Cigarette smokers have exaggerated alveolar barrier disruption in response to lipopolysaccharide inhalation

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Online Data Supplement:

Methods:

The first cohort was comprised of healthy outpatients enrolled in an elective bronchoscopy study at the University of Colorado.[1] Subjects underwent bronchoscopy with BAL. The bronchoscope was wedged into a subsegment of either the right middle lobe or the lingula. Three to four 50 mL aliquots of 0.9% saline were then instilled and aspirated with low suction. The first aspirated aliquot was discarded, while the second and subsequent aliquots were combined and used in experiments as representative of the distal airspaces. BAL fluid was immediately centrifuged (900 g, 5 min) after collection to separate cellular and acellular components. Specimens were aliquoted and stored at -80°C.

The second cohort consisted of healthy non-alcoholic volunteers enrolled in a study at Queen's University, Belfast, United Kingdom.[2] Subjects were randomized to either simvastatin or placebo for 4 days and then exposed to 50 μg inhaled LPS (Escherichia coli serotype O26:B6; Sigma Chemicals, Poole, Dorset, UK). LPS was dissolved in sterile 0.9% saline and inhaled via an automatic inhalation–synchronized dosimeter nebuliser (Spira, Hameenlinna, Finland). This technique delivers particles of a mass median aerodynamic

diameter (MMAD) of 10 μm as described previously.[3] Each subject performed five successive inhalations of the LPS solution (1.25 mg/mL) through a mouthpiece with a nose clip in place, for a total inhaled dose of 50 μg. The bronchoscope was wedged into a subsegment of the right middle lobe, followed by three 60 ml aliquots of 0.9% saline, which was aspirated immediately with low suction. BAL fluid was immediately placed on ice and transferred to the laboratory for processing. BAL fluid was centrifuged (900g, 5 minutes) and the supernatant was removed and stored at - 80^{o}C for subsequent analysis.

Table S1: Bronchoalveolar lavage biomarkers of lung injury and inflammation in unstimulated subjects and after LPS inhalation

Biomarker	Unstimulated – Colorado Cohort			Post-LPS – Belfast Cohort		
	Nonsmokers (n=10) (Median, IQR)	Smokers (n = 10) (Median, IQR)	P-value*	Nonsmokers (n = 21) (Median, IQR)	Smokers (n = 9) (Median, IQR)	P-value*
Neutrophils (%)	1 (0 – 2)	1 (0 – 2)	0.79	31 (24 – 48)	42 (41 – 59)	0.02
Neutrophils (x 10^5 cells)	1 (1 – 2)	4 (1 – 7)	0.14	3 (2 – 8)	9 (6 – 16)	0.02
Total Protein (μg/mL)	72 (52 – 176)	81 (38 – 101)	0.41	208 (177 – 264)	274 (246 – 326)	0.04
VEGF (pg/mL)	294 (190 – 400)	38 (20 – 61)	0.001	219 (167 – 308)	1 (1 – 7)	<0.0001
sVEGFr-1 (pg/mL)	0 (0 – 0)	1 (0 – 13)	0.08	31 (19 – 34)	99 (84 – 129)	0.0001
IL-1β (pg/mL)	0 (0 – 0)	1 (0 – 1)	0.007	37 (25 – 58)	108 (52 – 136)	0.002
IL-8 (pg/mL)	n/a	n/a	n/a	349 (219 – 467)	346 (287 – 415)	0.57
SP-D (ng/mL)	413 (373 – 447)	331 (203 – 477)	0.82	1347 (675 – 3104)	724 (483 – 737)	0.02

* p value from Mann Whitney U test

Table S2: Plasma biomarkers of lung injury and inflammation in unstimulated subjects and after LPS inhalation

