Online Data Supplement

Wood smoke exposure alters human inflammatory responses to viral infection in a sex-specific manner: A Randomized, Placebo-Controlled Study

Meghan E. Rebuli, Adam M. Speen, Elizabeth M. Martin, Kezia A. Addo, Erica A. Pawlak, Ellen Glista-Baker, Carole Robinette, Haibo Zhou, Terry L. Noah, Ilona Jaspers

Supplementary Methods:

Subject Recruitment

Subjects were identified from our internal database as well as self-identification from emails, flyers, and a website.

Inclusion Criteria

Inclusion criteria included normal lung function (as defined by FVC>75% predicted for gender, ethnicity, age, and height; FEV1>75% predicted; FEV1/FVC ratio >0.70 and <0.90), oxygen saturation of >94%, normal blood pressure (systolic between 140-90, diastolic between 90-60 mm Hg), Symptom Score no greater than 6 (out of 39), body temperature of no greater than 37.8°C (measured orally).

Exclusion Criteria

Exclusion criteria included a history of significant chronic illness (including diabetes, autoimmune diseases, immunodeficiency, known ischemic heart disease, chronic respiratory disease), positive pregnancy test within 48 hours of the time of challenge, use of any inhaled substance (for medical or recreational purposes; nonsmokers must have been abstinent from smoking for the prior 12 months, receipt of LAIV in the current season, history of allergy to eggs, acute (non-chronic) medical conditions (including but not limited to: pneumonia or bronchitis requiring antibiotics, febrile illnesses, flu-like symptoms), unspecified illnesses, expected exposure of subject to immunocompromised individuals for 3 weeks following inoculation.

WSP Generation

WSPs were generated according to the protocol previously described (5). Briefly, red oak wood, harvested in 2007 and used from 2014-2015 in this study, was heated on an electric heating element to generate wood smoke. The smoke was extracted from the chimney and injected into the chamber which was maintained at 22°C and 40% humidity. Smoke concentration was maintained and measured using a tapered element oscillating microbalance (TEOM; Thermo Fisher Scientific, Franklin, Massachusetts, USA) and a Model DR-4000 DataRAM™ (Thermo Fisher Scientific). The target concentration for the TEOM was set to be 500 $\mu g/cm^3$. To quantify subject exposure conditions, a DataRAM™ aerosol monitor, a TEOM, a Condensation Particle Counter (CPC) aerosol monitor, and a Versatile Air Pollution Sampler (VAPS), configured with two Teflon® and one Nuclepore® filters sampled inlet air to the chamber. Particle mass and size were maintained and verified with a Model 3022A CPC (TSI, Shoreview, Minnesota, USA), a Model 3080L SMPS (TSI), and a versatile air pollution sampler (VAPS; URG, Chapel Hill, North Carolina, USA). A Scanning Mobility Particle Sizer™ (SMPS™) also sampled the inlet air to verify that the particle size distribution did not vary significantly over the course of the study. In addition, the Pollutant Control System (PCS) monitored critical operating parameters (temperature flow, relative humidity, background CO, and total hydrocarbon concentrations) in the chambers. The DataRAM™ was used as a secondary monitor. As a safety precaution, the system was programmed to automatically shut down if the concentration exceeded 1200 $\mu g/cm^3$ or 40 ppm CO. A summary of particulate and pollutant gas exposure amounts is reported in Table E1. Additional information on the characterization

of particulates and gases from red oak smoke can be found in studies previously published (5, 61).

Data Sharing Statement

Individual participant data including online dictionaries will not be made available. Data that underlie the results reported in this article after de-identification (text, tables, figures) will be available in GEO (Figure 3 and 4 and Tables E2-E5) and upon request by those defined below. Study protocol, statistical analysis plan, analytic code will also be available upon request by those defined below. Data will be available starting 6 months following publication and ending 18 months following publication. Data will be shared with researchers who provide a methodologically sound proposal for analyses to achieve aims in the approved proposal. Proposals should be directed to the corresponding author. To gain access, data requestors will need to sign a data access agreement.

Supplementary Tables:

Table E1: Particulate and Pollutant Gas Exposure Summary

	Particulate	Gas				
Exposure	**$PM_{2.5}$**	**CO**	**Total Hydrocarbon**	**O_3**	**NO_x**	**SO_2**
FA (n=19)	Max = 3.1 $\mu g/m^3$	0.1 ± 0.1 ppm	1.9 ± 0.1 ppm	0 ppm	0 ppm	0 ppm
WSP (n=20)	488 ± 22 $\mu g/m^3$	2.3 ± 0.6 ppm	3.9 ± 0.4 ppm	0 ppm	0 ppm	0 ppm

Table E1: Particulate and Pollutant Gas Exposure Summary. Average of particulate and pollutant gas conditions during 2 hour WSP exposure, except for FA $PM_{2.5}$, where maximum exposure is noted. Mean ± SD is reported.

