was a slight decrease in phagocytic activity, however it was not significant
- There were no detectable TGF-beta levels in the lavage fluid of either instillation or inhalation studies (data not shown)
- Instillation of PM2.5 causes an increase in TNF-alpha levels in comparison to the other treatments 24 hours post instillation
- WS instilled mice demonstrated the lowest levels of both TNF-alpha and IFN-gamma 24 hours post instillation

Conclusions

- Inhalation of woodsmoke particles appears to depress macrophage function at both 2 hours post exposure and 24 hours post exposure
- Suppression of macrophage function 2 hours post exposure suggests a direct effect of the particle on the macrophage population
- The results from the instillation and inhalation studies suggest that the bioactive portion of woodsmoke is the particulates
- The instillation model also demonstrated a difference in effects between urban particulate matter and woodsmoke

Further Research

- More research will be conducted to explore the mechanism that is suppressing macrophage function *in vivo* after exposure to woodsmoke
- Further research will be conducted to establish how far post exposure the effects of woodsmoke persist
- There will also be further development in linking the effects of woodsmoke *in vitro* to the effects of instilled particles and inhaled woodsmoke
- Another study may be conducted to explore how instillation of particles effects bacterial clearance
- There is now also speculation that woodsmoke may be more similar in its effects to cigarette smoke than urban PM

Acknowledgments

The project described was supported by grants ES 016247 and RR-017670, and its contents are solely the responsibility of the authors and do not necessarily represent the official views of the NCRR or NIH.