Title: Examination of the exposome in an animal model: the impact of high fat diet and rat strain on local and systemic immune markers following occupational welding fume exposure

Introduction: The fundamental goal of this study was to develop an *in vivo* model of the exposome that could be used to evaluate exposure-induced alterations in local and systemic immune markers as a function of various exposomal factors. Accordingly, the model was designed to mimic an occupationally-relevant scenario in which inhalation of welding fumes (WF) constituted the primary occupational/environmental exposure of interest. Immune endpoints were assessed at three different time points representative of different stages in the exposure/response timeline (before WF exposure, directly after, and following a 12 wk recovery period). Moreover, the potential impact of genetic variation and lifestyle factors on the immune response to WF exposure and subsequent recovery was addressed by incorporating two different rat strains (Sprague-Dawley [SD] and Brown Norway [BN]) and two variations in diet (regular and high fat) into the model.

One of the primary objectives of this study was to determine whether consumption of a HF diet is associated with increased immunological responsivity following exposure to a common respiratory toxicant. The study was also executed with the goal of identifying which experimental factor (genetics/strain, diet, occupational exposure) is most influential in the modulation of local and systemic markers of immune status following WF exposure, and subsequently, the efficacy of inflammation resolution. The information obtained from these analyses will help elucidate which exposomic determinants may be particularly relevant in the context of immunotoxicity, and accordingly, warrant special attention in future investigations. The results will also contribute to the development of other *in vivo* exposome models and direct future efforts related to the exposome and its impact on human health.

Methods Collection:

- Male Sprague-Dawley (SD) and Brown Norway (BN) rats were maintained on high fat (HF) or regular (Reg) diets for 7 weeks. After the 7 weeks of diet maintenance, a set of rats from each strain was euthanized for collection of baseline parameters prior to WF exposure (7 wk time point). At the same time point, the remaining groups of rats were exposed by inhalation to stainless steel WF (target concentration of 20 mg/m^3 × 3 h/day × 4 days/week × 5 weeks) or filtered air (control) until week 12, at which time, half of the remaining animals from each strain was euthanized (12 wk time point). Finally, the last set of SD and BN rats was allowed to recover from welding fume exposure for 12 weeks, and then euthanized (24 wk time point). Following euthanasia, whole blood was collected from each rat, bronchoalveolar lavage (BAL) was performed, and lymphoid tissues were collected for subsequent evaluation of different immune markers.
- Total leukocyte number and leukocyte subsets (monocytes, eosinophils, lymphocytes, basophils, and neutrophils) were quantified for each animal using an IDEXX Procyte Dx Hematology Analyzer.
- BAL was performed on rats and total BAL cell number was quantified. Immune cells present in the BAL (alveolar macrophages, neutrophils, lymphocytes, eosinophils) were differentiated by flow cytometry.
- The lung-associated lymph nodes were harvested from each animal and total cell number was quantified. Spleens were also harvested. Lymph node and spleen cells were then phenotypically differentiated by flow cytometry to determine absolute number and proportionality of T-lymphocytes (CD4+ and CD8+), B-cells, NK cells, and non-lymphoid cells.

Citations:

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