EAACI Research Fellowship Final Report

Project title: Susceptibility factors for herpes simplex infections in patients suffering from atopic dermatitis

Research fellow: Kristin Boykova Boteva, EAACI Junior Member

Type of fellowship: Short-term research fellowship

Host supervisor: Professor Thomas Werfel

Location: Division of Immunodermatology and Allergy Research, Hannover Medical School, Hannover, Germany

Duration: 3 months (mid-August 2020 – mid-November 2020)

Questions addressed

The aim of my work in the undertaken project is to characterize cellular immune responses towards herpes viruses (namely Herpes Simplex Virus 1 and Varicella Zoster Virus) in patients with atopy versus patients without atopy within the frame of patient cohorts as part of the Cluster of Excellence RESIST (EXC 2155, Hannover Medical School, Hannover, Germany; https://www.resist-cluster.de/en/) that aims to investigate infection susceptibility in vulnerable patients.

Individuals suffering from atopic dermatitis(AD) are often more susceptible to viral infections. A subgroup of patients with AD have increased susceptibility towards Herpes Simplex Virus (HSV-1) which can result in recurrent, severe and disseminated infections (''eczema herpeticum''). In previous studies at the Division of Immunodermatology and Allergy Research, Hannover Medical School, involving affected patients, increased frequencies of Th2 cells directed towards HSV-1 were found. This suggests that an atopy-biased inflammatory milieu in these patients leads to a probably ineffective immune response (Traidl et al. 2018).

Varicella Zoster Virus (VZV) is another widely spread herpes virus (>90 % people worldwide infected) whose reactivation (especially in the elderly) can alongside with shingles lead to postherpetic neuralgia, uveitis, encephalitis and vasculitic stroke. So far, two VZV vaccines are available. While a live vaccine with an attenuated strain leads to protection in about 51 % of cases, a new vaccine based on a recombinant protein in combination with two adjuvants works significantly better (>90 % protection). However, a subgroup of subjects who did not respond in clinical studies remains and this group has not been characterized to date.

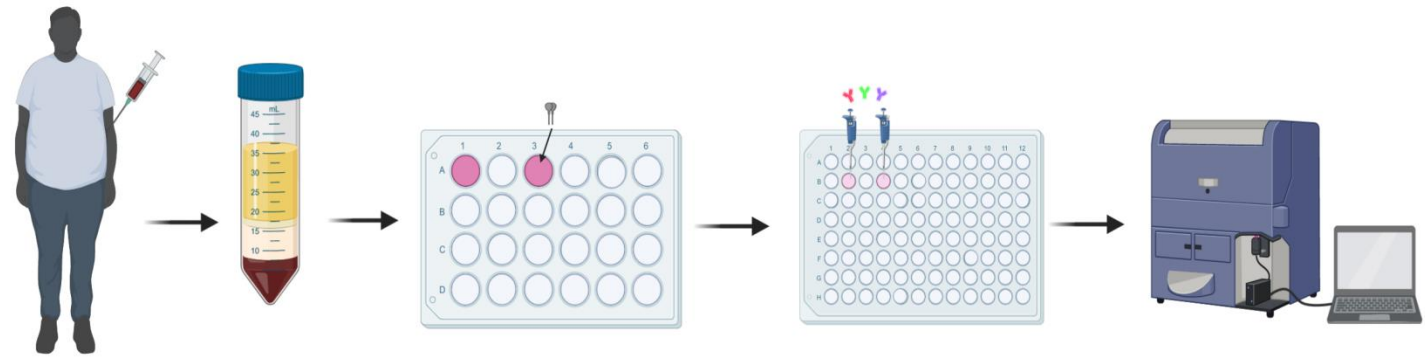
We would like to find and further describe how the atopy-biased immune system can contribute to severe herpes virus manifestations like eczema herpeticum and severe forms of VZV.

