**An *in vitro* Three-Dimensional Hydrogel Model to mimic Epithelial and Dendritic Cell Crosstalk in Allergic Sensitization**

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**INTRODUCTION:** The global population is growing and, therefore, the demand for sustainable and novel food proteins is rapidly increasing. However, allergy incidences are expected to rise as well as for newly introduced food proteins. These novel proteins sources will take up a larger share in human nutrition, hence adequate prediction of their tolerability is of importance. Food sensitization assays are mainly focusing on transport of the allergen over the epithelial barrier in two-dimensional (2D) *in vitro* systems. However, these systems lack the three-dimensional (3D) environment as well as the epithelial cell and immune cell crosstalk. Here, we aimed to combine the gold standard Transwell models with a 3D hydrogel environment, that allows studying monocyte derived dendritic cell (moDC) and T cell migration and activation as first events in food allergen intestinal epithelial cell (IEC) activation.
**METHODS:** The human gut epithelial cell line, Caco-2, was seeded and cultured for 21 days on 24 wells Transwell filters. Monocytes were isolated from healthy donor buffy coats and differentiated into moDCs using a GM-CSF and IL-4 cytokine cocktail. After 6 days of differentiation, moDCs were co-cultured in the basolateral compartment, in either medium (2D environment) or a collagen I hydrogel (3D environment). Epithelial cells were pre-incubated with Toxin A, to pre-prime the barrier permeability, for either one hour followed by 48 hours of epithelial exposure to egg ovalbumin allergen (OVA), or were directly exposed to OVA, peanut (Ara h 2) and milk beta-lactoglobulin (BLG) allergens for 48 hours. Basolateral supernatant was collected after 24 and 48 hours of co-culturing and moDCs were collected and characterized using flow cytometry. Barrier integrity was evaluated using transepithelial electrical resistance (TEER) followed by assessment of barrier permeability (4kD FITC-Dextran) and cell metabolic activity (PrestoBlue). Epithelial as well as immune cell derived mediators were measured by ELISA.
**RESULTS:** Food allergens did not decrease barrier integrity (TEER) neither increased barrier permeability in both medium and hydrogel conditions. One hour of Toxin A pre-incubation reduced barrier integrity in both medium ($p<0.05$) and hydrogel ($p<0.05$) cultures and increased permeability in hydrogel cultures ($P<0.01$) after 48h hours, which was not further affected by epithelial exposure to OVA. Epithelial exposure to food allergens Ara h 2 ($p<0.0001$) and BLG ($p<0.001$) alone, however, enhanced type 2 driving Chemokine (C-C motif) ligand 22 (CCL22) concentrations in IEC/moDC hydrogel cocultures. Chemokine (C-C motif) ligand 20 (CCL20) levels remained unaffected. Flow cytometry revealed slightly improved moDC differentiation ($CD14^-$/HLA-$DR^+CD209^+$) in hydrogels, however costimulatory molecule expression was not enhanced on moDCs in medium nor hydrogels in the allergen exposed co-cultures.
**CONCLUSION:** Specific type 2 moDC phenotypes were not observed, however, increased CCL22 production was found in 3D co-culture environments, which is a chemokine known to direct towards type 2 responses. Therefore, further characterization of the moDC's functionality, in medium and hydrogel cultures, towards the induction of a type 2 T cell activation should be evaluated.

**Information for submission:**

2. Topic/ submission categories: Mucosal & other organ specific Immunology