Mechanisms of Particulate Toxicity: Health Effects in Susceptible Humans

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Introduction

- Particulate air pollution is an issue of global health importance (outdoor and indoor environments; undeveloped and developed regions)
- Epidemiology: Increased particulate levels are associated with increased human cardiorespiratory morbidity and mortality
- Specifically, particulate air pollution has been associated with negative health effects pertaining to respiratory disease, including asthma

Introduction

- Ambient air pollution is a combination of particles and gases
- Particles:
  - Inflammation: mechanical presence or chemical composition
  - Epithelial cells, macrophages, neutrophils
  - Carbon (C); main constituent of many ambient particles
  - Ammonium-nitrate (AN); high in coastal or urban areas
- Gases:
  - Inflammation: oxidation
  - Ozone (O_3); major ambient gaseous toxin

Introduction

• Individuals with asthma could be particularly susceptible to the respiratory health effects of particulate air pollution, due to the airway inflammation and hyper-reactivity components of the disease

• Currently the mechanisms controlling particle-induced airway inflammation in asthma are incompletely understood

Hypothesis

This project was designed to test the hypothesis that:

Airway inflammation would be increased, and spirometric pulmonary function would be decreased as a function of a single particle exposure, and to a larger degree, as a function of a combined particle and ozone exposure and a serial particle exposure, compared to filter air exposure

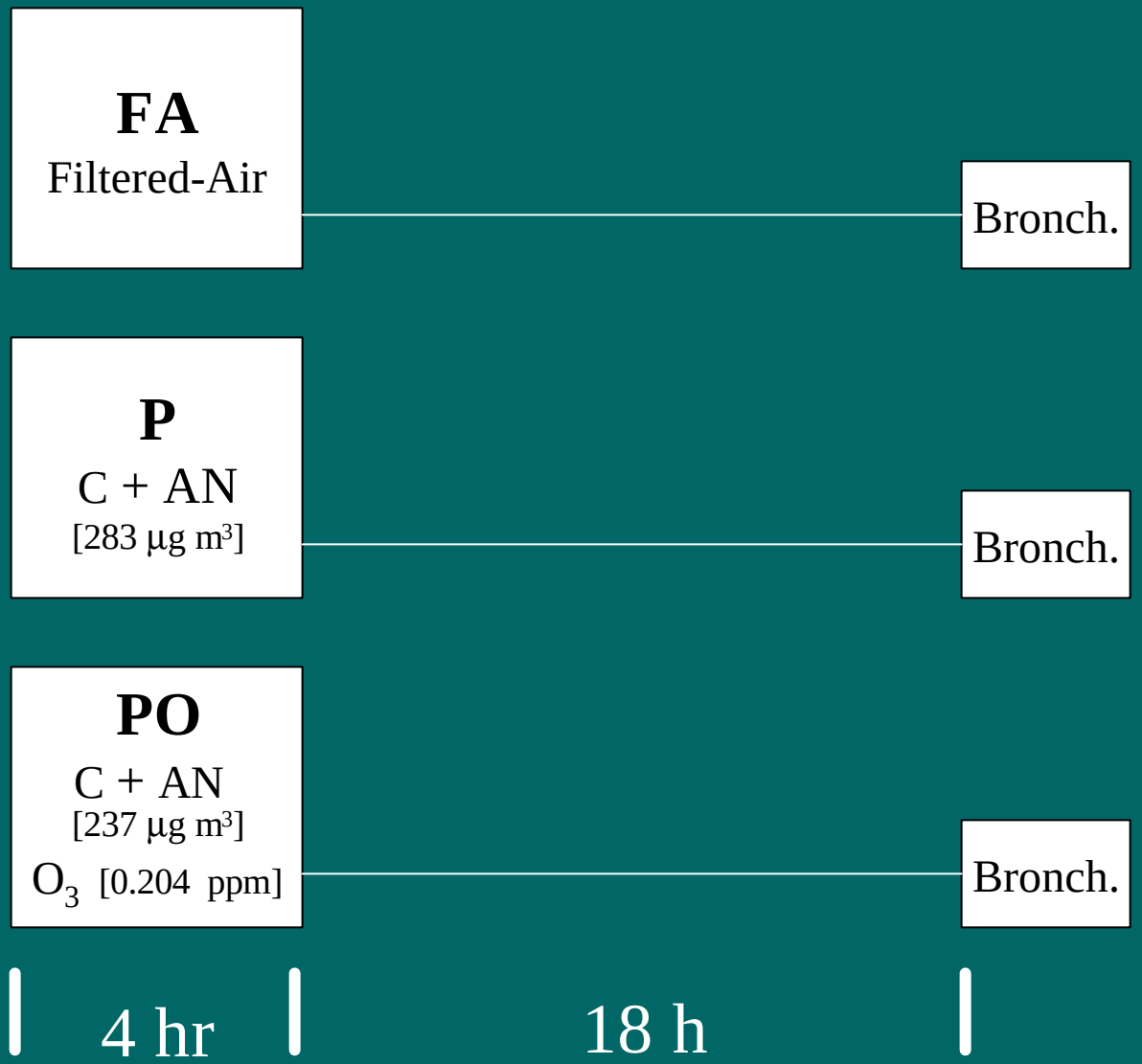
Design

- Two controlled human exposure experiments
- Single-blind, repeated-measures, counter-balanced
- Subjects:
  - Asthma (meth. PC_{20} <10 mg ml); allergic
  - Experiment One: N = 15; 9 females 6 males; Age = 36.8 ± 9.6 yr
  - Experiment Two: N = 10; 6 females 4 males; Age = 38.7 ± 10.2 yr
  - Medications controlled; asthma, allergy, anti-inflammatory

