EAACI Research Fellowship – Final report

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Project title: Systemic effects of atopic dermatitis in skin- and oral-mediated sensitization to food allergens

Type of Fellowship: Medium Term Research Fellowship

Duration: 6 months (1 September 2020 - 28 February 2021)

Location: Research Group for Food Allergy, National Food Institute, Technical University of Denmark, Kongens Lyngby, Denmark.

What questions were addressed and why?

Recent findings implicate the skin as a site of allergic sensitization to food allergens. Several models of Atopic Dermatitis (AD) demonstrate the development of food allergy following topical application of food allergens in the context of AD-like skin inflammation. However, it remains unknown if active AD affects the sensitization to food allergens via the oral route due to systemic effects of the skin inflammation. In individuals suffering from food allergy, intestinal permeability of food allergens is known to be increased.
This study aimed at analyzing changes in allergic sensitization to wheat allergen and the development of clinical food allergy in rats with and without active AD.
In this study, we aimed to answer these research questions:

- Can AD promote food allergic sensitization via both skin and oral routes?
- Is AD-like skin inflammation and oral sensitization associated with changes in systemic, skin and intestinal immune responses and increased intestinal permeability?
- Do active AD-like skin inflammation induce changes in the *ex vivo* immune response to bacterial lysates, food allergens, and M1/M2-polarising cytokines peritoneal-derived macrophages?

What was the nature of the research?

- The studies were conducted in Brown Norway rats that were kept on wheat-free diet for more than 10 generations. AD-like inflammation was induced in the skin using vitamin D3 analogue MC903, and sensitization was induced using Enzyme hydrolyzed gluten (EHG) administrated via the oral and skin routes.

-Allergic sensitization was evaluated by serum EHG-specific IgE using an in-house developed IgE-capture ELISA, ear swelling test, and by evaluating clinical symptoms after oral challenge with EHG. Furthermore, the immunogenicity of EHG was analyzed by EHG-specific indirect IgG1 and IgA ELISAs.

-Numbers of eosinophils, neutrophils, macrophages, T cells phenotypes and B cells were analyzed in blood, skin, small intestine, skin-draining LNs, and mesenteric LNs samples using flow cytometry.

- Mast cells, goblet cells and Eosinophiles was analyzed in small intestine and colon sections using histology staining toluidine blue, PAS and Hematoxylin-Eosin, respectively.

-Investigation of the intestinal permeability was assessed by protein uptake evaluation using a commercial BLG ELISA kit, in serum, as well as, in fractions of the small intestine (Peyer's patches, lamina propria, and epithelium), after *in vivo* challenge by Whey protein extracts.

- Peritoneal Macrophages were harvested by lavage of the peritoneal cavity, then cultured and stimulated *ex vivo* using bacterial lysates, food allergens, and M1/M2-polarising cytokines. Furthermore, the immune response to these stimuli on M1/M2 polarisation was analysed by flow cytometry and pro-inflammatory and regulatory cytokines levels were assessed using commercial cytokines ELISA Kits.

What was the result?

AD was found to promote sensitisation to EHG via the skin route. Sensitization to EHG via the oral route was not achieved in this model.

Increased AD-mediated skin sensitization was associated with an increased ear swelling response to EHG, and increased numbers of clinical symptoms following oral challenge with EHG.

AD had little effect on the composition of immune cells in the skin, blood and intestine. Interestingly, AD was found to promote Treg cells proliferation and activation in the skin.

Peritoneal macrophages derived from rats with AD exhibited alterations in the response to bacterial lysates compared to macrophages from rats without skin inflammation.

AD was associated with altered protein uptake in the intestinal compartments.

How will the findings impact future research?

Our findings show that AD can promote allergic sensitization to wheat via the skin. AD drives alterations in systemic immune function and intestinal permeability, which rise interesting research questions:

How AD is implicated in the induction of food allergy sensitization?

Does AD induced changes in microbiota composition in the skin can lead to changes in microbiota composition in the gut which lead to food tolerance disruption?

Personal reflection on what I have learned and how we can improve for the future

This fellowship gave me the chance to be part of the Research Group for Food Allergy at the National Food Institute at the Technical university of Denmark and have permitted this successful collaboration. I have learned so much on allergy animal model development and I was able to contribute to the group work with my ideas and scientific vision. This stay couldn't have been possible without EAACI contribution as well as Katrine Lindholm Bøgh and Jeppe Madura Larsen's supervision. It was a very enriching stay both scientifically and personally.

Acknowledgments

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I thank EAACI for believing in me and giving this opportunity to improve myself as a researcher as well as to have this experience which opened my mind to other horizons of allergy research.