Nature of research and work course

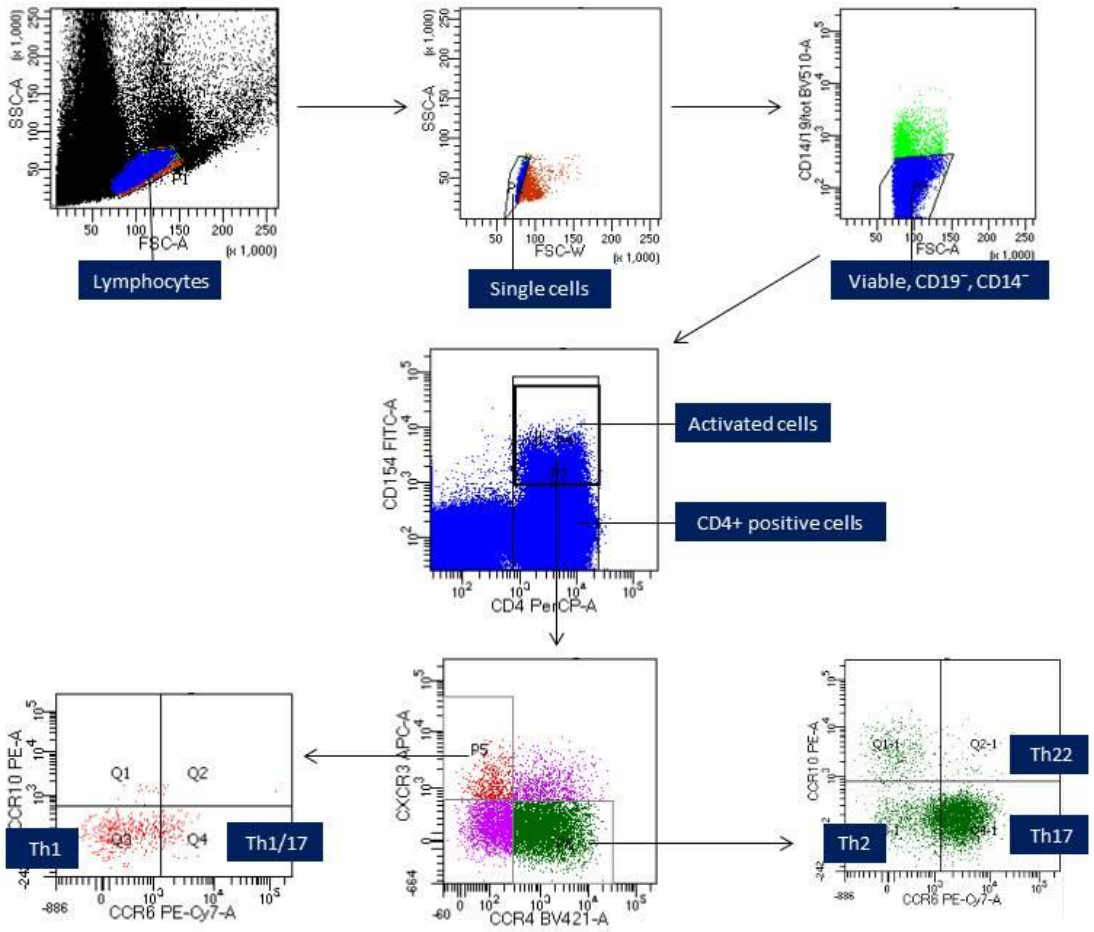
Focusing on the patient cohorts of the RESIST cluster, the nature of the research was descriptive. We stimulated peripheral blood mononuclear cells (PBMCs) of the patients from the HSV cohort and the VZV cohort with respective virus antigens. Then we detected via flow cytometry the specific T helper cells by the upregulation of CD154 (CD40L) and characterized them via surface markers. Then we calculated the percentage of activated Th1, Th2, Th17, Th22 and Th1/17 cells.

The protocol in brief followed the order:

- Blood drawal from cohorts' patients
- Isolating PBMCs via Ficoll gradient
- For HSV cohort patients: overnight stimulation of cells with HSV-1 gD recombinant protein (in the presence of CD40 Antibody for extracellular CD40L stabilization) and preparing a non-stimulated control;
  For VZV cohort patients: overnight stimulation of cells with VZV gE recombinant protein (in the presence of CD40 Antibody for extracellular CD40L stabilization) and preparing a non-stimulated control
- Surface staining of cells with fluorescently-labelled antibodies for flow cytometry analysis
- Flow cytometry analysis (BD FACSCanto II)



Our gating strategy in FACS Diva Software (on a VZV gE-stimulated sample) looked like this:



The data from the flow cytometry analysis was used to calculate the percentages of cells of interest in the unstimulated control versus the specific-antigen-stimulated sample. Then several graphs in GraphPad Prism were created (not shown for reasons of confidentiality for future publications in this report) and the next step is to correlate the laboratory findings with the clinical characteristics of patients by using the extensive questionnaires and physician assessments filled on patients' visits.

