Acute Woodsmoke Exposure Decreases Murine Alveolar Macrophage Activity Via the Non-canonical NF-κB Pathway

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Abstract

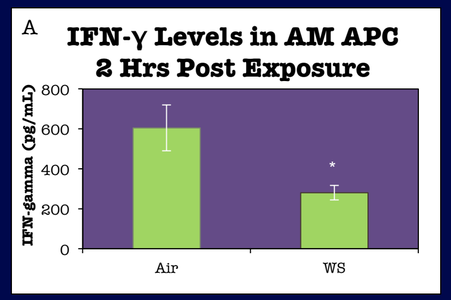
Alveolar macrophages (AM) are the first line of defense in fighting infections in the lung. AM are responsible for clearing particles, such as silica or bacteria, and are key regulatory cells of the immune response in the airways. Recently we observed decreased pathogen clearance following acute exposure to wood smoke (WS). In addition, after exposing mice to WS for 2 hrs, there were no signs of classic inflammation, such as TNF-α, neutrophil influx, and increases in total protein. There were, however, decreases in overall AM function including antigen presentation and bacterial clearance. These effects were observed after exposure to smoke from both EPA-certified and non-certified stoves. This suggests that differences in smoke composition between stoves did not alter the adverse health effects associated with exposure. One potential explanation for these decreases lies in the similarities of WS to cigarette smoke (CS). CS and WS both contain many polycyclic aromatic hydrocarbons (PAH) that are potential ligands for the aryl hydrocarbon receptor (AhR). Recent studies have linked components of CS to activation of the AhR resulting in the activation of the non-canonical (p52: RelB) nuclear factor κB (NF-κB) pathway. The non-canonical pathway is associated with decreases in inflammation and apoptosis, both of which are seen after exposure to WS. In order to determine whether the non-canonical pathway is being activated, AM were analyzed for RelB translocation to the nucleus using immunohistochemistry. Nuclei positive for RelB were exclusively in WS-treated mice. The lack of inflammation, decrease in APC activity, decrease in bacterial clearance, and the nuclear translocation of RelB in WS-exposed AM suggest that acute WS exposure decreases AM function via the non-canonical NF-κB pathway.

Introduction

Many epidemiological studies have shown a correlation between wood smoke (WS) exposure and respiratory infections, especially in children and individuals with preexisting conditions (Naeher et. al., 2007). The first line of defense for fighting off such respiratory infections in the lung is the alveolar macrophage. The macrophages are specialized immune cells that are responsible for responding to pathogens and regulating localized immune responses (Mosser, 2003). It has been previously shown that the lung macrophage population is responsible for clearing and responding to particles including particulate matter (PM), silica, and asbestos. In the case of silica and asbestos, their response is inflammation. (Holian et.al., 1997) Following an acute exposure to WS, we have observed that AM have a decreased ability to clear pathogens in conjunction with a lack of inflammation, which is in contrast to particulates such as silica and asbestos. New generation, EPA-certified stoves are hoped to help qualm some of the potentially adverse health effects caused by WS-derived PM by utilizing a more efficient combustion system and releasing lower levels of PM. Recent evidence suggests that in addition to lower PM levels, the chemistry of the WS from EPA-certified stoves is altered (Ward, unpublished). However, we observed some of the same effects regardless of whether the stove supplying the WS was EPA-certified or not.

