Effects of inhaled carbon nanoparticles on the mouse lung

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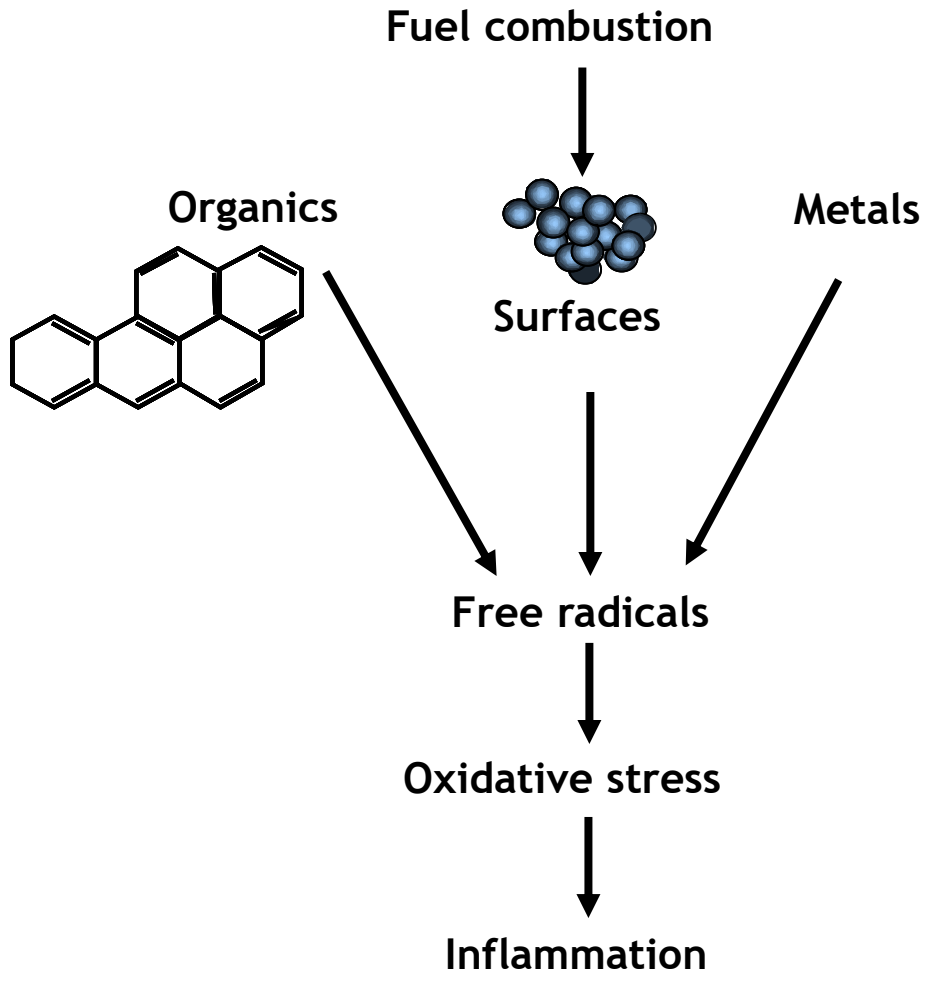
Background - UFP

- Traffic-related air pollution is characterized by high number concentrations of carbonaceous ultrafine particles (UFP) and has been associated with increased risks of respiratory diseases.
- Oxidative stress and inflammation have emerged as main mechanisms involved in the pathogenesis of particle-induced lung diseases.
- Several epidemiological studies have addressed the link between prenatal/early-life exposure to air pollution particles and lung disease development or progression
- In this context, studies have also explored the role of epigenetic mechanisms (i.e., DNA methylation, microRNAs, histone acetylation)



Donaldson *et al. Part Fibre Toxicol 2005*

Expression analysis of microRNAs / DNA methylation

- Epigenetic changes are strongly associated with environmental exposures and are mechanistically linked to adverse health effects.