In November we shifted from using conventional flow cytometer (BD FACSCanto II) to using spectral flow cytometer (Cytek Aurora). This machine captures the whole spectral signature of a fluorochrome and enabled us to design a panel with 22 markers and to extend our analysis to include also NK cells, NKT cells, T cell subtypes (CD4+, CD8+, T_{FH}), T cell differentiation (naïve, central

memory, effector memory, T_{EMRA}), activation markers, exhaustion markers and skin-homing markers.

The atopic status of the patients was checked by Thermo Fisher Scientific ImmunoCap Specific IgE towards inhalant allergens.

Results

We performed the experiment for 21 patients from the HSV Cohort and for 34 patients from the VZV Cohort and calculated the frequencies of specific Th cell subtypes.

As stated above, the results are to be further correlated to the clinical features of subgroups of patients exhibiting different disease severity and to their atopic status. Due to data protection reasons we are expected to start this next part of the project in January 2021.

Impact of the findings on future research

As mentioned above, the project is part of the Cluster of Excellence RESIST (Resolving Infection Susceptibility), which is a collaboration between Hannover Medical University and 5 other institutions and consists of 40 research groups that investigate different aspects of infection with major pathogens and immune system response. Physicians working with patients in the clinic and researchers collaborate for translational research outcomes. In the case of our project, we are characterizing the immune responses of patients in the Herpes Simplex Virus Cohort and Varicella Zoster Cohort towards HSV-1 and VZV. However, there are also further investigations performed with the same patients. Our measurements are supposed to be worked into a larger set of different experimental approaches, thereby building up a big data set including multiple omics data (transcriptomics, proteomics, virus and host genomics, epigenomics, bacterial metagenomics). Additionally, patients are filling in extensive questionnaires regarding lifestyle, previous and accompanying diseases and infection susceptibility and physicians are performing detailed checks on them. Further down the line of the project big data scientists will be involved to correlate and summarize the results from these research segments.

Adaptation from original plan

The start of my work in the lab was postponed by 3 months due to the COVID-19 pandemic. Participation of the Division of Immunodermatology and Allergy Research at MHH in the Cluster of Excellence RESIST allowed for research being performed on a grander scale. Instead of handling patients that have only AD and history of eczema herpeticum, the project was extended to all the patients in the Herpes Simplex Virus Cohort and the Varicella Zoster Virus Cohort. Because of the

opportunity to work on a spectral flow cytometer and to extend our analysis beyond T helper cells, we didn't follow the initial plan to include in the protocol the ARTE (Antigen-reactive T cell Enrichment) Technique. After a pilot experiment phase, we optimized the ARTE workflow to identify antigen-specific T cells by CD154. Precisely, we extended the number of cells being analyzed per donor (10 x10^6) and in parallel omitted the magnetic enrichment.

Personal reflection

Undertaking a project in the field of immunology has contributed to vast expansion of my understanding of the pathogenesis and treatment options of not only AD but all the diseases involving the immune system. I learned the technique of flow cytometry which is profound in the field of immunology, as well as to analyze the generated data in software like BD FACSDiva, SpectroFlo and FlowJo. Alongside with upgrading my lab skills, I broadened my horizons on the research designs and methods used up to date in the area of immunodermatology by participating in the department seminars and in the Journal Club with the members of our research group. Attending German classes on the campus of Hannover Medical School, I upgraded my language level so that I would be able to work with the database of RESIST questionnaires to connect our laboratory outcomes with the clinical findings. My work in the department continues after the end of the EAACI Fellowship with the goals of generating substantial results and completing a doctoral degree.

Acknowledgements

I would like to thank Professor Thomas Werfel for supporting my application and providing an interesting project with many learning opportunities. I am glad I joined the Division of Immunodermatology and Allergy Research which has an outstanding reputation in the field of AD and got the chance to absorb some of their experience. Special thanks to Dr Lennart Rösner who as immediate supervisor has initiated me to the lab protocols, spent time on introducing me to the complex method of flow cytometry and pointed me to the prominent literature pieces in the field. Thanks to Petra Knielin and Gabriele Begemann who have been constant support in the daily lab tasks. Acknowledgements to the researchers and students at the department for the fruitful discussions and ideas.

Finally, but yet as importantly, I would like to thank EAACI for enabling me to take on this experience and for the many other opportunities it provides junior members. I would strongly recommend both basic scientists and clinicians to participate in an EAACI Research Fellowship because with a good choice of project they would gain plenty of valuable insights to use in their further work, regardless if it is in the lab or in the clinic.