Exposure to wildfire particulate during infancy influences adolescent innate immune responses and lung growth

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Abstract

Rationale: The long-term health effects of acute wildfire smoke exposure are not known.

Objective: To determine if exposure to wildfire smoke during infancy is associated with alterations in innate immunity and lung function.

Methods: We evaluated a cohort of adolescent outdoor-housed rhesus macaque monkeys that were exposed as infants to ambient wood smoke from a series of Northern California wildfires in the summer of 2008. A second cohort of animals born in 2009 served as a control group. Peripheral blood mononuclear cells (PBMC) and pulmonary function measures were obtained when animals were 3 years of age. PBMC were cultured with either lipopolysaccharide (LPS) or flagellin, followed by measurement of secreted IL-8/IL-6 protein or toll-like receptor (TLR) pathway mRNA analysis.

***Measurements and Main Results*:** LPS or flagellin-induced IL-8 protein synthesis was significantly reduced in PBMC cultures derived from female animals with a prior history of wildfire smoke exposure. In contrast, LPS or flagellin-induced IL-6 protein synthesis was significantly reduced in PBMC cultures derived from male animals with a prior history of wildfire smoke exposure. Baseline and TLR ligand-induced expression of the transcription factor RelB was globally modulated in PBMC from wildfire smoke-exposed female monkeys, with additional TLR pathway genes affected in a ligand-dependent manner. Wildfire smoke-exposed animals displayed a reduction in lung volume relative to control animals.

Conclusion: Our findings suggest that ambient wildfire smoke exposure during infancy can alter innate immunity and lung function in a sex-dependent fashion that is maintained into adolescence.

The Environment, the Immune System, and Lung Development

- A large body of evidence shows associations between wildfire smoke exposure and increases in hospitalization, emergency room visits, doctor's visits for respiratory illness or heart disease, and asthma inhaler use.
- Children are particularly susceptible to the negative effects of air pollution because their undeveloped lungs are more vulnerable to injury. No data have been published specifically on wildfire smoke particulate.
- Current studies describing the effects of air pollution on the immune system are biased toward acute effects immediately following experimental exposures. Long-term sequelae are rarely described.
- In June 2008, an outbreak of wildfires in Northern California caused a spike in particulate air pollution levels. A cohort of macaques studied here were 3 months old and housed outside at the time of the wildfires. Differences in lung volume and immune function compared to unexposed animals were still apparent 3 years later.

The 2008 wildfires caused a large peak in air particulates in Yolo County

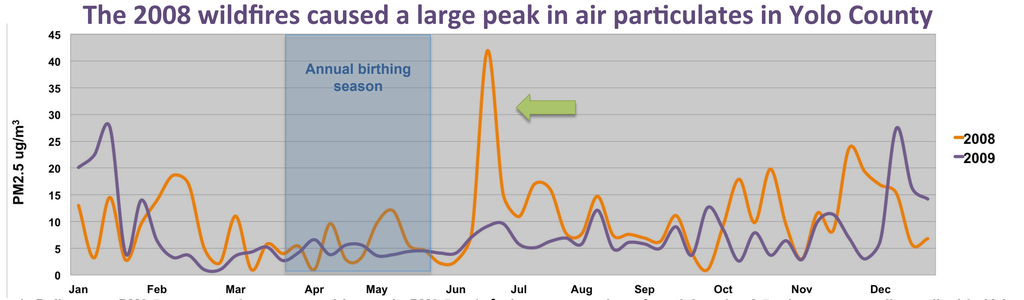

Fig. 1: Daily mean PM2.5 concentrations averaged by week. PM2.5 ug/m^3, the concentration of particles size 2.5 microns or smaller, spiked in Yolo County in June 2008 as a result of the wildfires. The population of Yolo and the primates used in this study were exposed to particulate concentrations exceeding the Federal Ambient Air Quality Standard for 6 consecutive days.

Wildfire-exposed animals exhibit altered innate immune responses 3 years later

Peripheral blood mononuclear cells produce less cytokine in response to bacterial products

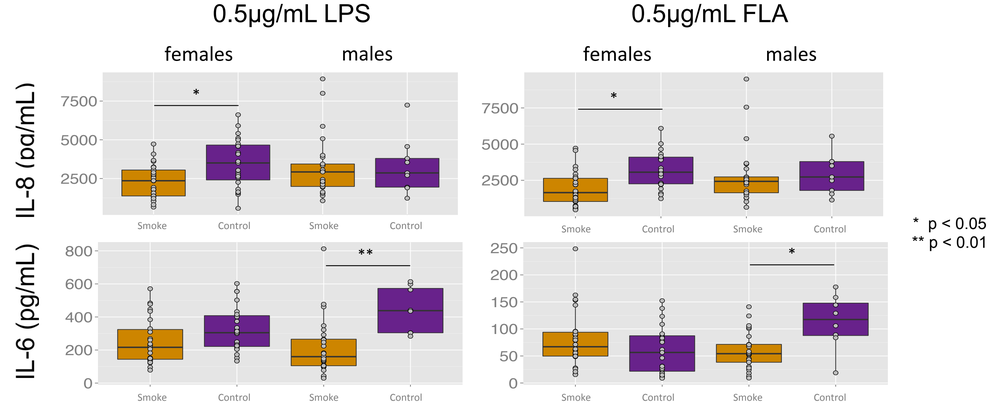

Fig. 2: Peripheral blood mononuclear cells (a group of circulating immune cells that includes T cells, B cells, and monocytes) from females exposed to wildfire smoke produce less IL-8 in response to the bacterial products lipopolysaccharide (LPS) and flagellin (FLA) *in vitro*, while PBMC from males produe less IL-6. IL-8 and IL-6 are important molecules in the innate immune response because they recruit neutrophils, promote phagocytosis, and are associated with inflammation.

