2277 Alterations in the Mouse Skin and Gut Microbiome following Dermal Exposure to the Antimicrobial Chemical Triclosan

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It is increasingly being recognized that the microbiome plays an important role in human health. Dysbiosis of the microbiome has been shown to alter immune responses and has been associated with increased risk of allergic disease. Triclosan is an antimicrobial chemical used in the healthcare field as a high level disinfectant. In humans, triclosan exposure has been associated with an increase in food and aeroallergy and asthma exacerbation. Although not directly sensitizing, dermal exposure to triclosan has been shown to augment allergic responses to experimental allergens in mouse models. However, the impact of dermal exposure to antimicrobials, such as triclosan, on the microbiome is unknown. This study investigated the impact of dermal exposure to triclosan on the skin and gut microbiome in mice. Mice were dermally exposed to 2- 3% triclosan or acetone vehicle control for either 7 or 28 consecutive days. Swabs were used to collect skin commensal bacteria prior to exposure and over the course of the exposure period and fecal pellets were collected following the last triclosan exposure to assess gut commensal bacteria. Following bacterial DNA extraction from skin swabs and fecal pellets, composition of the skin and gut microbiota was determined by 16S ribosomal RNA sequencing. Sequences were grouped into operational taxonomic units and given taxonomic assignments. Analysis of changes in relative abundance identified decreased Proteobacteria and increased Firmicutes in the triclosan exposed group compared to the vehicle control. The skin and gut diverged on the class taxonomic level; Clostridia increased in skin samples, whereas Bacilli increased in the fecal pellet samples. Within the class of Clostridia, Lachnospiraceae and Ruminococcaceae were both increased in relative abundance in the skin swab samples. Lactobacillaceae within the class Bacilli was increased in abundance in the gut. Taken together, dermal exposure to triclosan altered the composition of commensal bacteria in both the skin and gut of mice, suggesting that triclosan can induce dysbiosis of the microbiome and this may contribute to the observed alternations in immune function.

2278 Investigation on the Possible Role of microRNAs in the Regulation of Chemical Allergen Potency

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Allergic contact dermatitis (ACD) is an immunological mediated inflammatory disease and is one of the most common occupational diseases in industrialized countries. ACD is a T-cell mediated skin inflammation caused by repeated skin exposure to low molecular weight chemicals. Chemical allergy is of considerable importance to the toxicologist, who has the responsibility of identifying and characterizing the allergenic potential of chemicals. While incredible progresses have been made in the development of non-animal tests, currently it is not possible to estimate the sensitizing potency of chemical allergens. Using THP-1 cell line, a model for primary human monocytes, and assuming that the extent of chemical allergen-induced dendritic cells activation/maturation and lifespan may drive the quality and magnitude of T cell activation, we have conducted a study using allergens of different potency to verify this hypothesis. Up- regulation of CD80, CD86 and HLA-DR, and the release of several cytokines were evaluated in THP-1 cells. Results indicate that the extreme allergens induce a more rapid CD86 up-regulation (24h) compared to the moderate and weak allergens. Furthermore, HLA-DR was up regulated at 72h only by the extreme sensitizers. Overall, results suggest that allergens of different potency differently activate DCs, with extreme allergen inducing a higher degree of maturation compared to moderate and weak allergens. Based on these results, we moved to the analysis of miRNAs expression in response to chemical allergens as valuable explanation to understand allergenic potency. MVs release by immune system cells may be induced by soluble agonists or in response to physical or chemical stress. MicroRNAs are non-coding RNA molecules that regulate gene expression at the post-transcriptional level. Recent findings indicate that miRNAs contained in MVs may determine reprogramming of gene expression in target cells. Although ACD has been studied extensively, there are few studies conducted to investigate miRNA expression. Using miScript miRNA PCR Array (Qiagen) we identified few miRNAs involved in ACD. These include let-7, miR-142 and miR-155. We are currently investigating the possible correlation between the miRNA expression obtained with the screening and the effective potency of selected contact allergens tested. *Acknowledgements: This study was supported by funding from Colgate-Palmolive Grant for Alternative Research (SOT Award 2017–2018).*