- **MicroRNAs** (miRNAs) are single-stranded, short non-coding RNA sequences (~22 nucleotides) that post-transcriptionally can regulate gene expression.

- Deregulations of miRNAs have been observed humans in association with exposure to $PM_{2.5}$ and diesel exhaust particles (e.g. Motta et al., Toxicol Sci 2013; Yamamoto *et al.* Environ Health Perspect 2013)

- **DNA methylation** refers to the bonding of a methyl (CH_3) group to a cytosine base to form 5-methyl-cytosine (predominantly at CpG dinucleotides) and this dynamic modification has been implicated in the regulation of gene expression

Adverse health effects resulting from gestational exposure to UFP?

- Development of fibrotic lung pathology in adult mice following prenatal/early-life exposure to tobacco smoke → Associated with persistent alterations in DNA methylation
  (Cole *et al*. Inhal Toxicol 2017)

- Increased susceptibility to allergic airway disease (ovalbumin-challenge) in offspring of dams that received intranasal instillation of diesel exhaust particles or concentrated urban particulate matter → Associated DNA methylation changes (generations F1 to F3)
  (Gregory *et al.,* Am J Physiol Lung Cell Mol Physiol 2017)

- Reduced inflammatory response to house dust mite allergen challenge in mice that were exposed in utero to UFP → immunosuppressive effect!
  (Rychlik et al., PNAS 2019)

Adverse health effects resulting from gestational exposure to UFP?

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ARTICLE

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Ambient black carbon particles reach the fetal side of human placenta

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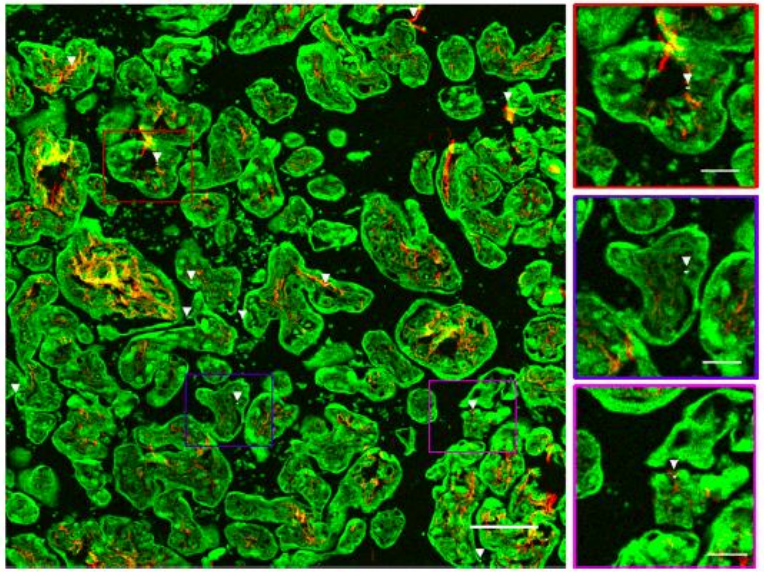

