Final Report – Jelle Folkerts
EAACI Long-Term Research Fellowship
12 Months (+ 3-month extension)
Erasmus MC, Rotterdam, The Netherlands.

The aim of this Long-Term Research Fellowship was to identify the mechanisms underlying the effects of butyrate on mast cell function, by generating genome-wide data sets of SCFA-dependent epigenetic control of gene expression. The obtained data will be brought back to the **home institute** and further utilized, e.g., by computationally integrating these data sets with asthma-associated genes derived from genome-wide association studies (GWAS) to directly link candidate butyrate-regulated genes or pathways in mast cells to allergic asthma and identify putative novel therapeutic targets to suppress mast cell activity.

In earlier studies we identified that butyrate treatment inhibited allergen-induced histamine release and airway contraction in guinea pig PCLS. Propionate and butyrate, but not acetate, inhibited IgE- and non–IgE-mediated human or mouse mast cell degranulation in a concentration-dependent manner. Notably, these effects were independent of the stimulation of SCFA receptors GPR41, GPR43, or PPAR, but instead were associated with inhibition of histone deacetylases. Transcriptome analyses revealed butyrate-induced downregulation of the tyrosine kinases BTK, SYK, and LAT, critical transducers of FcεRI-mediated signals that are essential for mast cell activation. In our initial epigenome analyses we found that butyrate redistributed global histone acetylation in human mast cells, including significantly decreased acetylation at the BTK, SYK, and LAT promoter regions.

To better understand and characterize the pathways between the butyrate-induced inhibition of HDAC activity (within 1 hour) and reduced mast cell activation (after 24 hours), we aimed to:

- Analyse butyrate-induced histone modifications in resting and anti-IgE stimulated human mast cells, using **ChIP-Seq**.
- Assess if butyrate-induced epigenetic changes cause altered genome-wide chromatin accessibility, using **ATAC-Seq**.
- Confirm that butyrate-induced histone modifications and chromatin accessibility alterations result in different gene expression profiles, using **RNA-Seq**.

Unfortunately, obtaining useful ATAC-Seq samples from primary human mast cells did not succeed in our hands. Yet, we were able to perform successful ChIP-Seq experiments using the epigenetic markers H3K27Ac and H3K4me3 after 0, 3, 12 and 24h of butyrate treatment. In addition, we obtained RNA-Seq profiles on the same time-points, although we only analysed 0h vs. 24h butyrate in our initial studies.

In brief, we found that butyrate increased global acetylation in primary human mast cells, but decreased acetylation at selected TSS regions of the genome. Additionally, and in accordance with recent literature, butyrate preferentially targeted genes that are under control of super enhancers. Butyrate did not have any significant effects on methylation at any of the measured timepoints. Interestingly, butyrate downregulated many genes pivotal to Fc-receptor mediated mast cell activation. Acetylation at the TSS and Super Enhancers regulating

these genes were significantly downregulated. Counterintuitively, acetylation at the TSS of upregulated genes was also slightly reduced. Yet, peaks associated with such genes, which were not on a TSS, were significantly enriched. Taken together, butyrate downregulates the expression of genes associated with Fc-receptor mediated mast cell activation, likely via strong downregulation of acetylation at the TSS of such genes, while upregulation of genes is likely to be caused by increased acetylation in the enhancer landscape.

Therefore, known health benefits of SCFAs in allergic disease can, at least in part, be explained by epigenetic suppression of human mast cell activation. Although counterintuitive, inhibition of HDAC activity by butyrate downregulates acetylation at TSS of a selected group of genes. Further studies are needed to confirm why butyrate has a preference for these genes, and an effort should be made to apply our findings in the development of new therapeutics targeting the epigenetic suppression of human mast cell activation.

During the Long-Term Fellowship I learned how to prepare and preform a ChIP-Seq and RNA-Seq experiments. More importantly, I learned how to analyse such data sets on the computer, calculate significance and compare the different conditions we tested. Although guides are available to learn such complex types of analyses, my host-supervisor, who is an expert in epigenetic analysis, greatly catalysed my efforts to acquire the required skills needed for this project.

I wish to thank the host-institute and EAACI for my fruitful and productive year at the Erasmus MC in The Netherlands.