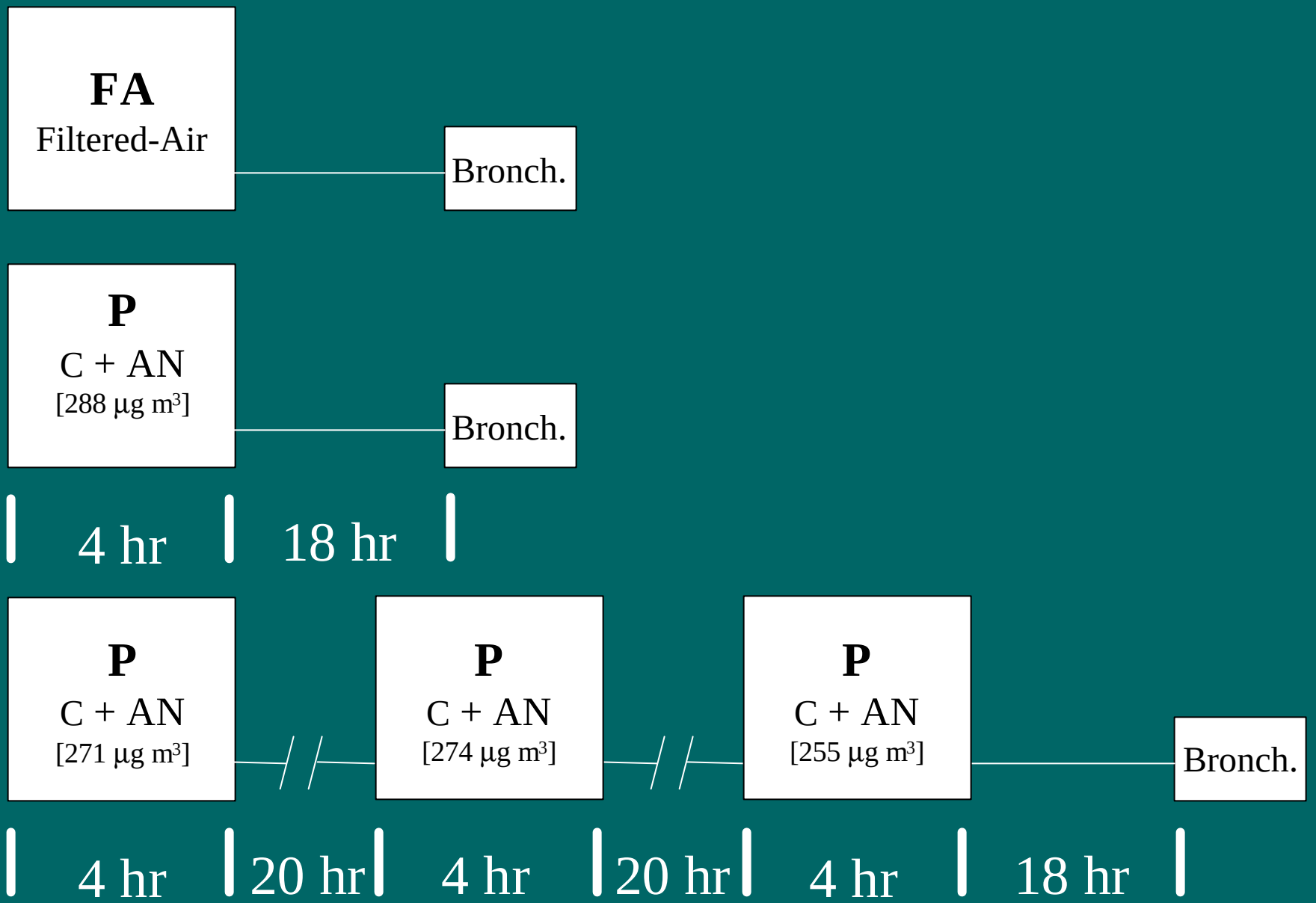
Exposure System

- Exposure Chamber (Airflow = 300 cfm)
- Particles:
  - Suspension of C and AN
  - Series of five air-based nebulisers
  - Particles; 95% < 1.0 μm; MMAD = 0.61 μm;
  - C = 91.6% mass; AN = 8.6% mass
- Ozone:
  - Oxygen (10%) argon mix
  - Corona discharge ozone generator

