**Using an In Vitro Co-culture Setup to Study the Effects of Dietary Components on Immune Cell Interactions**

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Diet has more and more been recognized as a mean to prevent disease by e.g. influencing the immune system. Food contains a variety of functional components, including dietary fibers, and bioactive peptides. These compounds can come into direct contact with immune cells in the intestine, which may then lead to specific immune effects. However, more research is needed to increase our understanding of the effects of specific food components on immune cell functioning and interactions between immune cells.

In our studies we first investigated the effects of different food components on cytokine production of human intestinal epithelial cells (IECs), dendritic cells (DCs), and T cells in monocultures. Subsequently, we cocultured IECs and DCs to study the effects of food components on their interactions. Finally, we tested the effects of coculture conditioned medium on T cell polarization. Cytokine levels in the supernatant were measured using a Luminex 100 System.

Different food components were found to have different effects on intestinal immune cell functioning. In our first study it was shown that some dietary fibers induced a slightly more tolerogenic status of DCs after stimulation (decreased levels of IL-1RA, IL-10, IL-6, and MCP-1 by arabinoxylan, decrease of MCP-1 by pectin and β-glucan), which was most pronounced when DCs were treated with fiber treated IEC conditioned medium (decrease of IL-1β, IL-1RA, IL-8, MCP-1, MIP1α, RANTES, and TNFα by inulin and pectin, decrease of IL-1β, IL-1RA, IL-8, MCP-1, and RANTES by GOS, and a decrease of IL-1RA, IL-8, MCP-1, MIP1α, and RANTES by arabinoxylan and β-glucan). Conditioned medium of the fiber treated IEC-DC coculture increased the Th1 (increased level of IFNγ, IL-2, and/or TNFα by GOS, inulin, arabinoxylan, or β-glucan) and Treg response (increased level of IL-10 by GOS), and decreased the Th2 response (increased level of IL-6 by GOS and β-glucan). On the other hand, when studying the effects of soy and cow’s milk peptide mixtures in the same setting, it was found that DCs showed a strong pro-inflammatory cytokine response after stimulation (soy and milk peptides increased IL-12, IL-8, TNFα, IL-10, IL-6, MCP-1, MIP1α, RANTES, IL-1RA, and TSLP). However, IECs were only slightly affected by these peptides (only soy peptides increased IL-8 and MCP-1), and also in the IEC-DC coculture the peptides did not affect the cytokine production. Conditioned medium of the peptide treated IEC-DC coculture did also not affect the cytokine production of T cell.

Overall, we showed that the *in vitro* coculture setup is a useful tool to study the effects of different food components on IECs, DCs, T cells, and their interactions, which are the main regulators of intestinal immunity. The gained knowledge may ultimately contribute to improving health by providing the optimal diet to specific target groups.