The lack of inflammation indicates that the mechanism of action for WS differs from that of many other particles. One type of particulate, cigarette smoke (CS), does however have some important similarities to WS. CS and WS have both been shown to decrease the activity of pulmonary macrophages (Thomas, et.al., 1978). CS and WS also contain many polycyclic aromatic hydrocarbons (PAH) which are known ligands of the aryl hydrocarbon receptor (AhR) (Platzer et.al., 2009). Recently CS smoke has been shown to activate the non-canonical nuclear factor kappaB (NF-κB) via the AhR (Baglole et.al., 2008). Since both CS and WS contain AhR ligands, a logical assumption is that WS should also activate the non-canonical NF-κB pathway. The NF-κB pathway is a quick-acting pathway that mediates many immune responses (Hiscott et.al., 2001). The non-canonical NF-κB pathway involves a heterodimer of two transcription factors, RelB and p52, which, when activated, usually results in immune suppression. The canonical NF-κB pathway involves the heterodimer p65/p50, which, when activated, usually plays a role in immune activation. In both pathways, activation results in translocation of the appropriate transcription heterodimer into the nucleus of the cell. (Beinke, Ley, 2004) Thus activation of the non-canonical NF-κB pathway via the AhR may be key in the mechanism of decreased bacterial clearance caused by WS. This study will be testing the hypothesis that decreased bacterial clearance post WS exposure is due to down-regulated alveolar macrophage function; and that decreased function is due to activating of the non-canonical NF-κB pathway.

Results



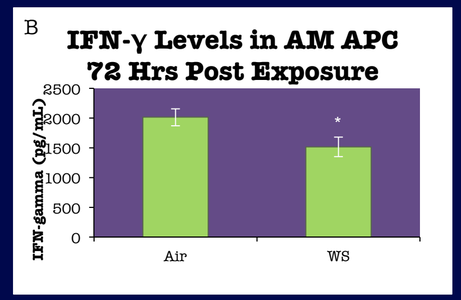


Figure 1: IFN-gamma levels present in APC assay supernatants. Figures show the average IFN-gamma level (pg/mL) ± the SEM (n=5) for each treatment group. Co-cultured AM 2 hour post WS exposure (A) show a significant decrease ($p<0.05$) in IFN-gamma as compared to co-cultured AM from air control mice , AM 72 hour post exposure (B) show a significant difference ($p<0.05$). The decrease in IFN-gamma levels helps support the conclusion that lung macrophage function is decreased post WS exposure.

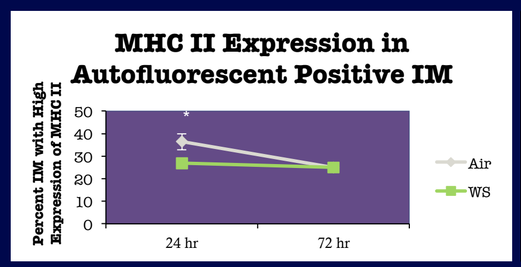
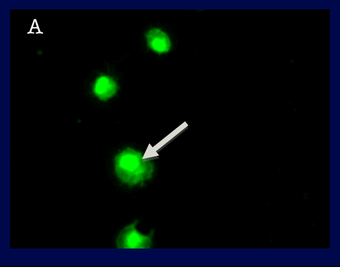


Figure 2: Flow analysis of IM 24 and 72 hours post exposure. Figure shows the average percentage of MHC II positive macrophages ± the SEM (n=5) for each treatment group. At 24 hours post exposure there was a significant difference in MHC II expression ($p<0.05$) between WS and air treated mice. This decrease indicates a decrease in IM activation following acute WS exposure.



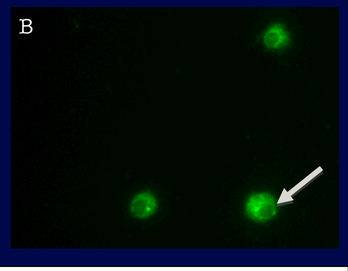
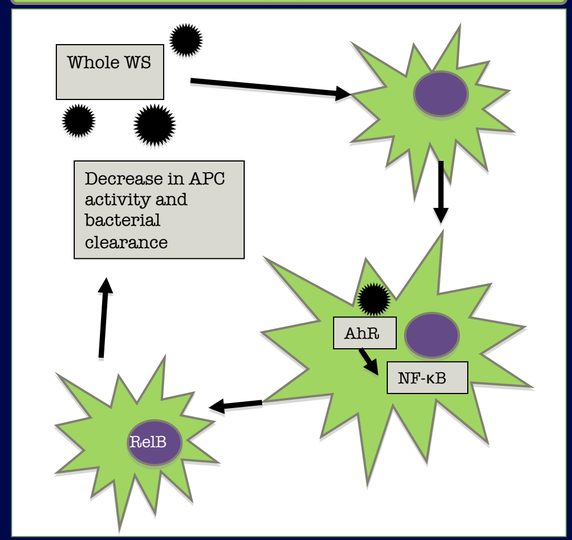


