Title: Assessment of dermal sensitization by nickel salts in a novel humanized TLR-4 mouse model_Dataset

Introduction: Nickel is one of the most common contact allergens worldwide. Despite this prevalence, many of the underlying immunological mechanisms responsible for nickel allergy remain unclear. This knowledge gap is partially attributable to the inherent resistance of laboratory rodents (mice and rats) to dermal sensitization with nickel salts. The fundamental goal of this study was to assess the potential utility of the novel humanized Toll-like receptor-4 (hTLR-4) mouse model for use in future *in vivo* studies of nickel allergy. Accordingly, hTLR-4 positive and negative mice of both sexes were first incorporated into a Local Lymph Node Assay (LLNA) to evaluate dermal sensitization following topical exposure to soluble nickel salts ($NiSO_4$). The ensuing immune responses were then characterized further by incorporating female and male hTLR-4 positive mice into a non-radioactive endpoint-based assay. Utilizing the same exposure scheme as in the LLNA, mice were euthanized following exposure and the auricular lymph nodes, spleen, and blood were collected to assess various immunological parameters associated with allergy and ACD.

Methods Collection:

- Local Lymph Node Assay- Three dosing solutions containing $NiSO_4$ were prepared at concentrations of 2.5, 5.0, and 10% (w/v) in mineral oil. Mice (n = 3-4 per group) were exposed topically to vehicle control (mineral oil) or $NiSO_4$ on the dorsal sides of both ears for three consecutive days (days 1, 2, and 3). Following two days of rest, on day 6, mice were injected intravenously via the lateral tail vein with 20 µCi tritiated ([^{3}H])-thymidine. Five hours after the [^{3}H]-thymidine injection, mice were euthanized via CO_2 asphyxiation. The left and right auricular lymph nodes were excised from each mouse and processed for assessment of radioactivity. Stimulation indices (SI) were then calculated by dividing the mean disintegrations per minute (DPM) for each test group by the mean DPM for the corresponding vehicle control group.
- 10d Study- hTLR-4 positive female and male mice (n = 5-10 per group) were exposed to either vehicle control or 10% $NiSO_4$ using identical procedures as those employed for dosing in the LLNA study. After three consecutive days of exposure (days 1, 2, and 3), mice were rested for six days and euthanized on day 10 by CO_2 asphyxiation. Following euthanasia, whole blood was collected, the lungs underwent bronchoalveolar lavage (BAL), and the auricular lymph nodes (right and left) and spleen were harvested.
- Total leukocyte number and leukocyte subsets (monocytes, eosinophils, lymphocytes, basophils, and neutrophils) were quantified using whole blood from each animal using an IDEXX Procyte Dx Hematology Analyzer.
- Total lymph node cell number was determined using a Coulter Counter and spleen wet weight was recorded. 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. Activation status was also determined for lymphocyte subsets.
- To quantify circulating total immunoglobulin (Ig)E levels, serum was diluted 1:10 and assessed by enzyme-linked immunosorbent assay (ELISA). Absorbance was determined at 450 nm using a Spectramax Vmax plate reader. The concentration of IgE in each sample was interpolated from a standard curve using multipoint analysis.
- Concentrations of various cytokines were also assessed in the serum of mice. Cytokines were quantified using a Milliplex MAP Kit magnetic bead panel and analyzed on a Luminex 200 system. The specific analytes of interest included a panel of T-helper (Th) type 1-associated cytokines (IL-2, IL-12, interferon [INF]-g) and several major Th2-related cytokines (IL-4, IL-5, IL-13). In addition, IL-6 and IL-10 levels were also assessed.

Citations:

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