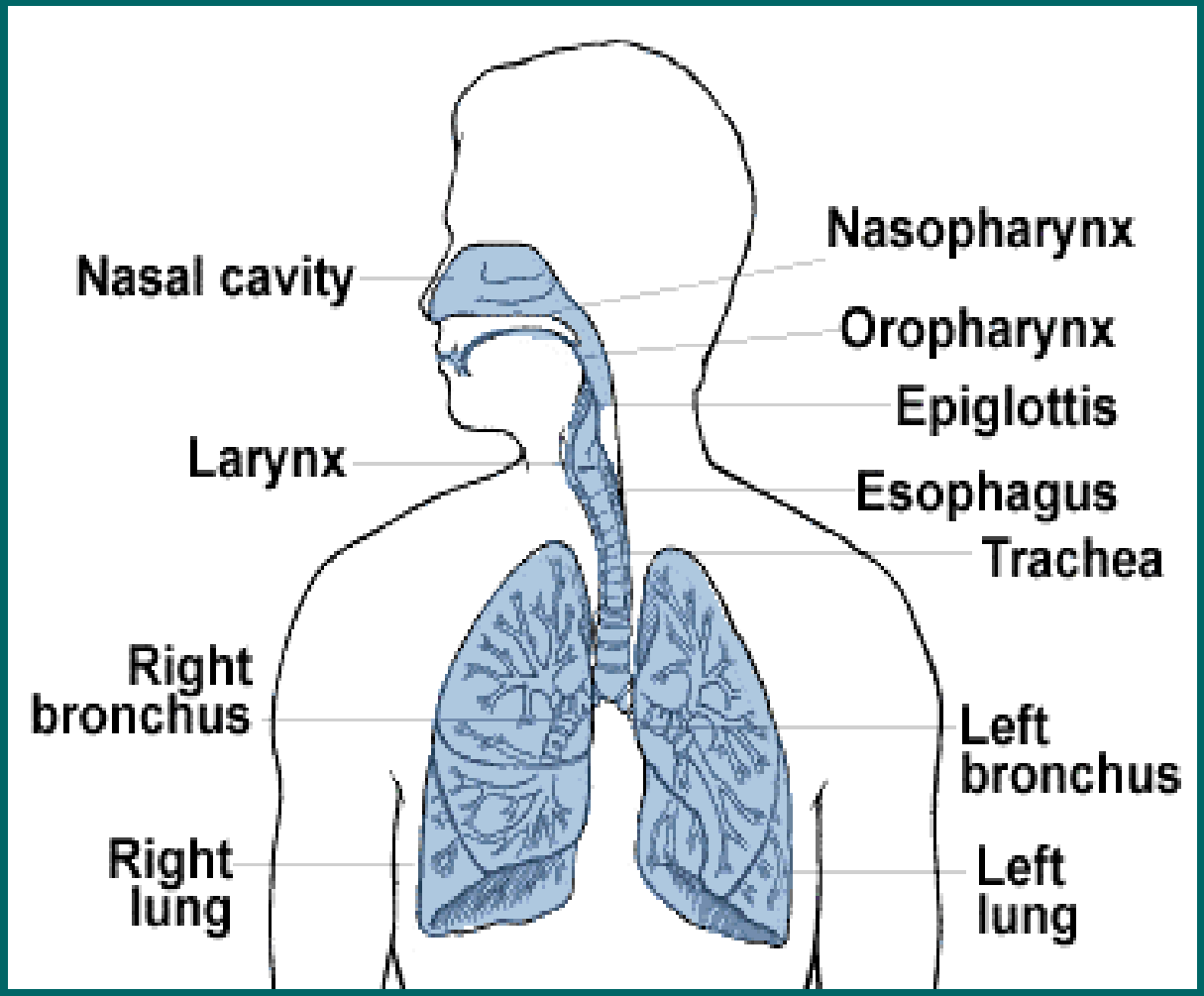
Design: Experiment One



Design: Experiment Two



Bronchoscopy



Lavage (Bfx amd BAL): Cells and proteins

Brushes: Epithelial cells

Biopsy: In vitro allergen exposure

Leukocytes (Differential)

Cell	Exposure Condition					
	FA		P		PO	
	Bfx	BAL	Bfx	BAL	Bfx	BAL
Macrophages (%)						
Median	92.5 [A]	92.4 [B]	91.6 [C]	86.1	72.8 [AC]	85.3 [B]
25-75% range	90.4 - 95.5	89.2 - 96.0	85.9 - 96.4	84.0 - 95.8	66.6 - 75.8	77.4 - 95.0
Neutrophils (%)						
Median	4.5 [A]	3.7	3.9 [B]	2.9	17.2 [AB]	5.4
25-75% range	2.5 - 5.4	1.5 - 5.3	2.4 - 5.6	1.2 - 5.2	10.7 - 23.8	1.9 - 9.4
Lymphocytes (%)						
Median	2.7 [A]	2.8	1.9 [B]	4.1	7.7 [AB]	4.5
25-75% range	0.0 - 3.6	0.3 - 5.5	0.0 - 5.9	0.0 - 8.6	0.3 - 8.9	0.3 - 8.4
Eosinophils (%)						
Median	0.3	0.0 [A]	0.6	0.6	1.7	1.3 [A]
25-75% range	0.0 - 0.3	0.0 - 0.3	0.3 - 2.4	0.3 - 1.9	0.6 - 3.8	0.3 - 2.3
Epithelial Cells (%)						
Median	16.8	4.8	10.5	4.8	10.3	5.0
25-75% range	9.3 - 26.8	1.8 - 9.0	9.0 - 22.9	2.5 - 12.8	7.0 - 17.5	2.0 - 5.5