Reductions in lung volumes associated with wildfire smoke exposure

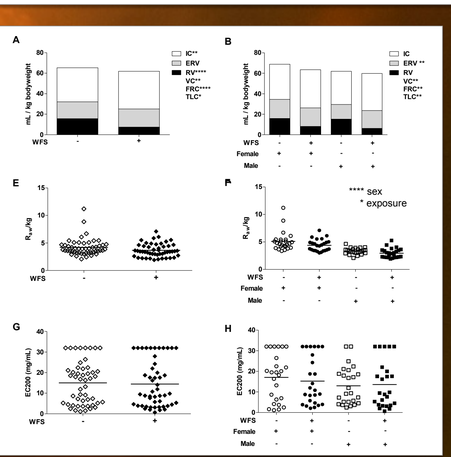

Figure 3. Pulmonary function measures in adolescent monkeys. *(A)* Lung volumes standardized to body weight and expressed as milliliters per kilogram of bodyweight in unexposed (-) or wildfire smoke exposed (+) monkeys at 3 years old. Statistics displayed for wildfire-smoke-exposed vs. unexposed, t-test *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001. *(B)* Lung volumes in females vs males. Statistics displayed for females vs. males, two-way ANOVA with exposure **p<0.01. *(C)* Airways resistance (R_{aw}), normalized by body weight. *(D)* Airways resistance (R_{aw}), normalized by body weight in females and males, two-way ANOVA sex vs. WFS exposure *p<0.05; ****p<0.0001. *(E)* Airways hyperresponsiveness to methocholine challenge (EC200), expressed in concentration of methocholine needed to achieve doubling of baseline airways resistance, with a maximum dose of 32mg/mL. *(F)* EC200 in females vs. males.

Altered TLR signaling pathway

	LPS			FLA		
WFS	-	+		-	+	
CCL2	3.27	1.46		1.85	0.70	
CHUK	1.18	1.31		0.89	1.18	*
FADD-like	0.59	0.98	*	0.62	0.74	
FOS	0.98	0.60	*	0.81	0.53	
IL12B	0.37	0.14		0.72	0.83	
MAL	0.10	0.17		0.05	0.15	
MAP4K4	0.14	0.27		0.10	0.24	
MYD88	0.59	0.88	**	0.51	0.57	
REL	8.53	10.35		7.69	9.75	
RELB	3.81	4.72	***	3.42	4.36	**
TLR9	0.08	0.03		0.06	0.03	

Table 1. RT-PCR array analysis on 84 mRNA associated with TLR signaling pathways. Assessment was focused on cultures generated from a subset of the primary cohort of wildfire smoke-exposed (n=6) and control (n=6) female monkeys. Profiles of genes significantly affected or differentially expressed in PBMC cultured for 3 hours with LPS or flagellin versus media controls are summarized. *p<0.05; **p<0.01; ***p<0.0001 Specific polycyclic polyaromatic hydrocarbons found in wood smoke activate *RelB*, a non-canonical NFkappaB molecule, suggesting a role for the RelB/aryl hydrocarbon receptor complex in mediating the effects of wood smoke exposure.

Summary

- Animals born in 2008 were exposed to a peak in particulate material during development, while animals born in 2009 were not.
- Different sex-specific changes in the immune response were seen in males and females.
- PBMCs taken from wildfire-exposed animals **3 years after exposure** exhibited significantly reduced IL-8 responses to bacterial components.
- PBMCs taken from wildfire-exposed males **3 years after exposure** exhibited significantly reduced IL-6 responses to bacterial components.
- Wildfire smoke exposure was associated with reduced lung volumes in both males and females without changes in airways resistance or airway hyperresponsiveness.
- There is increased RelB expression in PBMC from wildfire smoke-exposed animals, suggesting that transcription factor regulation might be a target for air pollutant exposures

Conclusions

We conclude from this study in rhesus monkeys that exposure to wildfire PM during the postnatal period of development can result in dysfunction of innate immune responses towards infectious agents and lung function decrements at adolescence. Our data suggests that children who underwent similar exposures to wildfire smoke $PM_{2.5}$ in 2008 as infants/toddlers may exhibit a similar health profile, with the caveat that unlike local children, the animals in this study were housed completely out of doors for the duration of the study. Furthermore, because rhesus macaques develop at a faster rate than children, the relative impact of a short environmental exposure may differ. Nevertheless, the ability to quantitatively assess exposure impact on immune parameters using a non-invasive peripheral blood assay also makes our approach developed for rhesus monkeys feasible for a large population-based human study.

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