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

Emily X. Kobos, Virginia Porter, Tony Ward, and Christopher T. Migliaccio
Department of Pharmaceutical Sciences, Center for Environmental Health Sciences
The University of Montana, Missoula, MT

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, and bacteria, and are key regulatory targets 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 levels. They 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 affect 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 polyaromatic hydrocarbons (PAHs) that are potential ligands for the aryl hydrocarbon receptor (AhR). Recent studies have linked components of WS to activation of the non-canonical (p52:RelB) NF-kB 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. Nuclear 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-kB 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 (Makker et al., 2007). The first line of defense for fighting off such respiratory infections in the lung is the alveolar macrophage. The macrophages are specialised immune cells that are responsible for fighting off pathogens and regulating localized immune responses (Mossner, 2000). 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 calm 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 smoke support was an EPA-certified or not. The lack of inflammation indicates that the mechanism of action for WS differs from that of many other pollutants. One type of particulate, black carbon (BC), 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., 1976). CS and WS also contain many polyaromatic hydrocarbons (PAHs) 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) (Platzer et al., 2009). Recent studies have linked components of CS to activation of the AhR resulting in the activation of the non-canonical (p52:RelB) nuclear factor kappaB (NF-κB) pathway.

Materials and Methods

Woodsmoke Exposure

Lentivirally delivered woodsmoke was burned (350 ft, headwind, every 5-10 minutes) and vented through aluminium 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.

Flow Cytometry Analysis

Lung macrophages from mice were stained with anti-CD3, anti-CD4, anti-CD8 and anti-CD25, and then coverslipped and viewed using a Nikon Eclipse E800 microscope and a Nuance CRI camera.

Results

Figure 1: IFN-γ levels present in APC assay supernatants. Figures show the average IFN-γ levels (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-γ 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-γ levels helps support the conclusion that lung macrophage function has decreased post WS exposure.

Figure 2: Flow analysis of IM 24 and 72 hours post exposure. Figures 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 decrease 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 indicate a striking nuclear staining pattern, indicated by the arrow, (A.) that is known to be absent in the air control samples (B). Nuclear staining of RelB in WS-exposed AM indicates that RelB has been activated via the non-canonical NF-kB pathway.

Discussion

The decrease in APC activity 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 affect 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 polyaromatic hydrocarbons (PAHs) that are potential ligands for the aryl hydrocarbon receptor (AhR). Recent studies have linked components of WS to activation of the non-canonical (p52:RelB) NF-kB 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. Nuclear 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-kB pathway.

Conclusion

Decreases in IFN-γ levels in APC supernatants indicate decreased activity of AM following WS exposure from both EPA-certified and non-certified stoves.

• No differences in LDR levels were detected between mice exposed to WS versus Air and WS exposed supernatants in the APC assay, indicating that the decrease in IFN-γ levels was not due to cell death.

• No TNF-α was detected in the lavage fluid of either WS-exposed or Air-exposed mice, demonstrating that there was no inflammation post exposure.

• Recent decreases in APC expression in air-exposed positive IM also indicate a decreased level of activation following acute exposure to WS.

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 inclusions 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.

Acknowledgments

The project described was supported by grants ESR25 016247 and ESR25 05527 from the European Community’s Seventh Framework Program (FP7/2007-2013) under grant agreement no. 283390. The content is solely the responsibility of the authors and does not necessarily represent the official views of the Agency or the European Community.

Literature Cited


Working Hypothesis

• Decreases in IFN-γ levels in APC supernatants indicate decreased activity of AM following WS exposure from both EPA-certified and non-certified stoves.

• No differences in LDR levels were detected between Air and WS exposed supernatants in the APC assay, indicating that the decrease in IFN-γ levels was not due to cell death.

• No TNF-α was detected in the lavage fluid of either WS-exposed or Air-exposed mice, demonstrating that there was no inflammation post exposure.

• Recent decreases in APC expression in air-exposed positive IM also indicate a decreased level of activation following acute exposure to WS.

• No differences in LDR levels were detected between Air and WS exposed supernatants in the APC assay, indicating that the decrease in IFN-γ levels was not due to cell death.

• No TNF-α was detected in the lavage fluid of either WS-exposed or Air-exposed mice, demonstrating that there was no inflammation post exposure.

• Recent decreases in APC expression in air-exposed positive IM also indicate a decreased level of activation following acute exposure to WS.

• No differences in LDR levels were detected between Air and WS exposed supernatants in the APC assay, indicating that the decrease in IFN-γ levels was not due to cell death.

• No TNF-α was detected in the lavage fluid of either WS-exposed or Air-exposed mice, demonstrating that there was no inflammation post exposure.

• Recent decreases in APC expression in air-exposed positive IM also indicate a decreased level of activation following acute exposure to WS.

• No differences in LDR levels were detected between Air and WS exposed supernatants in the APC assay, indicating that the decrease in IFN-γ levels was not due to cell death.

• No TNF-α was detected in the lavage fluid of either WS-exposed or Air-exposed mice, demonstrating that there was no inflammation post exposure.