Table E2: Effect Measure Modification – Exposure and Exposure*Sex interaction using AUC

Gene Name	p-value (WSP vs. FA)	p-value (Exposure*Sex)	Fold Change (WSP vs. FA)	Fold Change (M vs. F)
CD4	0.065406	0.003305	1.36983	1.19543
ARG1	0.546377	0.007978	-1.18975	-1.18331
MAFF	0.861526	0.008522	1.03227	1.45941
LTB4R	0.789467	0.008734	1.05639	1.71373
CD55	0.383938	0.014967	1.25122	1.68746
C1S	0.676886	0.016879	-1.14073	-1.38377
TBXA2R	0.699581	0.017826	-1.16967	-1.402
PIK3C2G	0.836377	0.022538	1.08806	-1.47858
TGFBR1	0.920132	0.023716	-1.01056	1.3175
HMGB2	0.714777	0.024107	-1.06897	1.54209
C4A	0.436519	0.030747	-1.29897	-1.48316
C6	0.999092	0.033212	-1.00053	-1.38121
C8A	0.945154	0.033302	-1.03504	-1.42154
MAP3K5	0.655475	0.03358	-1.07004	1.15964
TGFB1	0.344201	0.035437	1.29108	2.33352
MASP1	0.769095	0.035625	1.12889	-1.36591
NOD2	0.34118	0.036881	-1.28437	1.01632
TNFSF14	0.713696	0.040306	-1.15059	1.09137
FASLG	0.34914	0.041163	1.35263	-1.39114
HDAC4	0.998504	0.041418	1.00034	1.24218
MAP2K6	0.987699	0.041807	1.00296	-1.09873
CCL16	0.763436	0.042055	-1.13348	-1.52762
MYL2	0.513148	0.044467	1.32503	-1.20074
IL21	0.584261	0.048331	1.28682	-1.0572
IL12B	0.325895	0.048969	-1.66355	-2.32097

Table E3: Effect of WSP on response to LAIV using AUC

Male:

Gene Name	p-value(Exposure)	Fold Change (WSP vs. FA)
CD4	0.01385	2.2334
CCR7	0.01713	3.06097
CSF2	0.021554	4.10801
PLCB1	0.022174	2.01468
MASP1	0.026872	4.02212
IL10	0.036279	2.67509
CCR4	0.036974	4.05198
IL9	0.038358	3.76082
CSF1	0.041238	2.43764
IRF5	0.042387	1.67746
IL23R	0.044703	2.95218
FASLG	0.04758	3.76355
C8A	0.049139	4.67001

Female:

Gene Name	p-value(Exposure)	Fold Change (WSP vs. FA)
HMGB2	0.001364	-1.91603
LTB4R	0.001376	-2.06824
DEFA1	0.014651	-3.01252
MAFF	0.015025	-1.82179
CYSLTR1	0.018493	-2.17949
IFIT2	0.021802	-2.76806
DDIT3	0.026489	-2.13474
TLR8	0.027336	-2.4758
MAPK14	0.028024	-1.69729
IL10RB	0.028125	-1.55563
ARG1	0.028729	-2.63883
MAP3K5	0.029296	-1.55263
IL6R	0.038415	-2.65308
HSH2D	0.039313	-1.80766
PPP1R12B	0.040401	-1.94256
AGER	0.042347	-1.74823
RAF1	0.044097	-1.57108

BCL6	0.048679	-1.68466

Table E4: Sex-specific LAIV effects (pre- vs post-inoculation) in FA group.

Male:

	Day 0 vs. Day 1			Day 0 vs. Day 2	
Gene Name	p-value (LAIV)	Fold Change (Pre- vs Post-)	**Gene Name**	p-value (LAIV)	Fold Change (Pre- vs Post-)
TREM2	0.007813	2.04916038	*CCL8*	0.007813	37.41135458
CXCL1	0.007813	1.82940651	*IL1A*	0.007813	3.154977969
FLT1	0.007813	1.682213013	*TLR7*	0.007813	3.107220217
CCL2	0.01563	2.820317666	*HLA-DRB1*	0.007813	2.697422642
IL8	0.01563	1.969357808	*CXCL9*	0.01563	25.43810562
DDIT3	0.02344	2.136475954	*CCL2*	0.01563	19.40880389
MRC1	0.02344	1.893308702	*HLA-DRA*	0.01563	3.037885422
IL1A	0.03906	2.605211393	*CD40*	0.01563	2.828447025
DEFA1	0.03906	1.823248736	*CCR2*	0.01563	2.764976637
MMP9	0.03906	1.645016841	*OAS2*	0.01563	2.022748378
			CXCL10	0.02344	39.03072232
			Infl_B_NA	0.03906	6.505346042
			NOS2	0.03906	3.320672148
			MRC1	0.03906	3.178906456
			IRF3	0.03906	-1.57405723
			HSPB1	0.03906	-1.848761258

