Abstract

Dissertation Dipl. troph. Eva Sewekow "Sojabohnenprotein P34: Aufreinigung, Verdauung und epithelialer Transport im enteralen Zellkulturmodell"

Many people do suffer from allergies (Bischoff 2006). Allergenic proteins have special properties, which allow them to reach the immune cells of the gastrointestinal tract and elicit adverse reactions. The protein P34 is the most important soybean allergen for humans (Ogawa et al. 1991). In this work, P34 was used as an example antigen to study how antigens reach the cells of the gut immune system. It was of interest to study the immune modulatory effects of P34 on immune cells, and before how P34 survives digestion and is transported in and through enterocytes. As model cells, the IPEC-J2 cell line of polarized enterocytes (Berschneider 1989) and blood derived peripheral blood mononuclear cells (PBMCs) and monocyte derived dendritic cells (MoDCs) from the pig were used.

P34 could be successfully purified with hydrophobic interaction chromatography using a one step elution protocol. Proteins were bound to Butyl Sepharose 4 FF in 0.6 M ammonium sulphate and P34 was eluted with 0.25 M salt in the elution buffer. The purification procedure could be up-scaled twice. The protein survived an *in vitro* digestion procedure. Part products of 20 kDa and the whole protein were found after two hours of pepsin and two hours of pancreatin (and bile) digestion. Antibodies to P34 could be found in pigs sera. P34 was endocytosed concentration- and time-dependent in IPEC-J2 cells and adsorbed to their surface. Adsorption was probably partly dependent on the proteins glycosylation. That P34 is taken up by these enterocytes via caveolae/lipid rafts was found using density gradient ultra centrifugation and raft isolation. The inhibition of this pathway to 44 % was verified with methyl-β-cyclodextrin (MβCD) – a cholesterol depleting substance necessary for caveolae formation. Transcytosis of P34 was possibly affected by MβCD. Finally, P34 increased epithelial IL-6 mRNA, but not IL-8 and TGF-β mRNA concentration dependent. The protein had no influence on the proliferation of PBMC cultures. The antigen presenting MoDCs endocytosed P34 up to a concentration of 100 µg/mL in a dose dependent manner.

In conclusion, purified P34 is a very good model protein with its properties like stability during digestion, glycosylation and size to study the uptake and transport of antigenic proteins and follow their way to immune cells which elicit adverse reactions in the case of allergy. Due to the fact, that P34 antibodies could be found in different pig sera, it can be assumed, that the used *in vitro* models deliver a realistic picture of *in vivo* situations. It could be shown, that P34 passes absorptive enterocytes. No immune modulatory effects could be found in PBMC cultures incubated with P34 which leads to two conclusions: first, P34 addresses to less cells and comes without co-stimulatory molecules and second, P34 was not included into co-culture cell systems where cells interplay. Further steps could involve co-culture systems to study whether P34 induces cell mediated cross-talk and more detailed studies on the receptors with which P34 is endocytosed.

Bischoff, S. (2006): Food Allergies, *Current Gastroenterology Reports*, 8, 374-382

Berschneider, H. M. (1989): Development of normal cultured small intestinal epithelial cell lines which transport Na and Cl, *9th Annual Meeting of the American Gastroenterological Association*

Ogawa, T., Bando, N., Tsuji, H., Okajima, H., Nishikawa, K., and Sasaoka, K. (1991): Investigation of the IgE-binding proteins in soybeans by immunoblotting with the sera of the soybean-sensitive patients with atopic dermatitis, *J. Nutr. Sci. Vitaminol. (Tokyo)*, 37, 555-565.