Exposure to the immunomodulatory chemical triclosan differentially impacts immune cell populations in the skin of haired (BALB/c) and hairless (SKH1) mice – Dataset

Introductory Information

Workers across every occupational sector have the potential to be exposed to a wide variety of chemicals, and the skin is a primary route of exposure. Furthermore, exposure to certain chemicals has been linked to inflammatory and allergic diseases. Thus, understanding the immune responses to chemical exposures on the skin and the potential for inflammation and sensitization is needed to improve worker safety and health. Responses in the skin microenvironment impact the potential for sensitization and these responses may include the presence of proinflammatory cytokines, inflammasome activation, barrier integrity, skin microbiota, and the presence of immune cells. Selection of mouse strain to evaluate skin effects, such as haired (BALB/c) or hairless (SKH1) mice, varies dependent on the experimental design and needs of a study. However, dermal chemical exposure may impact reactions in the skin differently depending on the strain of mouse. Additionally, there is a need for established methods to evaluate immune responses in the skin. In this study, exposure to the immunomodulatory chemical triclosan was evaluated in two mouse models using immunophenotyping by flow cytometry and gene expression analysis. The flow cytometry panel reported in this manuscript in combination with gene expression analysis may be used in future studies to better evaluate the effect of chemical exposures on the skin immune response. These findings may be important to consider during strain selection, experimental design, and result interpretation of chemical exposures on the skin.

Methods Collection

1. Animals
    a. Female SKH1 mice (6-8 weeks old, Charles River) and BALB/c mice (8 weeks old, Taconic) were purchased and allowed to acclimate for at least one week
2. Triclosan Exposures
    a. Mice (5/group) were exposed once per day for 2, 4, and 7 consecutive days to acetone (0%, vehicle control) or to triclosan (2%) dissolved in acetone (w/v) on the entire dorsal back skin (100 µL/mouse)
3. Euthanasia and Skin Collection
    a. Back skin (~1 cm^2) was collected, fat removed, and weighed
    b. For immune phenotyping analysis, skin was placed into tubes containing 2 mL RPMI and kept on ice
    c. For gene expression analysis, skin was placed into tubes containing 500 µL RNAlater (Invitrogen) and frozen at -80 °C until processed
4. Immune Phenotyping Analysis
    a. Skin was minced and then digested
    b. Following incubation, samples were transferred to ice and 2 mL RPMI with 10% FBS was added to each tube to stop digestion
    c. Cells were passed through a 70 µm cell strainer and washed
    d. Live cells were counted on a Cellometer
    e. Cells were incubated with anti-mouse CD16/32 anti-FcγII and FcγIII Fc Block
    f. For staining, cells were incubated with a cocktail of fluorochrome-conjugated mouse antibodies

g. Following incubation, cells were washed and then fixed in Cytofix buffer
h. Cells were resuspended in phosphate buffered saline containing 1% bovine serum albumin and 0.1% sodium azide and events were collected on an LSR II flow cytometer (BD Biosciences) within 24 hours

5. Gene Expression Analysis
    a. Total RNA was isolated from the skin using the RNeasy kit
    b. A QIAcube (Qiagen) automated RNA isolation machine was utilized in conjunction with the RNA isolation kit
    c. The concentration and purity of the RNA were determined using a NanoDrop Spectrophotometer
    d. Reverse transcription was performed using a High-Capacity cDNA Reverse Transcription Kit
    e. TaqMan Fast Universal PCR Master Mix (Applied Biosystems), cDNA, and gene-specific primers (TaqMan Gene Expression Assays) were combined, and real-time quantitative PCR (qPCR) was performed

Citations

Baur R, Shane HL, Weatherly LM, Lukomska E, Kashon M, Anderson SE (2022) Exposure to the immunomodulatory chemical triclosan differentially impacts immune cell populations in the skin of haired (BALB/c) and hairless (SKH1) mice. Toxicology Reports 9(2022):1766-1776. https://doi.org/10.1016/j.toxrep.2022.09.005.

Acknowledgements

This work was supported by NIOSH.

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