The goal of this project was to investigate the role of antigen presenting cells, dendritic cells (DCs) and Langerhans cells (LCs), in the complex intercellular communication network of the skin. We planned to determine the receptor-arsenal of *in vitro* differentiated DCs and LCs with Next-Generation Sequencing, and to use these generated data to find possible targets of keratinocyte- and sebocyte-derived factors. The putative communication between these cells is bidirectional, so we also planned on finding LC and DC-derived factors that influence the major biological functions of keratinocytes and sebocytes.

In the first year in the context of Task 1, we planned to expand and validate the preliminary results from the parallel FK_17 grant (FK 125053), where we had already investigated the receptor arsenal of skin APCs. In these preliminary experiments we focused on receptors that were found to be of interest in dermatological disorders, and many of the investigated genes showed minimal or no expression in either dendritic cells (DCs), or Langerhans cells (LCs). To validate and expand the possible receptor arsenal that could be investigated in subsequent experiments we also performed an extensive RNASeq analysis of donor-matched immature DC and LC cultures. The RNASeq data supported our preliminary results regarding the limited number of receptors we have already investigated, while also giving is a very broad overview of the total exome of these cells: with this data we systematically identified receptors of interest both in our current and project, and as a starting point for possible future investigations. We found that in LCs and DCs differentiated from the monocytes of the same donor the LC marker CD207 shows greatly increased expression in our LC cultures, supporting the validity of the model. We also found that although our LCs typically express higher levels of proinflammatory cytokines (e.g. IL1A, IL1B, IL12B, CXCL8), the expression of costimulatory molecules (CD80, CD86) is not as high as we would expect if the cells were already mature antigen presenting cells. Perhaps the most striking result in this respect is that our LCs express around half as much CD74 as immature dendritic cells, which suggests that these cells are still at least partly immature.

During our experiments in this period, we decided to focus on the most promising receptor uncovered by the above experiments, namely TRPV4. TRPV4 is part of a group of thermosensitive transient receptor potential ion channels that, while nonselective cation channels, are mostly calcium permeable. TRPV4 is an especially interesting candidate to influence LCs' function, since two of its major activating factors (hyperosmolarity and UV radiation) are characteristically found in the epidermis, where they reside under steady state conditions. In the first year of the FK_17 project we showed that the activation of TRPV4 with its specific agonist GSK1016790A dose-dependently increased the intracellular calcium concentration of Langerhans cells in the nanomolar range, which effect could be completely abrogated by the addition of a TRPV4-specific antagonist HC 067047. In long term treatments we found that TRPV4 activation also stimulated the differentiation of LCs by effectively doubling the expression of CD207 found on the cells.

Continuing these experiments, we investigated the secretome of our cells with the help of cytokine arrays. We identified several proteins secreted by LCs upon their stimulation with peptidoglycan, and have started the validation of these results by ELISA, as well as the investigation of the effect of TRPV4 activation both in parallel with peptidoglycan and if used as a singular treatment.

In parallel to Task 1 we also began our experiments under Task 2 as planned in the grant submission. In these experiments we investigated the role of factors secreted by DCs and LCs on sebocytes and keratinocytes respectively. In the first year we determined the proper dilution of conditioned media to use without influencing the viability of these cells, and we collected conditioned media from 12-14 donors (depending on the cells type).

As well as the experiments detailed above we also completed experiments related to previously started projects. In these experiments we showed that the expression of pattern recognition receptor TLR3 increases in keratinocytes in dermatoses characterized by chronic itch, and that the activation of these receptors results in the production of inflammatory cytokines such as IL-6 and IL-8, which can influence the functions of neighboring immune cells. We showed that the endothelin produced by keratinocytes activates nerve endings in their close proximity, and that this may form the basis of a positive feedback loop that underlies chronic pruritus. The manuscript based on these results was submitted and accepted in the current period to the Journal of Investigative Dermatology.