Figure 3: Cellular Fluorescent staining of RelB in WS versus Air exposed AM. WS exposed AM show a striking nuclear staining pattern , indicated by the arrow, (A,) that is shown to be absent in the air control samples (B). Nuclear staining of RelB in WS exposed mice indicates that RelB has been activated via the non-canonical NF-kappaB pathway.

Working Hypothesis



Summary

- Decreases in IFN-gamma levels in APC supernatants indicates decreased activity of AM following WS exposure from both EPA-certified and non-certified woodstoves
- No differences in LDH levels were detected between Air and WS exposed samples in the APC assay, indicating that the decrease in IFN-gamma levels was not due to cell death
- No TNF-alpha was detected in the lavage fluid of either WS (from an EPA-certified stove) or Air control mice , demonstrating that there was no inflammation post exposure
- Significant decreases in MHC II expression in autofluorescent positive IM also indicate a decreased level of activation following acute exposure to WS
- Current data indicate no differences in health effects between certified and non-certified woodstoves at the same PM-level of exposure; more experiments must be conducted to evaluate whether or not EPA-certified woodstove smoke is less harmful compared to non-certified woodstove smoke at the reduced PM levels
- Nuclei positive for RelB were found exclusively in AM exposed to WS, indicating that the non-canonical NF-kappaB pathway is indeed being activated following acute wood smoke exposure

Further Research

- Further experiments will be conducted to confirm that smoke produced by the EPA-certified stove does indeed have the same effects on AM that the non-certified stove does; experiments will include inoculations and evaluations of bacterial clearance, and evaluations of the phagocytic ability of AM post exposure
- Experiments will be designed to block RelB activity in an attempt to eliminate the decreases in overall activity of AM post exposure to WS
- Once the link between the non-canonical NF-kappaB pathway and WS exposure has been well established, further experiments will be needed to link the AhR to activation of that pathway

Literature Cited

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Materials and Methods

Woodsmoke Exposure
Locally-derived wood was burned (50g bundles, every 5-10 minutes) and vented through aluminum tubing to an exposure chamber. PM levels were monitored in real time using a TSI DustTrak.

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.

APC assay
AM were co-cultured with transgenic (OVA-specific, DO11.10) T cells in a 96-well plate at a ratio of 4:1 (TC:AM), and incubated at 37°C for 48 hours. Supernatants were collected for additional analyses.

Cytospin Analysis
AM were cytocentrifuged (1500 rpm for 5 minutes), fixed in 4% paraformaldehyde, and then air-dried overnight prior to immunocytochemistry

Immunocytochemistry
After blocking for 1 hr at room temp, cytospin slides were incubated with anti-RelB antibodies overnight at 4 °C. Slides were incubated with secondary antibody (AlexaFluor 488) for 1 hr at room temp, then coverslipped and viewed using a Nikon Eclipse E800 microscope and a Nuance CRI camera

Flow Cytometry Analysis
Leukocytes collected from gradient centrifugation were stained for flow cytometry analysis. T cells populations were assessed by staining with anti-CD3, anti-CD4, anti-CD8, and anti-CD25; while macrophage populations were assessed by staining with anti-F4/80, anti-CD11b, anti-CD11c, and anti-MHC II antibodies.
analysis of florescence was performed on a FACSAria system (BD)

Acknowledgments

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