2279 The Effects of Pristine and Carboxylated Multiwalled Carbon Nanotubes on Phagocytic Function of Macrophages

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The global production and applications of multi-walled carbon nanotubes (MWNTs) have increased in recent years despite evidence that MWNTs cause pulmonary fibrosis in lab animals that may lead to mesothelioma. Studies to understand the pathological mechanisms of MWNTs often focus on macrophages as they are first responders to invaders in the body. Recent work in our lab shows that both human and mouse macrophages preferentially accumulate ~100X more carboxylated MWNTs (C-MWNTs) than pristine MWNTs (P-MWNTs). Also, Class A scavenger receptors expressed in macrophages may be involved in the selective accumulation of C-MWNTs (Wang et. al., *Nanotoxicology*, 2018, DOI: 10.1080/17435390.2018.1472309). To investigate the potential impacts of accumulated C-MWNTs and P-MWNTs on phagocytic function of macrophages, this study focused on two approaches: 1. Mouse macrophage RAW 264.7 cells were pre-exposed to C-MWNTs or P-MWNTs at 37 °C for 2h and 24h, washed and challenged with 1 µm fluorescent polystyrene beads to monitor the phagocytic activity of the cells. This was done quantitatively through flow cytometry and qualitatively using confocal fluorescence microscopy. The fluorescent intensities of the cells treated with either C-MWNTs or P-MWNTs were compared to that of the control cells. The results demonstrated that 24h exposure to C-MWNTs reduced phagocytosis of beads by 30-40% versus the control while exposure to P-MWNTs did not, suggesting that 24h pre-exposure to C MWNTs impaired phagocytic function in macrophages. 2. To further investigate the impact of C-MWNTs on macrophages, the second approach will employ GFP expressing *E. coli* as a phagocytic marker. Cells treated with C-MWNTs or P-MWNTs will be washed and then challenged with *E. coli*. The phagocytosed bacteria will be detected using confocal fluorescence microscopy. The difference in bacteria uptake with and without pre-exposure to either C-MWNTs or P-MWNTs will be quantitatively measured using flow cytometry. The approaches developed in this study will facilitate the assessment of potential impacts of various MWNTs on macrophages' defensive function to phagocytose pathogenic bacteria.

2280 Development of a Zebrafish Larvae-Screening Assay to Identify Compounds with Immunotoxicity and Anti-inflammatory Activity

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Zebrafish is a unique model for pharmacological manipulation of the innate and adaptive immune response. They are small and permeable to many small compounds and there are several transgenic lines available to visualize cells from the innate immunity (neutrophils and macrophages) and adaptive immunity (B and T cells). Taking advantage of zebrafish embryo transparency, we can test the toxicity of pharmacological, agrochemical and cosmetic compounds to the immune system by quantifying these cell populations. Additionally, this model can also be used to identify new anti-inflammatory compounds, by following leukocyte recruitment to inflammation induced by sterile tissue injury. We have developed an assay in zebrafish larvae to detect compounds with specific toxicity for the immune system and to screen and identify new anti-inflammatory drugs. For these purposes two transgenic lines have been used: neutrophil-specific Tg *(mpx:GFP)i114* and macrophage specific Tg *(mpeg:mcherry)*. Different reference compounds with known anti-inflammatory effect were chosen and their doses selected after an MTC (Maximum Tolerated Concentration) assay carried out in 3 days post fertilization (dpf) embryos (when the innate immune system is already in place). To determine compounds toxicity at the innate immune system level, embryos were exposed to high dose for 48 h and the population of neutrophils and macrophages was quantified by fluorescence microscopy. Inflammation was induced by sterile injury of the tail fin and neutrophil recruitment to the wound site was assessed at 4, 6 and 12 h post injury in the presence of reference compounds using a partially automated platform. The ability of the compounds to suppress the expression of inflammatory genes (il1b, tnf-a) was also evaluated by quantitative PCR. This zebrafish assay shows to be a cost-effective assay over mammalian models for the identification of new anti-inflammatory drugs as well as for the evaluation of immunotoxicity.

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