Protein

Protein	Exposure Condition					
	FA		P		PO	
	Bfx	BAL	Bfx	BAL	Bfx	BAL
Total Protein (µg ml)						
Median	85[A]	158[B]	93[C]	174	216[AC]	179[B]
25-75% range	46 - 160	125 - 169	39 - 188	115 - 174	114 - 319	138 - 240
IL-8 (pg ml)						
Median	24.2	8.1	35.4	13.7	24.3	18.9
25-75% range	8.4 - 44.8	5.3 - 16.8	22.2 - 89.3	10.1 - 27.7	16.0 - 47.2	5.5 - 30.3
GMCSF (pg ml)						
Median	0.7	4.1	0.6[A]	3.3	1.6[A]	3.7
25-75% range	0.4 - 1.1	3.7 - 4.5	0.2 - 0.9	2.9 - 4.1	1.5 - 1.8	1.3 - 6.9
CRP (µg ml)						
Median	0.002[A]	<0.00035	0.002	<0.00035	0.005[A]	<0.00035
25-75% range	0.001 - 0.003		0.001 - 0.003		0.004 - 0.006	

Gene Expression

Gene	Exposure Condition								
	FA			P			PO		
	Bfx	BAL	Epi	Bfx	BAL	Epi	Bfx	BAL	Epi
IL-1β									
Mean	7.1	5.7		7.2	5.7		6.9	6.0	
±SE	0.3	0.8		0.7	0.7		0.7	1.0	
IL-6									
Mean	12.6	10.4		11.2	10.8		11.9	10.5	
±SE	0.3	0.6		0.8	0.1		0.5	0.5	
IL-8									
Mean	8.9[A]	9.8	9.3	9.6[B]	7.7	8.5	7.5[AB]	8.3	8.6
±SE	0.3	1.5	0.3	0.5	0.7	1.0	0.5	0.8	1.1
IL-10									
Mean	15.8[A]	15.5	15.3	16.0[B]	14.1	14.5	14.4[AB]	14.0	15.1
±SE	0.4	1.4	0.5	0.4	0.2	0.5	0.4	1.0	0.2
HIN-1									
Mean	17.7	10.9	5.4	16.4	8.3	5.0	18.1	10.5	5.9
±SE	2.0	2.3	2.8	1.2	0.6	2.5	2.5	1.3	2.7