Female:

	Day 0 vs. Day 1			Day 0 vs. Day 2	
Gene Name	p-value (LAIV)	Fold Change (Pre- vs Post-)	**Gene Name**	p-value (LAIV)	Fold Change (Pre- vs Post-)
Infl_B_NA	0.003906	9.241010156	*CCL3*	0.003906	51.11179507
CCL23	0.003906	8.016063548	*CCL4*	0.003906	27.64120375
CCL8	0.003906	6.244748105	*CXCL10*	0.003906	20.6824397
CCL7	0.003906	5.945413521	*CCL2*	0.003906	15.3966243
Infl_A_Tex_M 1	0.003906	5.921301288	*CXCL9*	0.003906	7.589648458
IL10	0.003906	5.881293239	*IL1A*	0.003906	6.518482522
CCL2	0.003906	4.925099043	*CCL8*	0.003906	6.446695724
IL12A	0.003906	4.785271906	*CXCR4*	0.003906	5.937900187
CCL3	0.003906	4.659278875	*CSF1*	0.003906	5.722438352
IL3	0.003906	4.124752983	*IL8*	0.003906	4.579654919

PTGS1	0.003906	3.744985862	*IL1RN*	0.003906	3.859965046
CCL22	0.003906	3.404143238	*C3AR1*	0.003906	3.641615968
MYL2	0.003906	3.378943739	*IFIT1*	0.003906	3.219023805
CCL4	0.003906	3.130882703	*IL1RAP*	0.003906	2.972443638
TLR9	0.003906	3.005630534	*TNFAIP3*	0.003906	2.812820594
CCR7	0.003906	2.995848138	*PTGER4*	0.003906	2.792303015
GRB2	0.003906	2.961689133	*IRF7*	0.003906	2.665142036
IL1A	0.003906	2.838743642	*RIPK2*	0.003906	2.544761017
CSF1	0.003906	2.831061418	*TLR2*	0.003906	2.453384271
PTGDR2	0.003906	2.80505909	*MX2*	0.003906	2.437732698
SMAD7	0.003906	2.525189697	*CXCL1*	0.003906	2.392780205
CCR4	0.003906	2.500589929	*HIF1A*	0.003906	2.367771194
C9	0.003906	2.466775395	*TLR4*	0.003906	2.29578065
CRP	0.003906	2.352060815	*TNF*	0.003906	2.262662251
CCL19	0.003906	2.337546417	*OASL*	0.003906	2.250103643
CCL21	0.003906	2.232478883	*CXCR2*	0.003906	2.112985494
IL7	0.003906	1.968441815	*TGFB1*	0.003906	2.089056194
NLRP3	0.003906	1.961513372	*RELB*	0.003906	2.081345794
IFNB1	0.003906	1.572055806	*NFKB1*	0.003906	1.992217883
IFNA1	0.003906	1.555518767	*PRKCB*	0.003906	1.969201836
CSF2	0.007812	5.844004388	*PTGS2*	0.003906	1.836447223
CCL16	0.007812	4.049074241	*MMP9*	0.003906	1.816765413
HSPB2	0.007812	4.007860363	*PGK1*	0.003906	1.54136075
IL12B	0.007812	3.090095132	*PTK2*	0.003906	0.3650952
IL2	0.007812	2.394964306	*Infl_B_HA*	0.007812	9.083687881
PTGER2	0.007812	2.126168291	*IL6*	0.007812	4.970110161
CXCL10	0.01172	10.67338276	*CCR1*	0.007812	3.417586177
IL22	0.01172	4.361853282	*IL1B*	0.007812	3.363155418
LTA	0.01172	2.701166936	*MAFF*	0.007812	3.109968538
DEFA1	0.01172	2.674307978	*CCL7*	0.007812	2.896815066
CYSLTR2	0.01172	2.595014725	*DDIT3*	0.007812	2.767150296
CD86	0.01172	2.35001837	*IL6R*	0.007812	2.318650061
LIMK1	0.01172	1.752905397	*CEBPB*	0.007812	2.290620169
Infl_A_Tex_HA	0.01427	9.93340872	*FLT1*	0.007812	2.144770916
CD55	0.01953	2.082457393	*IL18RAP*	0.007812	2.107655338
GNGT1	0.01953	2.067370605	*IFI44*	0.007812	1.791161703
MEF2C	0.01953	2.001308491	*IL1R1*	0.007812	1.714933354
IL13	0.01953	1.943371445	*Infl_B_NA*	0.01172	33.5156007
AREG	0.01953	1.943120324	*IFIT3*	0.01172	4.68865956
IL21	0.01953	1.88821308	*NLRP3*	0.01172	3.089140361
PTGER4	0.01953	1.735326728	*STAT1*	0.01172	2.844395051

