ImmunoTools *special* Award 2024

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Study of allergenicity of food allergens in micro and nanoplastic corona

Background: Submillimitere-sized plastic particles (microplastics and nanoplastics) are widespread contaminants present in nature. Initially considered a waste that does not interact with living organisms, it is now well known that these particles accumulate in marine species and can enter the food chain and be distributed to humans through seafood. These particles have been shown to interact with various biopolymers, including proteins, due to their expansive surface-to-mass proportion. The binding of proteins to MPs and NPs is proposed to be multilayered, with proteins tightly bound referred to as hard corona and those more loosely bound as protein soft corona. The binding of proteins to MPs has already been shown to affect protein structure, thereby possibly affecting its functional properties.

Objective: Considering that food allergens represent potential cargo of MPs/NPs and that simultaneous ingestion of MPs/NPs and allergenic food could modulate sensitization and allergic responses, the objective of our work is the investigation of allergenicity of food allergens ovalbumin (OVA) from chicken egg white and shellfish tropomyosin (TPM) originating from microplastic and nanoplastic corona.

Preliminary results: Interactions of purified food allergens OVA and TPM with polypropylene (PP) and polyethylene terephthalate (PET) MPs and NPs of different sizes were investigated. Binding affinities were studied using spectrophotometric techniques, while the formation of protein hard and soft corona was studied using electrophoretic and microscopic techniques. Changes in protein structure upon the interaction of proteins with MPs/NPs were studied using circular dichroism spectroscopy.

Research plan – Implementation of ImmunoTools **reagents**: OVA and TPM soft and hard corona will be isolated and compared with control, native proteins (unexposed to MP/NPs) concerning their behavior in processes that are involved in immunogenicity and allergenicity. Intestinal epithelial transport and uptake and processing in dendritic cells (DCs) and subsequent presentation to T cells of food allergens' corona will be investigated and compared to control proteins that were unexposed to MP/NPs. Additionally, allergen-specific IgE-sensitized basophil cell degranulation capacity of food allergens in MP/NP corona will be investigated.

Epithelial transport of MP/NP corona's OVA and TPM and native OVA and TPM will be investigated using the Caco-2 cell monolayer as a model system for the intestinal barrier. The transport of allergens will be quantified in time by quantification of allergens on the basal side of the Caco-2 cells using ELISA with allergen-specific antibodies and fluorescently labeled secondary antibodies. The uptake of FITC-labelled allergens by bone marrow-derived DCs (BMDCs) will be monitored in time by flow cytometry. Endolysosomal degradation of native allergens and corona allergens inside BMDCs will be investigated and compared through monitoring non-degraded allergens upon phagocytosis of allergens covalently coupled to polystyrene beads using allergen-specific antibodies and FITC/HRP labeled secondary antibodies.

Allergen-specific $CD4^+$ T-cells will be obtained from spleens of mice immunized with corresponding allergen (OVA/TPM). BMDCs from naive mice will be primed with different forms of allergen (native and corona allergen) and co-cultured with freshly isolated $CD4^+$ T-cells. T-cell cytokine response will be followed by measuring the levels of Th1 and Th2-type cytokines IFN-γ and IL-5, IL-10 and IL-13 in co-culture supernatants using ELISA.

Finally, the degranulation capacity of different forms of allergens (native and corona allergens) will be assessed using basophil activation assay as well as humanized rat basophil leukemia (RBL) cells sensitized with allergen-specific IgE.

The obtained results will help elucidate the effects of co-exposure to microplastics and nanoplastics and food allergens.

ImmunoTools *special* AWARD for **Mirjana Radomirović**

includes 10 reagents

FITC - conjugated goat anti-rabbit secondary antibody, goat anti-mouse secondary antibody

PE - conjugated goat anti-mouse secondary antibody

HRP - conjugated mouse anti-rabbit secondary antibody, goat anti-mouse secondary antibody

goat anti-human IgE

recombinant murine cytokines: rm IFN-gamma, rm IL-5, rm IL-10, rm IL-13

DETAILS more AWARDS