Spirometric Pulmonary Function

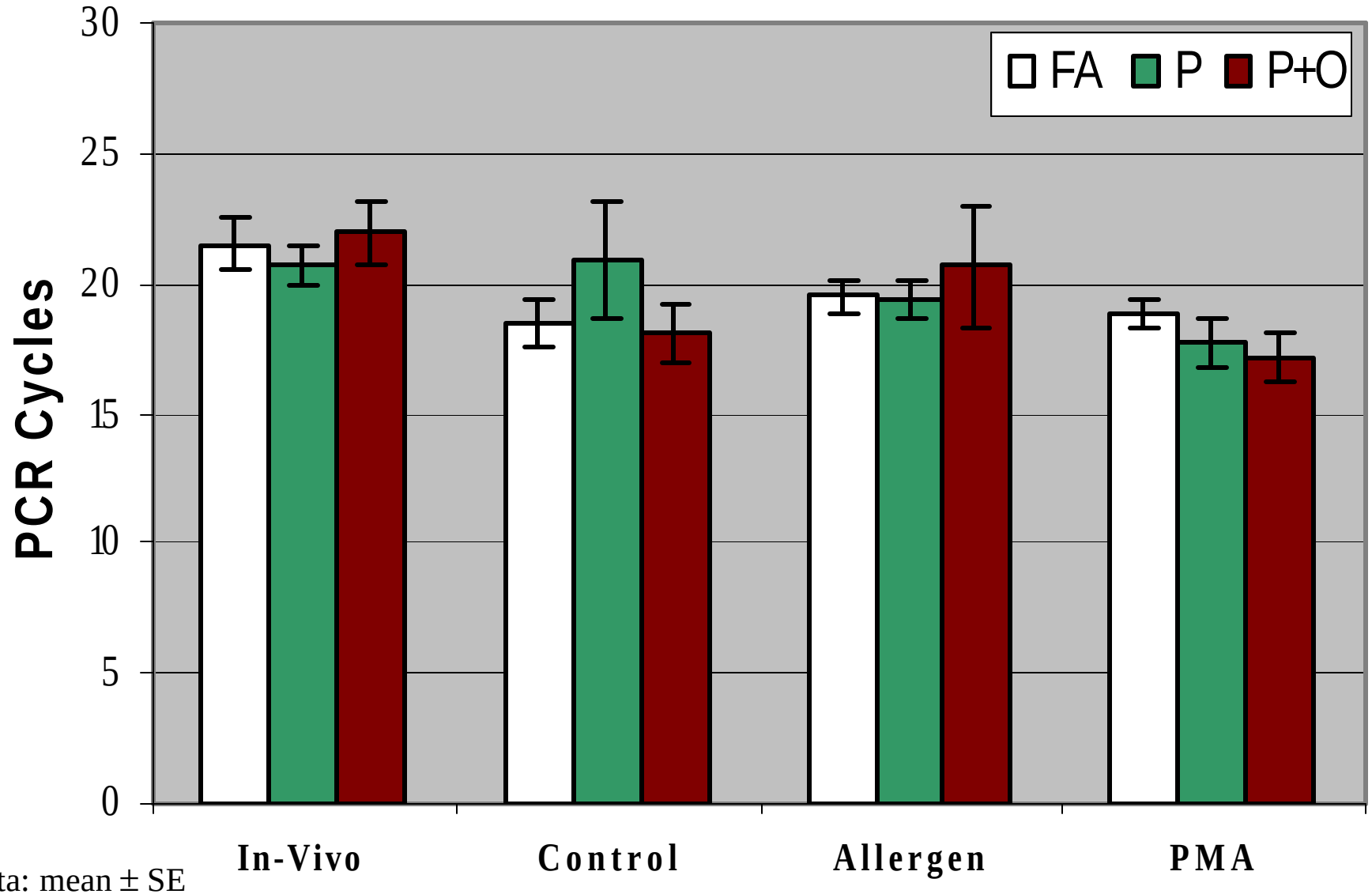
	Exposure Condition								
	FA			P			PO		
	Pre-Exp	Post-Exp	Post-18	Pre-Exp	Post-Exp	Post-18	Pre-Exp	Post-Exp	Post-18
FVC (l)									
Median	3.50	3.46[A]	3.56[B]	3.54	3.39[C]	3.50[D]	3.61[E]	3.23[ACF]	3.23[BDEF]
25-75% range	2.98 - 3.98	2.99 - 3.91	2.94 - 3.96	2.93 - 3.92	2.99 - 4.01	2.90 - 3.84	3.02 - 3.88	2.77 - 3.76	2.85 - 3.78
FEV_1 (l)									
Median	2.60	2.63[A]	2.67[B]	2.64	2.58	2.57[C]	2.56[D]	2.31[AD]	2.57[BC]
25-75% range	2.20 - 3.15	2.07 - 3.16	2.10 - 3.12	2.05 - 3.14	2.11 - 3.12	2.02 - 3.12	2.03 - 3.10	1.90 - 2.95	1.91 - 3.03
FEF_{25-75} (l s)									
Median	2.23	2.32[AB]	2.23	2.04	1.93[A]	1.97	2.28	1.61[B]	1.87
25-75% range	1.55 - 2.97	1.48 - 3.23	1.57 - 3.05	1.57 - 3.10	1.54 - 2.99	1.61 - 2.94	1.52 - 2.87	1.45 - 2.92	1.36 - 2.75

Method: Tissue

- Allows simultaneous investigation of allergy effects

- In-vivo response
  - No culture
- In-vitro allergen exposure
  - 24 hr exposure
  - Allergen; subject specific
  - No allergen (Neg. control)
  - PMA (Pos. control; internal)
- Quantitative Real-Time RT-PCR
  - Twelve cytokines involved in inflammation