TLR2	0.01953	1.610243036	*TYROBP*	0.01172	2.298562619
CFB	0.01953	0.601634495	*LTB*	0.01172	2.131023484
IL4	0.02734	3.955951735	*STAT3*	0.01172	1.581107521
IL9	0.02734	3.601163503	*RELA*	0.01172	1.53013144
C5	0.02734	3.529136704	*CCL20*	0.01953	3.700744919
C1S	0.02734	2.595762325	*LY96*	0.01953	2.424028501
TLR5	0.02734	2.365599543	*CCL22*	0.01953	2.423419158
HDAC4	0.02734	2.105602563	*IRF1*	0.01953	2.246912296
C4A	0.02734	1.921266989	*CXCR1*	0.01953	2.09639244
CXCL3	0.02734	1.897269868	*CD86*	0.01953	2.00691079
IFNG	0.02734	1.728705651	*ITGB2*	0.01953	1.876625252
IFIT1	0.02734	1.684730433	*NOD2*	0.01953	1.808692879
IL18	0.02734	1.625060076	*MAPKAPK5*	0.01953	0.502761281
NOD2	0.02734	1.563775625	*CXCL2*	0.02734	4.027751908
IL15	0.03906	3.470491365	*IFIT2*	0.02734	3.491921718
ARG1	0.03906	2.797587519	*HLA-DRA*	0.02734	2.000205579
CCL24	0.03906	2.592214441	*HLA-DRB1*	0.02734	1.746071379
PTGIR	0.03906	2.564713532	*NFE2L2*	0.02734	1.616362809
CXCL2	0.03906	2.461393515	*MEF2C*	0.02734	1.591370672
C8A	0.03906	1.912615337	*HMGN1*	0.02734	0.619129142
PRKCA	0.03906	1.854890899	*C2*	0.02734	0.583897024
TGFB1	0.03906	1.776271808	*CCR7*	0.03906	2.07455971
TNF	0.03906	1.730627211	*ALOX5*	0.03906	1.90361503
IL1RN	0.03906	1.715646595	*FOS*	0.03906	1.526512311
MAFF	0.03906	1.596056771	*CFB*	0.03906	0.654154003
OASL	0.03906	1.505086892	*PLA2G4A*	0.03906	0.621616209
CD163	0.03906	1.501528666	*HSPB1*	0.03906	0.588808602
Infl_A_Cal_NA	0.04232	6.128551467			

Table E5: Baseline sex differences in gene expression, Day 0 (no exposure).

Gene Name	p-value (Sex)	Fold Change (M vs. F)
RHOA	0.000203	1.54542
CXCL1	0.000291	3.18367
NOS2	0.000375	-2.49951
PRKCB	0.000557	4.8067
IL8	0.000592	5.6267
RELA	0.000613	1.48478
LY96	0.000661	4.30209
IL1RAP	0.000685	3.85854
HIF1A	0.000862	2.64982
AGER	0.000874	2.4876
TGFB1	0.000899	3.98756
TLR4	0.000972	4.88807
ALOX5	0.001023	4.09038
TLR2	0.001134	3.78333
MX2	0.001265	3.00611
TYROBP	0.001296	4.52392
STAT3	0.001371	1.91787
MMP9	0.001518	3.68616
HRAS	0.001525	-2.22978
PTGS2	0.00162	4.56818
CEBPB	0.001778	3.61829
ITGB2	0.001869	3.92476
IL6R	0.002001	3.66127
BCL6	0.002012	2.15153
CXCR2	0.002057	4.93035
NLRP3	0.002059	3.72431
IRF7	0.002068	2.29229
RELB	0.002077	2.66529
HSH2D	0.002174	2.69799
MAFF	0.002434	2.27959
TNFAIP3	0.002593	3.02043
CCR1	0.002604	3.68728
LTB	0.002923	4.66
CFL1	0.003024	1.58519