During the second year of the project we continued our research into the complex intercellular communication network. We continued our work on the first and second tasks as per the research plan, with some modifications based on our RNASeq results from the first year. According to our results monocyte-derived dendritic and Langerhans cells express several distinct receptors that can respond to sensory nerve ending-derived peptides (neuropeptides). Since Langerhans cells are commonly associated to free nerve endings in the epidermis in human skin, we expanded the focus of our research to neuropeptides to supplement our work with keratinocytes and sebocytes. We validated the expression of neuropeptide receptors found in our RNASeq results (RAMP1, CALCRL, SORT1, NPR1) on Langerhans cells with RT-qQPCR. To assess the functionality of these receptors we treated DCs and LCs with agonists of these receptors (calcitonin gene related peptide, neurotensin, B-type natriuretic peptide [BNP]), and observed their effects on the differentiation and maturation of the cells. Of the tested agonists BNP had the most striking effect, as it was capable of significantly increasing both the differentiation and maturation of Langerhans cells. In parallel to these exciting results we also continued our research into the role of the TRPV4 ion channel on Langerhans cells, the results of which presented in an international conference (49th Annual European Society for Dermatological Research (ESDR) Meeting Bordeaux, France 2019. September 18-21). We also investigated the effect of plant derived cannabinoids on the maturation and differentiation of monocyte-derived dendritic cells, which we presented at a national conference (48th Annual meeting of the Hungarian Immunological Society, Bükfürdő 2019. October 16-18.). Although this latter result is not directly outlined in the research plan, the results of these investigations are especially interesting in the context of skin-resident antigen presenting cells, since the phytocannabinoids tested (cannabidiol, cannabigerol, cannabinol and tetrahydrocannabinol) are increasingly common in topical treatments for dermatitises. Further information about their effect on the orchestrators of the immune response in skin, i.e. DCs and LCs, is of paramount importance to understand their possible therapeutic applications.

Also in the second year we further characterized the effect of TRPV4 agonism and antagonism on LCs. We validated the results of our cytokine array experiments with specific ELISAs (proving that the cells produce IL-6, CXCL-8, MIF, MMP-9, CCL-17), and have found that the overall TRPV4 agonist treated cells tend more toward anti-inflammatory, rather than a classical

inflammatory phenotype. This data was also supported by the decreased T cell stimulatory capability of the cells after TRPV4 activation.

In the context of Task 2 we have performed multiple indirect coculture experiments using conditioned media from both dendritic cells and Langerhans cells on keratinocyte and sebocyte cultures. Surprisingly the conditioned media has only minimal effect on the experimental endpoints outlined in the original research plan, and due to the high degree of inter-donor variability in our antigen presenting cell cultures not many of these modest effects observed were reproducible multiple times. Although this setback is unexpected, the shift towards neuroimmune interactions as detailed above managed to fill this void in the research plan. Indeed, although the FK-17 grant that supports this PD-18 grant was concluded at the end of the second year we were successful in securing an FK-20 grant that focuses on antigen presenting cell-nerve interactions (FK-134993).

In addition to the above detailed progress two co-authored papers were submitted in this period. The first is a review on the role of necroptosis (published in Cell Death and Disease, IF: 6.484), a type of programmed cell death, which could a significant factor in the development of dermatitises characterized by increased keratinocyte proliferation (e.g. psoriasis). The second publication is a research article (published in Biochemical Pharmacology, IF: 5.009) about the role of transient receptor potential melastatin 3 in the transduction of the effects of volatile anesthetics.

In the final year of the grant, we focused on finishing Tasks 3 and 4 based on the results from the previous year. We investigated the role of keratinocytes in inflammatory skin diseases on patient samples from atopic dermatitis patients. Biopsies taken from lesional and non-lesional skin of patients showed increased expression of TRPV3 in both sites. We also established keratinocyte cultures from non-lesional shave biospies, and found that not only was the expression of the channel higher, but ion currents determined with patch clamp were also significantly increased compared to healthy control cells. These results mark a significant step in our understanding of the pathological processes behind the development of atopic dermatitis lesions, and therefore a manuscript was submitted as a Concise Communication to Experimental Dermatology, a Q1 journal in the field of Dermatology.

In parallel to the above detailed experiments, we also completed our experiments on the TRPV4 ion channel on Langerhans cells, and have begun preparing a manuscript based on these results.. Expanding previous years' work we looked at the effect of TRPV4 activation on maturation, and found that neither the endocytic activity of the cells nor the expression of a costimulatory molecule was significantly affected by the TRPV4 agonist. Activation of TRPV4 mostly reduced the secretion of both inflammatory cytokines and allogeneic T cell proliferation. Based on these, and previous results TRPV4 supports the development and maintenance of a "resting state" on these cells. The manuscript under preparation is planned to be submitted to the D1-rated Journal of Investigative Dermatology later this year.

Finally, we also performed experiments on a specialized area of the integument, in which we investigated the role fo the endocannabinoid system on human corneal epithelial cells. We showed that, as in the skin, these cells are capable of synthesizing and degrading endocannabinoids, as the enzyme apparatus required for this is found both *in vitro* and *in situ* on the cells. The effect of the prototypical endocannabinoid anandamide was studied in inflammatory models, where it was

shown that, despite previous hypotheses, endocannabinoids are not necessarily anti-inflammatory, and may be proinflammatory under certain conditions. A manuscript summarizing these results was published in the Q1-rated International Journal of Molecular Sciences (IF: 5.923).

A total of six research articles were published over the course of this PD grant, in two of which the applicant was the first or last author. In addition, there are two more articles that are near acceptance or submission in which the applicant will be the last, or shared last author. With the help of this grant many promising new results were produced, which formed the basis of a new FK project (FK 134993).