Biomarker	Unstimulated – Belfast Cohort			Post-LPS – Belfast Cohort		
	Nonsmokers (n = 21) (Median, IQR)	Smokers (n = 9) (Median, IQR)	P-value*	Nonsmokers (n = 21) (Median, IQR)	Smokers (n = 9) (Median, IQR)	P-value*
MMP-8 (ng/mL)	1 (1 – 2)	1 (1 – 2)	0.61	6 (4 – 10)	11 (9 – 22)	0.006
IL-1β (pg/mL)	0 (0 – 0)	0 (0 – 0)	1.0	0 (0 – 0)	0 (0 – 0)	0.23
SP-D (ng/mL)	104 (66 – 124)	108 (76 – 136)	0.54	96 (69 – 128)	130 (114 – 201)	0.04
IL-8 (pg/mL)	0 (0 – 0)	0 (0 – 0)	1.0	2 (1 – 2)	2 (0 – 3)	0.003

* p value from Mann Whitney U test

Table S3: Linear regression coefficients of smoking for bronchoalveolar lavage biomarkers of lung injury and inflammation after LPS inhalation (Belfast cohort)

Biomarker	Unadjusted Coefficient	P value	Coefficient Adjusted for Statin	P value
Total Protein* (log units)	0.23	0.07	0.21	0.09
IL-1β (pg/mL)	63	< 0.001	61	< 0.001
Neutrophils (x 10^5 cells)	8	0.02	7	0.03
Neutrophils (%)	19	0.006	19	0.009
SP-D* (log units)	-0.49	0.03	-0.51	0.03
VEGF* (log units)	- 1.9	< 0.001	-1.9	<0.001
sVEGFr-1 (pg/mL)	80	<0.001	77	<0.001

* log transformed

Table S4: Linear regression coefficients of interaction between smoking and LPS for bronchoalveolar lavage biomarkers of lung injury and inflammation

Biomarker	Unadjusted Coefficient For Interaction Between Smoking and LPS	P value for interaction	Coefficient Adjusted for Statin + Age	P value for interaction, Adjusted for Statin + Age
Total Protein* (log units)	0.56	0.04	0.54	0.047
IL-1β (pg/mL)	62	< 0.001	58	0.001
Neutrophils (x 10^5 cells)	5	0.15	5	0.18
Neutrophils (%)	19	0.01	18	0.02
SP-D* (log units)	-0.41	0.13	-0.46	0.09
VEGF* (log units)	- 1.1	< 0.001	-1.1	<0.001
sVEGFr-1 (pg/mL)	77	<0.001	73	<0.001

* log transformed

Table S5: Linear regression coefficients of smoking for plasma biomarkers of lung injury and inflammation after LPS inhalation (Belfast cohort)

Biomarker	Unadjusted Coefficient	P value	Coefficient Adjusted for Statin	P value
MMP-8 (ng/mL)	7431	0.008	6480	0.01
IL-1β (pg/mL)	0	0.27	0	0.30
SP-D (ng/mL)	58	0.049	59	0.05
IL-8 (pg/mL)	1	0.003	1	0.001

