The following is the final report regarding my 3-month EAACI Research Fellowship. This was conducted from January 14th to April 12th, 2019 in the Department of Immunology at Hospital Fundación Jiménez Diaz, Madrid, Spain at the group of Dr. Vanesa Esteban.

**The questions addressed**
During anaphylaxis, endothelial cells (ECs) and smooth muscle cells (SMCs) in the blood vessel wall are activated to increase vascular permeability and vasodilation. Murine studies suggest that increased permeability primarily originate from microvessels i.e. ECs but whether this is also the case in humans is not yet known. It is also not known whether SMCs from different vessels (i.e. artery and vein) respond differently and impact the response of the ECs. Therefore, the aim of this research fellowship was to test if we could detect unique permeability patterns of ECs and SMCs from different human vasculature in response to known anaphylaxis mediators. Understanding this can help gain fundamental knowledge of the vascular permeability taking place during anaphylaxis in humans.

**The nature of the research**
Dr. Esteban and her group have long experience with isolation and culturing of cells from different vasculature tissues and have established several protocols for in vitro assessment of permeability, in order to investigate mechanisms controlling vascular leakage during anaphylaxis. For this project, a system was set up using transwells and FITC-labeled dextran beads. In this system, monolayers of either aortic ECs (+/- SMCs), ECs from vena saphena (+/- SMCs), or ECs from microvessels (either lung or dermal) was used. After monolayer formation, these were then stimulated with histamine, PAF or serotonin or no stimuli combined with FITC-Dextran and incubated for 60 min. Levels of FITC-Dextran was measured from lower compartment at 5 min, 30 min and 60 min and levels of FITC was read using a fluorescens plate reader.

**Overview of the final experimental setup**

<table>
<thead>
<tr>
<th>Isolation of cells*</th>
<th>2 Seed of cells</th>
<th>3 Cell monolayer formation</th>
<th>4 Stimulation</th>
<th>5 Measurements</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microvessels (lung, dermal)</td>
<td>Artery</td>
<td>EC</td>
<td>Semipermeable membrane</td>
<td>Mediators (PAF, histamine, serotonin)</td>
</tr>
<tr>
<td>Vein</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

---

Page 1 of 2
**Overview of the obtained results**
The first goal was to learn how to isolate, maintain and grow ECs and SMCs as well as setting up the transwells for testing.

After this, time was spent on adjusting the methods to best fit the experiments of this project. This included testing:

- the best method to measure the permeability (Transepithelial/endothelial electrical resistance (TEER) vs FITC-Dextran)
- the optimal concentration of SMCs
- the optimal days for monolayer formation
- the optimal concentration of each of the stimulants

Finally, the experiments were run for the different ECs (and SMCs). The huge amount of data acquired from these experiments is still being processed and plans for future experiments with the aim of a future publication are on their way.

**Changes from original plan**
Compared to the original plan, few changes were implemented. In the original plan, we intended to only test the two mediators PAF and histamine. As we later discovered both ECs and SMCs have the potential to highly respond to serotonin, we also included this mediator in our setup. On the other hand, we originally planned to also test response to serum from anaphylactic patients. This was omitted as we first wanted a clear view of the response to different anaphylaxis mediators (histamine and PAF) before testing with serum, which we did not have time for in the three months available. The plan was to measure the permeability with both TEER and FITC-Dextran. In our initial tests, we however found the TEER too unstable (due to the fairly low resistance in the EC monolayers) and this was therefore removed from the final setup. Finally, the original plan was to run test of three different types of ECs (from artery, vein and lung microvessel), n=5 for each. Due to high variation in the experiments, this was increased to n=6 for all three. Also, ECs from dermal microvessels were also included (n=3) to test if ECs from lung microvessel and dermal microvessel had similar response patterns.

**Future research**
Some of the responses found were unexpected. Therefore, we are currently trying to investigate the reasons for this further, possibly by including more experiments and testing these cells using other methods. This will both be in our laboratory in Denmark, but also still in close collaboration with the group of Dr. Esteban.

**Concluding remarks**
These three months have given me a great insight into the dynamics of the vascular wall as well as handling and growth of ECs and SMCs of different types of human blood vessels. I have also gained an increased knowledge into the possibility of testing permeability of these and some of the obstacles to be aware of in setting up these experiments. This knowledge will be very useful in designing future experiments regarding permeability. Also, the data acquired will lead to more research in this area in our group to further understand some of the processes taking place in the vascular wall during anaphylaxis.

I would like to finish off by deeply thanking Dr. Esteban and her group for welcoming me and helping me with this project. It has truly been a pleasure to be part of their team for three months. I would also like to thank EAACI for choosing this project to receive the fellowship thereby enabling this project to happen.