Protection of the preterm brain against inflammatory stress

Summary

Improved perinatal care increases survival of preterm infants and lowers the gestational age of survivors. Decreases in gestational age are associated with higher mortality and morbidity of preterm infants, including cerebral (Encephalopathy of prematurity, EoP), gastrointestinal and respiratory disorders. Morbidities cause enormous psychological and economic burden extending into adulthood.

Independently of preterm delivery, a crucial driving force behind the etiology of adverse outcomes in pre-term neonates are acute inflammatory processes. Immune triggers include antenatal infection (i.e., chorioamnionitis), hypoxia-ischemia (HI), and different postnatal injurious triggers (i.e., oxidative stress, sepsis, mechanical ventilation and hemodynamic instability). Systemic inflammation communicates via brain barriers, initiating a neuroinflammatory response that eventually results in detrimental structural changes and injury of the developing fetal brain. Thus, we hypothesized that novel therapies should balance systemic and local inflammation, protect brain barriers and act regenerative. Mesenchymal stem cells or their extracellular vesicles (EVs) meet those criteria.

The aim of this thesis was to test cell-based therapies and identify molecular downstream targets that could be exploited for novel therapeutic interventions to protect the fetal brain from inflammatory challenges. As reported previously and confirmed in this thesis, one of the trophic factors of stem cells and their downstream target is Annexin A1 (ANXA1). It was first known as downstream mediator of glucocorticoids acting in resolution of inflammation through regulating leukocyte migration in inflamed tissues. Neutrophils in the exudate are driven by ANXA1 to become apoptotic thereby driving monocytes/tissue resident macrophages to become phagocytic and release anti-inflammatory mediators. In the brain, it improves brain barrier integrity.

In chapter 2 and 3 we tested the immunomodulatory potential of Multipotent Adult Progenitor Cells (MAPCs), which are bone marrow-derived stromal cells with strong anti-inflammatory and regenerative capacities in our translational ovine model for chorioamnionitis.

Intravenous administration of MAPCs in an infectious context enhanced expression of ANXA1 by the cerebrovasculature and immune cells at brain barriers and in cerebral perenchyma (chapter 2). The ANXA1 expression and the number of brain barrier associated immune cells was decreased in untreated animals exposed to antenatal infection (chapter 2). MAPC treatment after infection increased expression of pro-inflammatory markers expressed by microglia/macrophages (MG/MΦ). This was

accompanied by a strong increase of several key anti-inflammatory mediators including IL-10 and ANXA1.
MAPCs seem to orchestrate the balance between pro- and anti-inflammatory MG/MΦ in antenatal inflammation. Further, increased expression of ANXA1 by the cerebrovasculature and immune cells at brain barriers and MG/MΦ following MAPC treatment in an infectious setting may indicate a MAPC driven early defence mechanism to protect the vulnerable brain against ongoing infection and additional pro-inflammatory insults in the neonatal period.

In chapter 4, we tested mesenchymal stem cell derived EVs in a preclinical ovine model of global HI.
BBB integrity was compromised after HI which was improved by MSC-EVs containing ANXA1. Treatment with these MSC-EVs or ANXA1 improved BBB integrity in an *in vitro* model for HI, an effect abolished by FPR inhibitors. Furthermore, endogenous ANXA1 was transiently depleted after induction of HI in cerebrovasculature and ependyma. Time-dependent changes of cerebrovascular and ependymal ANXA1 expression in the preterm brain in the course of antenatal inflammation confirmed these findings, indicating a delayed loss of ANXA1 (Figure 1 in General Discussion).
Targeting the FPR pathway by ANXA1 and potentially other ligands in the immature brain has great potential in preventing BBB loss and concomitant brain injury following HI (and potentially after infection).
We therefore tested intranasal vs intraperitoneal ANXA1 therapy in a mouse model for HI. We found neuroprotective potential of intranasal ANXA1 (decreased neuronal- and white matter- loss) in ANXA1 treated animals after HI which was more evident in male mice compared to female mice (Figure 2 in General Discussion).

In conclusion, the work described in this thesis indicates that stem cell-based therapies modulates the immune response and/or brain barrier properties in the presence of prenatal inflammatory stressors (i.a. LPS, HI). Within the context of antenatal inflammation, modulation of the immune response might be an attempt to balance the immune response in order to prepare the neonatal system towards postnatal challenges. ANXA1, which preserves the brain barrier integrity and induces resolution of inflammation by skewing immune cells towards an anti-inflammatory phenotype, was identified as an important downstream molecule of cell-based therapies. Its role in prevention of brain barrier breakdown via the ANXA1/FPR axis has been validated in the neonatal setting and is has neuroprotective effects when administered locally via the nose and pharmacologic effects were primarily seen in male mice. Hence, optimal dosing

and administration routes (i.n. vs iv.) in larger animal models should be tested for ANXA1. Subsequently, synergistic effects of pharmacologic ANXA1 and stem cells or standard therapies, such as hypothermia, should be tested in future. Accordingly, this thesis forms the base for additional preclinical research and clinical trials, leading to improved neonatal outcome after perinatal inflammatory stress