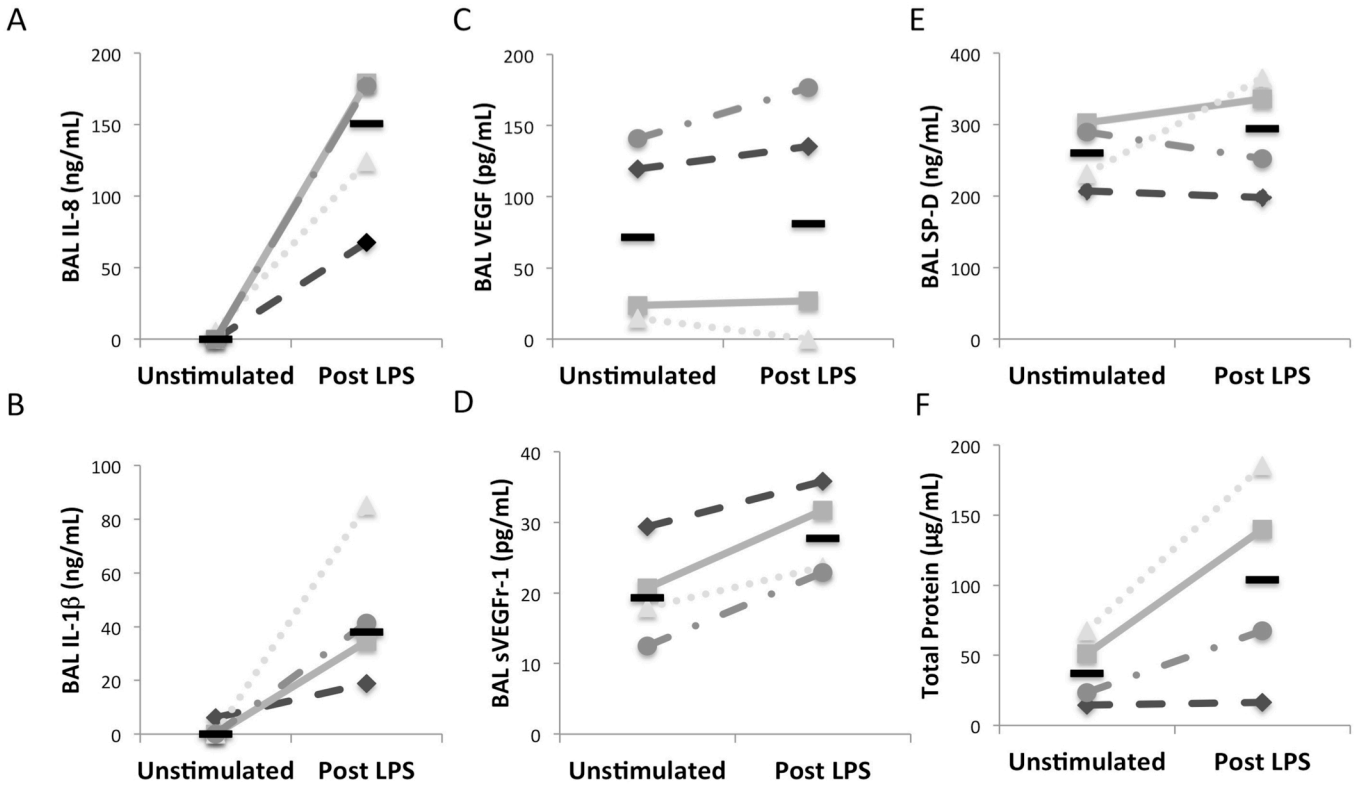
Table S6: Linear regression coefficients of interaction between smoking and LPS for plasma biomarkers of lung injury and inflammation

Biomarker	Unadjusted Coefficient for Interaction between Smoking and LPS	P value for interaction	Coefficient Adjusted for Statin	P value for interaction, adjusted for Statin
MMP-8 (ng/mL)	6329	0.045	6864	0.03
IL-1β (pg/mL)	0	0.26	0	0.26
SP-D (ng/mL)	36	0.31	36	0.31
IL-8 (pg/mL)	1	0.002	1	0.002

Table S7: Bronchoalveolar lavage biomarkers of lung injury and inflammation in 4 additional smoking Belfast subjects before and after LPS inhalation

Biomarker	Pre-LPS (median, IQR)	Post-LPS (median, IQR)
Total Protein (μg/mL)	37 (19 – 59)	104 (42 – 163)
IL-1β (pg/mL)	0 (0 – 3)	38 (27 – 63)
SP-D (ng/mL)	260 (219 – 296)	293 (225 – 351)
VEGF (pg/mL)	72 (19 – 130)	81 (13 – 156)
sVEGFr-1 (pg/mL)	19 (15 – 25)	28 (23 – 34)
IL-8 (ng/mL)	0 (0 – 3)	151 (96 – 178)

Figure S1: BAL biomarkers in 4 additional Belfast smokers that underwent bronchoscopy both before and 6 hours after 50 µg LPS inhalation. A) BAL IL-8, B) BAL IL-1β, C) BAL VEGF, D) BAL sVEGFr-1, E) BAL SP-D, F) BAL Total Protein. Each line connects individual data points from a single subject. — = median value.



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