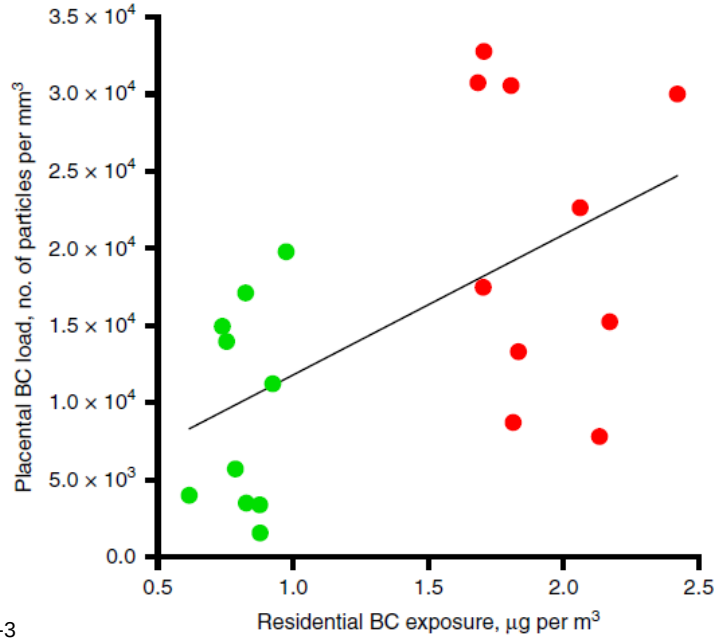

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Study aims/methods

AIMS

- To investigate the toxicity of clean ultrafine carbonaceous particles (UFPc) in the absence of associated chemical constituents such as metals and organic species
- To study the potential impact of prenatal/early-life exposure to UFPc on the susceptibility to UFP exposure in adult lung

APPROACH

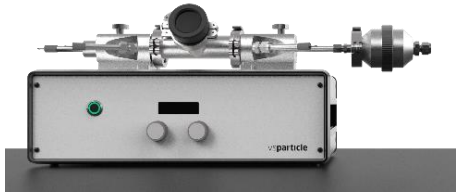
- Groups: (pregnant) female C57bl6 OlaHsd mice and/or their offspring (female or male).
- Repeated whole body inhalation exposure studies to UFPc (4h/day)
- Challenge of pre and/or postnatally UFPc exposed male mice at adulthood with an inflammatory dose of UF carbon black particles

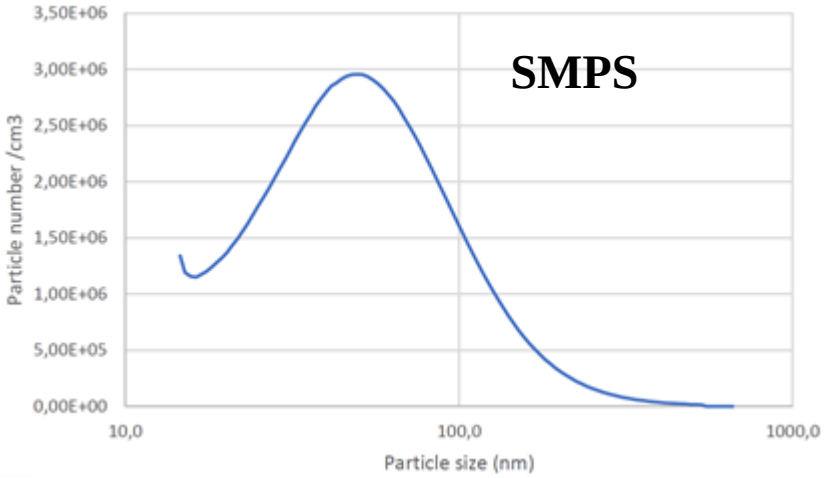
UFP exposures

Inhalations:

- UFPc exposures obtained by means of a VSP G1 generator (VSParticle, The Netherlands) equipped with hollow graphite electrodes, using N_2 as carrier gas

- Average mass concentration of 450 µg/m^3
- Median particle size of 55 nm





Challenge protocol:

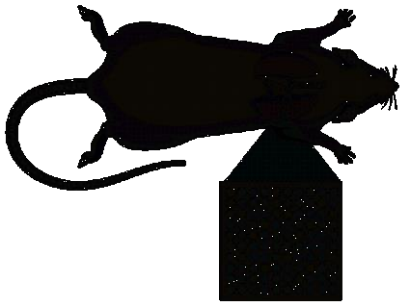
- Pharyngeal aspiration to a single relatively high-dose of carbon black (Printex 90; 5mg/kg BW in phosphate buffered saline) to quantify the inflammatory response to this challenge at t = 12 h.