CXCR4	0.003193	3.88143
IL18	0.003214	-1.754
IL1RN	0.003257	2.98848
CCR3	0.003342	4.46706
IFIT3	0.003666	2.90235
FOS	0.003712	4.14904
NFE2L2	0.003858	1.7471
PTK2	0.003935	-2.1961
DDIT3	0.003973	3.05621
CXCR1	0.004048	4.51161
MAPK14	0.004212	2.0444
STAT1	0.004343	2.20943
CCL24	0.004409	2.42766
NR3C1	0.00447	1.96393
RAPGEF2	0.00493	1.72072
MYD88	0.005096	1.77671
RPS6KA5	0.005295	2.3037
MAPK1	0.005351	1.96078
IFIT1	0.005426	2.38115
MAP3K7	0.005499	-1.28615
TLR1	0.005654	3.28904
PLA2G4A	0.005931	-1.54779
TLR3	0.005939	-1.8385
MKNK1	0.006556	2.1061
MAPKAPK5	0.007301	-1.75643
MYC	0.00735	-1.85249
HSPB1	0.008022	-1.7798
RIPK2	0.008297	2.19659
HDAC4	0.008451	1.85009
TLR8	0.008492	3.00011
NFKB1	0.008507	1.64848
CD55	0.00913	2.90335
MAP3K9	0.009158	-1.90767
IL18RAP	0.009601	4.79324
IL1B	0.010231	4.16997
C3AR1	0.010454	2.62651
KEAP1	0.01079	-1.58589
HMGB2	0.011177	1.82559
PTGER4	0.011537	1.72997
IL1A	0.011889	3.35446
HMGN1	0.012921	-1.57357

C2	0.014071	-1.89355
TLR6	0.014211	2.96286
DEFA1	0.014775	4.14247
IFIT2	0.016702	2.81224
IRF1	0.016787	1.88011
RAF1	0.016807	1.69921
CCL4	0.016869	8.58631
MEF2C	0.016974	2.11682
LTB4R	0.019573	1.76578
CYSLTR1	0.021013	2.22308
TNF	0.03083	2.61108
GRB2	0.031893	2.06566
CFD	0.037971	3.29263
TRAF2	0.043434	-1.57651
CXCL2	0.048547	1.65995

Table E6: Effect Measure Modification – Protein Exposure*Sex Interaction

Protein Name	p-value (M vs. F)	p-value (WSP vs. FA)	p-value (Exposure*Sex)	Fold Change (WSP vs. FA)	Fold Change (M vs. F)
IL13	0.449398	0.433414	0.044464	1.10541	1.10157
IL5	0.061459	0.710185	0.102629	-1.20371	2.82761
MDC	0.270784	0.731576	0.110874	1.06483	1.22631
IL10	0.10673	0.263933	0.189159	-2.01318	2.92646
IL8	2.78E-05	0.536215	0.199618	1.10633	2.29227
MIP-1α	0.458098	0.769743	0.287585	1.09043	1.2473
IL2	0.181377	0.758284	0.307595	1.10092	1.53604
MIP-1β	0.20502	0.312245	0.369393	1.35942	1.475
INF-γ	0.196202	0.715675	0.418136	1.44358	-4.74387
IL12p70	0.014553	0.686489	0.419449	-1.16367	2.83948
IL12p40	0.196038	0.665094	0.425741	1.13487	1.47175
IL16	0.000914	0.666951	0.457824	1.1414	3.48495
Eotaxin	0.002856	0.323257	0.459396	-1.30144	2.45387
GM-CSF	0.01412	0.903646	0.477808	1.01629	1.41453
VEGF	0.000417	0.581807	0.531825	1.06328	1.55223
Eotaxin-3	0.063634	0.986167	0.542495	1.01026	3.55312
TARC	0.003912	0.449599	0.589954	-1.19557	2.13096
MCP-4	0.044077	0.790822	0.604339	1.07593	1.80252
IL15	0.157089	0.818506	0.617067	1.03417	1.23498

IL7	0.061196	0.779845	0.67837	1.05208	1.42289
IL-1α	0.000644	0.869319	0.709925	1.0275	1.89178
IL1β	0.028658	0.555485	0.832339	-1.16536	1.83038
MCP-1	0.028044	0.140127	0.863453	1.34361	1.57356
TNFα	0.002477	0.827069	0.951545	-1.04298	1.91131