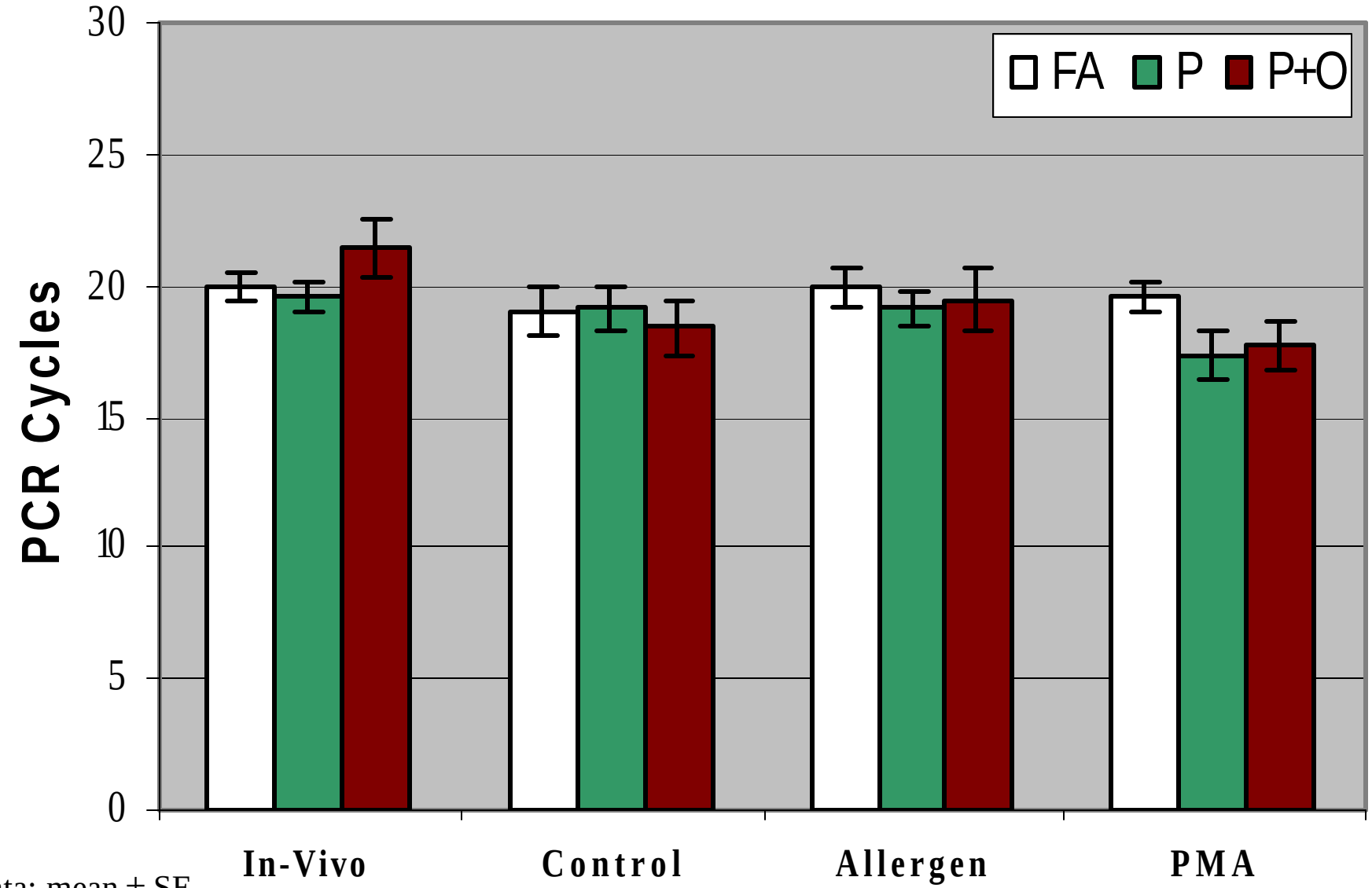
Tissue: IL-12 mRNA



Data: mean ± SE

↓ Ct = ↑ expression

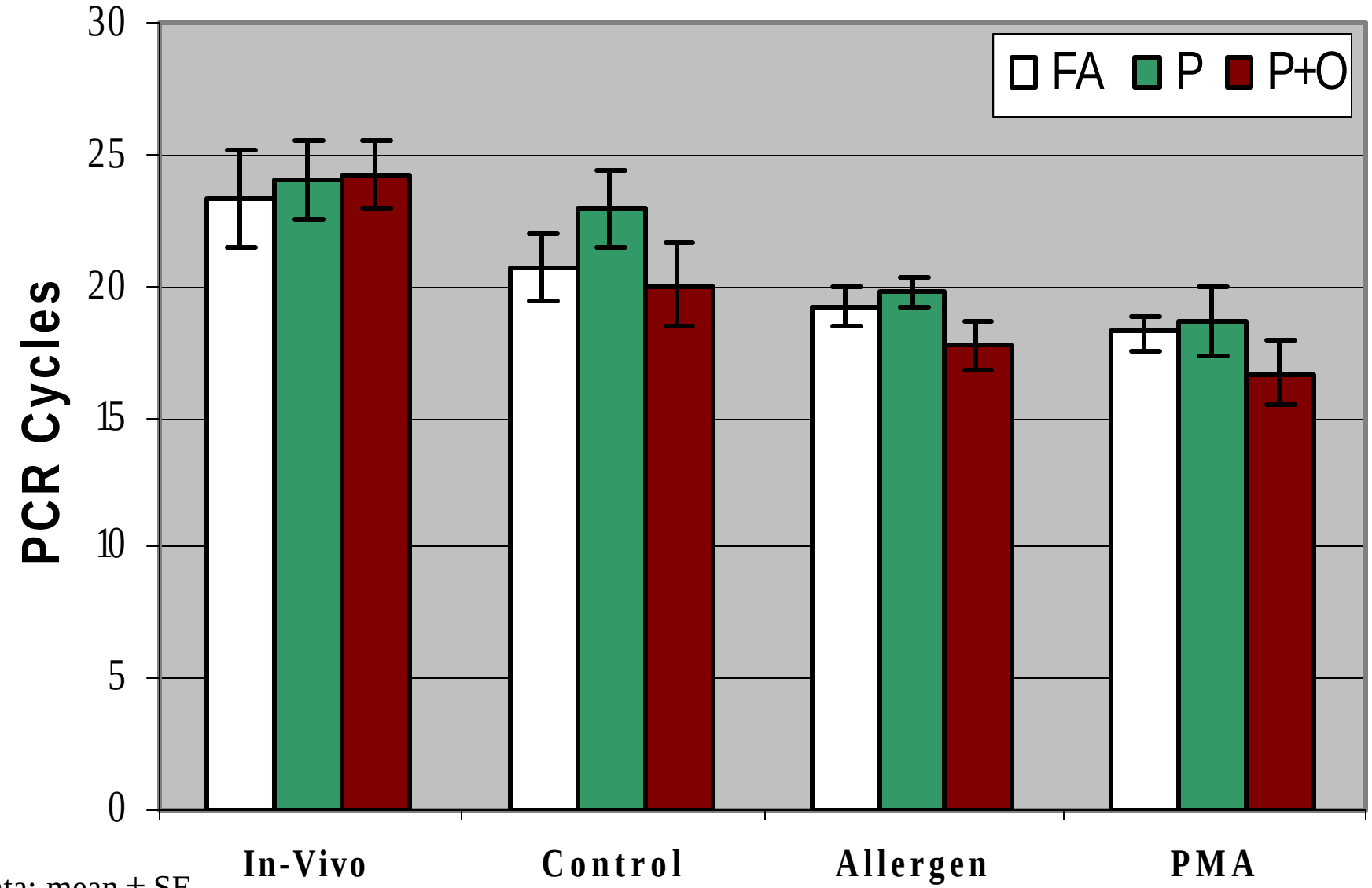
Tissue: IL-15 mRNA



Data: mean ± SE