Evaluation of effects in mouse lung

Bronchoalveolar lavage (BAL) analysis

- Flow cytometry based analyses; total/differential cells counts (GR-1, CD11c ; CD11b, F4/80)

RNA/DNA isolation from homogenized lung tissue



- Isolation of DNA and RNA: AllPrep DNA/RNA/miRNA Universal Kit (Qiagen)

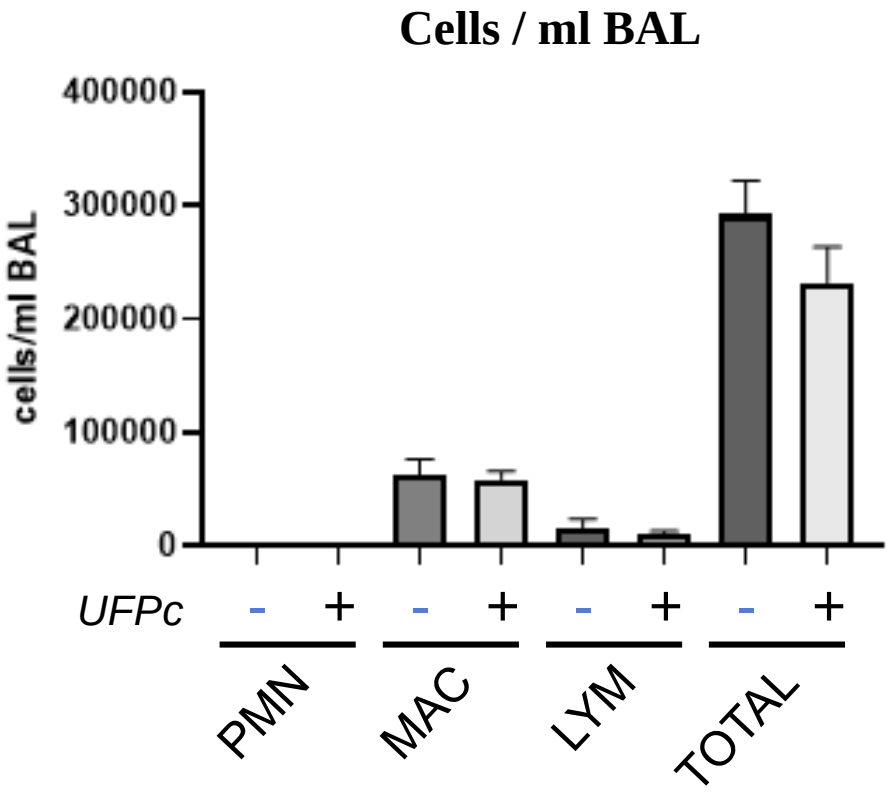
mRNA and miRNA profiling

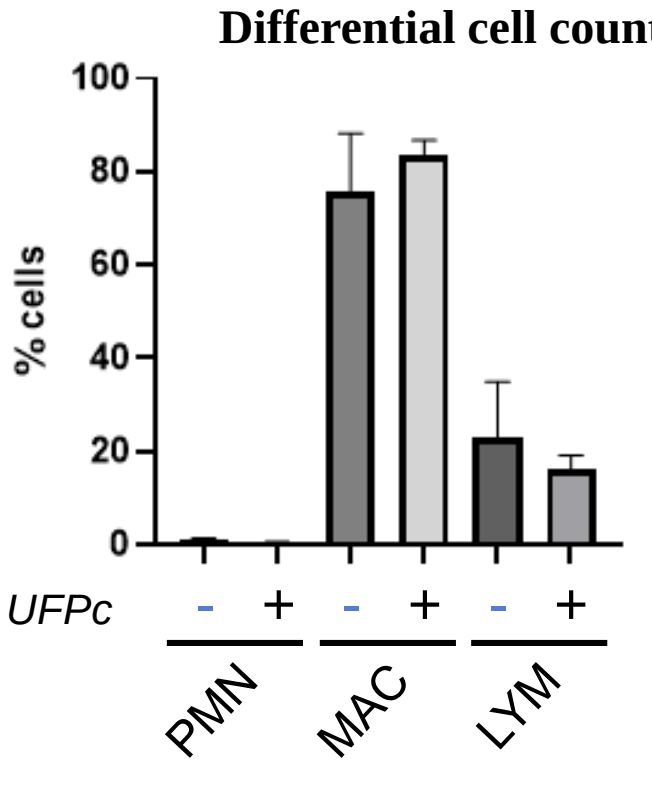
- mRNA libraries: NEBNext Ultra II Directional RNA Library Prep with Beads (NEBNext)
- miRNA libraries: QIAseq miRNA Library Kit (Qiagen)
- Sequencing: NovaSeq 6000 (Illumina)

