SYNOPSIS

Autism Spectrum Disorders (ASD) constitutes a spectrum of developmental disorders that share common phenotypes of social and communication deficits and repetitive behaviours. ASDs have many possible underlying causes including genetic and environmental factors. One of these factors is infection in the mother during gestation. Specifically, an increase in cytokines in the maternal circulation during pregnancy has been linked to ASD-like phenotype of male offspring in mouse models of maternal immune activation. Although, it has been established in the scientific literature that maternal cytokines can exert such detrimental effects on embryonic brain development and blocking the binding of cytokines to their receptors ameliorates this effect, the molecular mechanism and details of this signalling process are not yet clear.

The objective of the thesis was to ascertain whether inflammation in the mother can directly alter gene expression in the embryonic brain early in development and thereby lead to ASD. We started the study with the specific aim of setting up a working mouse model of MIA at gestation day E9.5, as this is the temporal window when gene expression in the embryonic brain corresponding to the start of neurogenesis occurs. We tested two models, each based on an immunogen viz. LPS and Poly IC. LPS is a component of bacterial cell membranes and thus acts as a proxy for bacterial infections whereas Poly IC is structurally similar to double-stranded RNA and acts as a proxy for viral infections.

We observed a significant increase ofcytokines in the plasma and liver of female mice 3 hours after injection of either LPS or Poly IC, thereby indicating a maternal immune response. We also observed an increase in the mRNA of the cytokine IL-6 in embryonic brains 24 hours after a 0.5mg/kg per body weight injection of LPS to pregnant females at E9.5. However, with the LPS based model we observed high degree of embryonic and perinatal mortality whereas with a dose of 2mg/kg body weight of Poly IC, mortality was much less. Therefore, further experiments on embryonic gene expression were only performed with the PolyIC based model. Two month old male offspring from Poly IC injected mothers were screened for social novelty preference in a 3-chamber behavioural set-up and

we observed that they show reduced social preference for a novel animal – a hallmark of ASD in mouse models.

Thereafter we began a pilot gene expression study, where we determined the expression of the following genes in the embryonic brain in response to MIA: BWR1 (Also known as Tssc3 or Ipl) which encodes a cytoplasmic protein containing a PH-domain which is expressed in the placenta, ATG5 which codes for a protein involved in autophagy, NEUROD2 which codes for a transcription factor that controls expression of neuron-specific promoters and plays a role in neuronal differentiation, and SMARCA4 which codes for a helicase that controls chromatin remodelling which is an important process controlling the expression of other key genes. We observed an increase in Neurod2 mRNA and a decrease in Smarca4 mRNA in the embryonic brain 24 hours after injection of 2mg/kg body weight of Poly IC to the mother at E9.5. We subsequentlywanted to determine if this difference in gene expression is only evident at 24 hours or lasts beyond this time point and we observed that thisdifference in expression was not observed for any time points beyond 24 hours that we studied viz. 48, 72 and 144 hours. Similarly we did not observe any difference in gene expression in the cortex of P0 neonates suggesting a transient change in embryonic gene expression post Poly IC induced inflammation in the mother that doesn't seem to last beyond 24 hours with the dose of PolyIC that we have used. This experimental resulttaken together with the results from social interaction studies of the offspring indicates that transient gene expression changes during embryonic development can have far reaching consequences on adult social behaviour.

Since it became clear from the study that Poly IC induced maternal immune activation can alter gene expression in the embryonic brain, we decided to further elucidate this process on a global scale. Therefore, we analysed the total mRNA profile of the embryonic brain by high throughput RNA sequencing at E10.5, i.e. 24 hours after pregnant females were injected with 2mg/kg body weight of Poly IC. We determined the differential gene expression profile of the embryonic brain from Poly IC injected and control pregnant females and observed a significant up-regulation of 12 genes and a significant down-regulation of 54 genes. Some of these genes possess well established roles in embryonic development and synaptic function, such as NetrinG1 and Neurexin1. Mutations in some of the genes which are down-regulated have been recently associated with cognitive disorders

and ASD. Some of the ASD associated genes for which we observed differential expression are Astn2, Ntng1, and Pcdh10. A few other differentially expressed genes have been linked to ID and schizophrenia in the literature e.g. Pde7b and Cdk5r1.We also performed a pathway analysis for investigating pathways that are affected by MIA and observed an effect on pathways associated with embryonic brain development, synaptic signalling and metabolism. A number of key developmental processes such as axon guidance, formation of cell-cell junctions and adhesions, and synaptic assembly and maturation seem to be affected in varying degrees. Interestingly, we observed a decrease in the expression of Frizzled and Notch that are involved in the Wnt signalling pathway and dorso-ventral axis formation, both of which are vital for embryonic development.

Taking all these findings into consideration, we have reached the conclusion that a single inflammatory prompt in pregnant females coinciding with the onset of neurogenesis in the embryos can perturb the expression of a number of different genes that are a part of the embryonic development program, which is under a complex regime of temporal and spatial regulation that ultimately lays down the foundation of the architecture for a fully functional adult brain in the future. Additionally, if the inflammatory prompt islargeenough, it can affect the development program to such a great extent that it becomes fatal for the embryo. Even within the same litter, the effect of MIA on the embryos can vary depending on the health and immediate environment of the individual embryo at the time of MIA such that some embryos might die off *in utero* while others survive but with permanent developmental deficits. This is reflected in the reduced litter size of MIA mothers.

Therefore, a single inflammatory prompt at E9.5 in pregnant mice, affects the health and development of the embryos. LPS induced MIA leads to high embryonic resorption and perinatal mortality whereas MIA induced by 2mg/kg body weight of Poly IC leads to a reduction in the litter size and adult offspring display deficit social novelty preference indicating an ASD phenotype. Upon investigating gene expression, we found that MIA alters the expression of a number of genes in the embryonic brain that are important for neurodevelopmental processes such as neurogenesis, differentiation, cell migration and axonal guidance and for maintaining synaptic integrity. Thus, maternal immune activation during the onset of neurogenesis can potentially alter gene expression in the embryo and affect the course of neurodevelopment such that it leads to ASD.