↓ Ct = ↑ expression

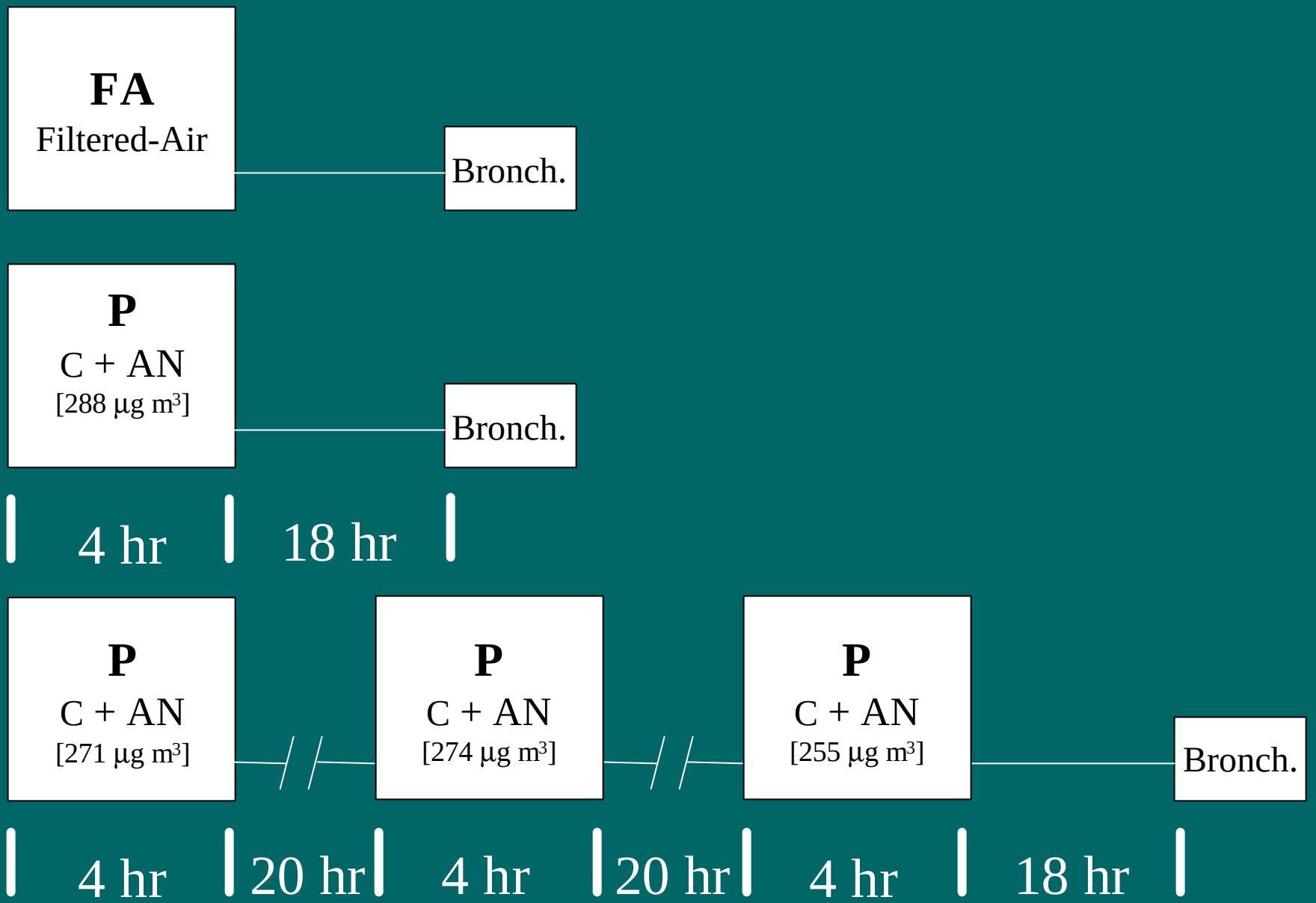
Tissue: IL-10 mRNA



Data: mean ± SE

↓ Ct = ↑ expression

Design: Experiment Two



Leukocytes (Differential)

