Effects of inhaled tier-2 diesel engine exhaust on immunotoxicity in a rat model: A hazard identification study. Part III. Immunotoxicology_Data set

Introductory Information

Diesel exhaust (DE) is an air pollutant containing gaseous compounds and particulate matter. Diesel engines are common on gas extraction and oil sites, leading to complex DE exposure to a broad range of compounds through occupational settings. The US EPA concluded that short-term exposure to DE leads to allergic inflammatory disorders of the airways. To further evaluate the immunotoxicity of DE, the effects of whole-body inhalation of 0.2 and 1 mg/m3 DE (total carbon; 6 h/d for 4 days) were investigated 1-, 7-, and 27-days post exposure in Sprague-Dawley rats using an occupationally relevant exposure system. DE exposure of 1 mg/m3 increased total cellularity, number of CD4+ and CD8+ T-cells, and B-cells at 1 d post-exposure in the lung lymph nodes. At 7 d post-exposure to 1 mg/m3, cellularity and the number of CD4+ and CD8+ T-cells decreased in the LLNs. In the bronchoalveolar lavage, B-cell number and frequency increased at 1 d post-exposure, Natural Killer cell number and frequency decreased at 7 d post-exposure, and at 27 d post-exposure CD8+ T-cell and CD11b+ cell number and frequency decreased with 0.2 mg/m3 exposure. In the spleen, 0.2 mg/m3 increased CD4+ T-cell frequency at 1 and 7 d post-exposure and at 27 d post-exposure increased CD4+ and CD8+ T-cell number and CD8+ T-cell frequency. B-cells were the only immune cell subset altered in the three tissues (spleen, LLNs, and BALF), suggesting the induction of the adaptive immune response. The increase in lymphocytes in several different organ types also suggests an induction of a systemic inflammatory response occurring following DE exposure. These results show that DE exposure induced modifications of cellularity of phenotypic subsets that may impair immune function and contribute to airway inflammation induced by DE exposure in rats.

Methods Collection

1. Animal Exposures
    - Male Sprague-Dawley rats (200-275 g at arrival)
    - Diesel exhaust (DE) (0.2 and 1 mg/m3) whole-body inhalation for 6 hours/day for 4 days (filtered air used as control)
    - Tissues collected 1, 7, and 27 days following last DE exposure
2. Diesel exhaust exposures

- An eight-kilowatt (KW) diesel generator was used to produce diesel exhaust in real time during inhalation exposures
- Mobil 1 Delvac 1300 Super Motor Oil 15w40 was used as the engine oil
- The fuel used in the generator was ultra-low sulfur (15ppm or less sulfur), No. 2 dyed winter blend from Jacobs petroleum products in Waynsburg PA.
- A custom exposure system with software written in LabVIEW automatically controlled chamber air flows, particle concentration, and exposure duration.
- The count median electric mobility diameter was 120 nm.

3. Tissue Collection
    - Bronchoalveolar lavage fluid collected from left lung by filling the left lung with 3ml of phosphate-buffered saline (PBS), massaging for 30 seconds, withdrawing, and repeating process one more time
    - Lung lymph nodes (LLN) were collected in PBS and single cell suspensions were prepared by mechanical disruption between frosted microscope slides
    - Spleen were collected in complete medium and prepared using a 30ml syringe plunger and passing the homogenate through a cell trainer to obtain single cell suspensions
4. Immune Cell Subsets
    - Flow cytometry using BD LSRII Flow Cytometer and data was analyzed using FlowJo software.
5. Natural kill cell assay
    - NK cell activity was evaluated using Yac-2 cells as the target cells and splenocytes in single cell suspensions were used as the effector cells.
6. Hematology
    - Blood samples were collected via abdominal aorta of the rats. Selected complete blood counts were evaluated using IDEXX ProCyte Hematology Analyzer.
7. Spleen IgM response to SRBC
    - Primary IgM response to SRBC was conducted using a modified hemolytic plaque assay.

Publications

Dzubak L, Shane H, Baur R, Lukomska E, McKinney W, Roberts J, Fedan J, Anderson S [2024]. Effects of inhaled tier-2 diesel engine exhaust on immunotoxicity in a rat model: A hazard identification study. Part II Immunotoxicology. Toxicol Rep. Jan 15; 12:135-147. Doi: 10.1016/j.toxrep.2024.01.004.

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