DNA methylation

- Conversion of dsDNA to ssDNA: Bisulfite Conversion Kit (Zymo research)
- Methylation array: Infinium Mouse Methylation BeadChip, scanning chips on iScan (Illumina)

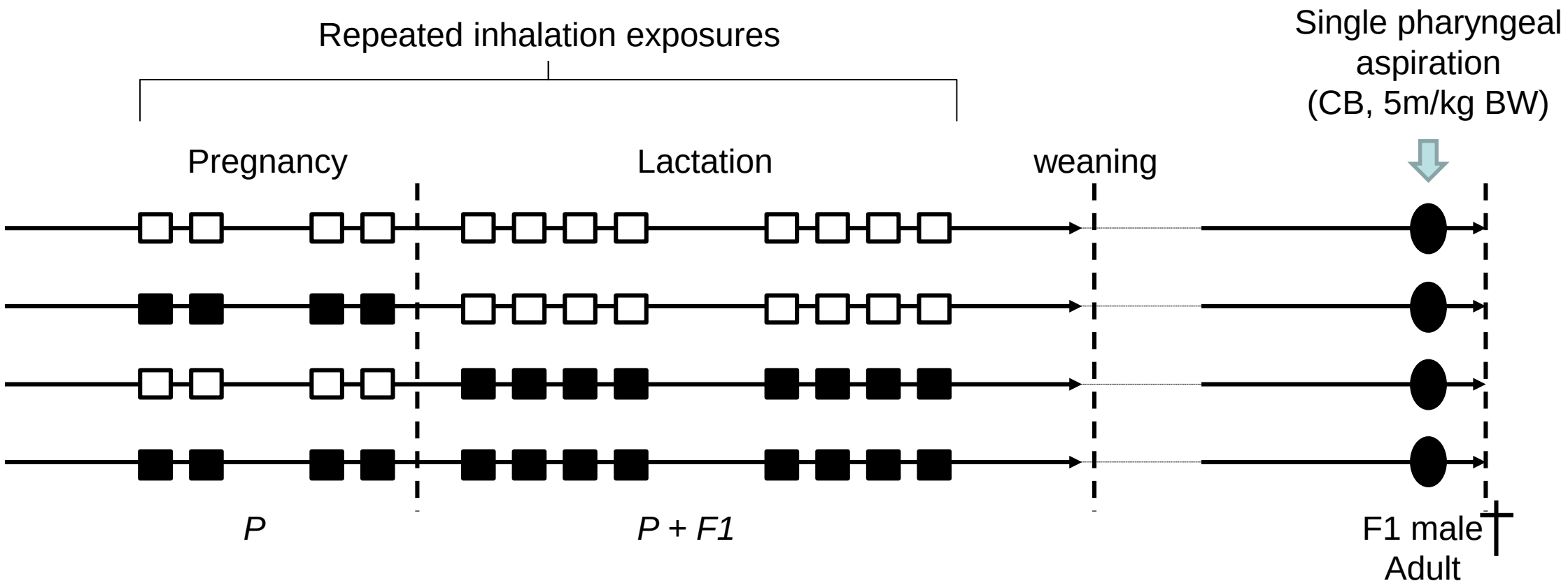
Results



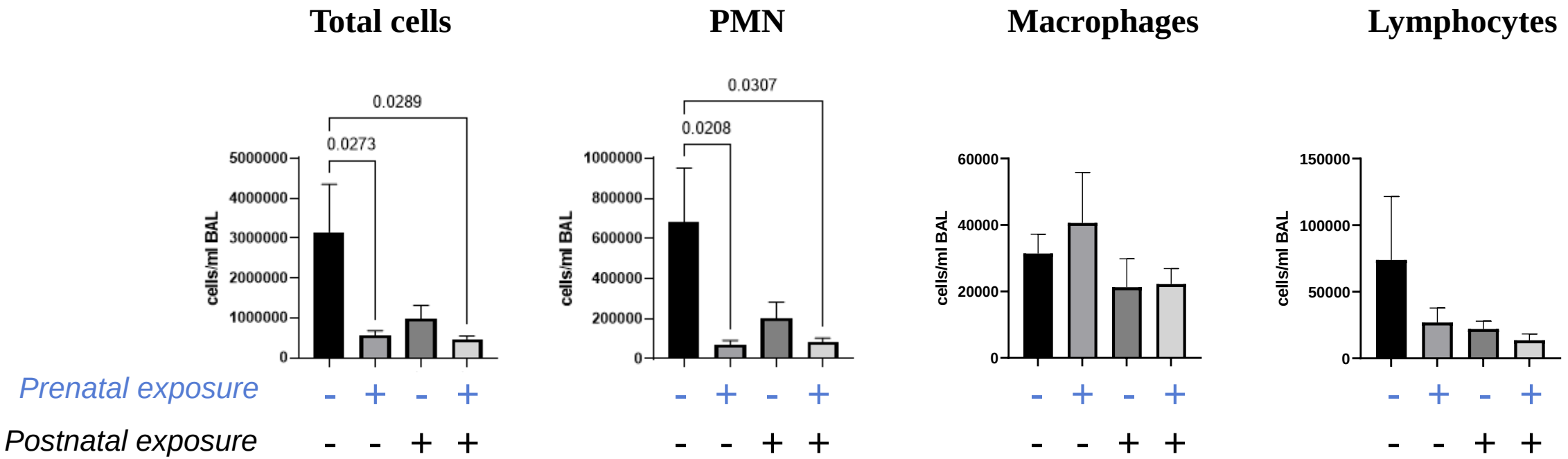


Lung tissue analysis → **no obvious differences in expression of mRNA or miRNA**

Design - challenge study



Results



Lung tissue analysis → **no consistent differences in methylation status**

Summary and conclusions

- Repeated whole body inhalation exposure to UFPc did not result in sustained lung inflammation and toxicity in mouse lung (BALF analysis, mRNA & miRNA profiling).
- Prenatal or pre-plus postnatal exposure to UFPc results in a significantly attenuated inflammatory response of adult mouse lung to UFCB
- Lung tissue DNA methylation analysis of lung provides no clues for potentially underlying mechanisms

⇨ The results of this experimental approach suggest that inhalation exposure to UFP during pregnancy may reduce offspring's susceptibility to subsequent pulmonary inflammatory insults.

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https://www.tube-project.eu/

Statistical analysis

DNA methylation:
Raw microarray data were processed in the R program using the minfi package (Bioconductor). Beta values for the determination of the methylation level were calculated. Significant beta values < 0.2 or > 0.7 were considered as hypomethylation or hypermethylation, respectively. Subsequent analyses using linear models in limma package, including covariates, were performed to detect differentially methylated sites between the studied groups.

mRNA/miRNA expression:
An NGI-RNAseq pipeline was used for RNA sequencing data. It pre-processed raw data from FastQ inputs, aligned the reads, generated gene counts and performed extensive quality-control on the results. DESeq2 with default parameter settings was applied to normalize read counts and to identify the differences in gene expression between sample groups.