Cell	Exposure Condition					
	FA		P		P3	
	Bfx	BAL	Bfx	BAL	Bfx	BAL
Macrophages (%)						
Median	95.2	92.6	91.6	89.0	96.3	93.0
25-75% range	92.5 - 96.1	89.2 - 97.5	86.4 - 96.4	79.4 - 95.8	90.7 - 97.1	88.9 - 97.8
Neutrophils (%)						
Median	2.6	3.6	3.9	2.6	2.7	2.4
25-75% range	2.2 - 4.5	1.5 - 4.5	2.4 - 5.3	1.2 - 5.2	1.0 - 5.8	0.8 - 4.7
Lymphocytes (%)						
Median	1.1 [A]	2.8	1.9 [B]	4.1	0.0 [A B]	0.3
25-75% range	0.0 - 3.6	0.3 - 5.5	0.3 - 5.9	0.0 - 8.6	0.0 - 0.4	0.0 - 0.8
Eosinophils (%)						
Median	0.0	0.0	0.6	0.6	1.0	1.1
25-75% range	0.0 - 0.3	0.0 - 0.3	0.0 - 1.0	0.4 - 1.3	0.0 - 2.1	0.8 - 1.4
Epithelial Cells (%)						
Median	9.8	3.8	17.3	5.5	12.3	7.5
25-75% range	8.5 - 22.3	1.8 - 5.3	9.8 - 27.5	4.3 - 15.3	10.3 - 27.5	3.8 - 8.8

Protein

Protein	Exposure Condition					
	FA		P		P3	
	Bfx	BAL	Bfx	BAL	Bfx	BAL
Total Protein (µg ml)						
Median	106	158	113	174	111	155
25-75% range	61 - 160	125 - 169	39 - 188	115 - 231	63 - 133	133 - 177
IL-8 (pg ml)						
Median	24.8	6.6	35.4	20.8	12.5	9.0
25-75% range	8.4 - 44.8	5.2 - 23.9	22.2 - 89.3	9.0 - 28.4	6.9 - 32.3	6.5 - 12.3
GMCSF (pg ml)						
Median	0.9	4.2	0.7	3.5	0.6	3.2
25-75% range	0.5 - 1.1	3.8 - 4.5	0.6 - 0.9	3.2 - 4.1	0.4 - 2.4	3.0 - 3.9
CRP (µg ml)						
Median	0.002	<0.00035	0.002	<0.00035	0.002	<0.00035
25-75% range	0.001 - 0.004		0.001 - 0.003		0.001 - 0.006	

Spirometric Pulmonary Function

	Exposure Condition						
	P3 Exp-1		P3 Exp-2		P3 Exp-3		
	Pre-Exp	Post-Exp	Pre-Exp	Post-Exp	Pre-Exp	Post-Exp	Post-18
FVC (l)							
Median	3.78	3.84	3.82	3.81	3.86	3.79	3.74
25-75% range	3.06 - 3.86	3.03 - 3.86	3.11 - 4.05	3.08 - 4.03	3.23 - 3.96	3.16 - 3.95	2.84 - 4.03
FEV_1 (l)							
Median	2.63	2.73	2.67	2.99[A]	2.73[B]	2.61[C]	2.57[ABC]
25-75% range	2.19 - 2.97	2.09 - 2.94	2.20 - 3.29	2.16 - 3.21	2.23 - 3.02	2.28 - 2.97	2.11 - 2.88
FEF_{25-75} (l s)							
Median	1.77	1.67	2.39[A]	1.91[B]	1.87	1.74	1.63[AB]
25-75% range	1.58 - 2.98	1.48 - 2.77	1.96 - 2.94	1.68 - 2.98	1.55 - 2.65	1.68 - 2.46	1.48 - 2.38

Discussion

- The results of this project indicate that in individuals with asthma, both single and serial exposures to carbon and ammonium nitrate particles results in:
  - Decreases in spirometric pulmonary function
  - No changes in inflammatory cells (neutrophils, macrophages)
  - No changes in cytokine protein or gene expression

- The absence of particle-induced inflammation could be due to the chemical composition of the particles (metals, acids)

Discussion

- Combined exposure to particles and ozone resulted in:
  - Increases in several inflammatory associated cells
  - Increases in protein and gene expression
  - Decreases in spirometric pulmonary function

- It is expected that for the combined exposure these changes are due, at least primarily, to the ozone component of the exposure

Future Directions

- Healthy subjects
- Other susceptible individuals
- Particle chemical composition (metals, acids)
- Ozone-only exposures

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