The preterm lungs and perinatal stress

SUMMARY

Perinatal stress is a major contributor to preterm birth and postnatal neonatal morbidities. Perinatal stress comprises prenatal and postnatal risk factors, of which the most common ones are prenatal infections that can induce inflammation and postnatal mechanical ventilation with oxygen supplementation. While the effects of postnatal stress on the preterm lungs have been extensively studied in the previous decades, the impact of prenatal inflammation on essential lung developmental stages is partially still unclear. Prenatal inflammation has been reported to influence developmental mediators and vascular markers of the developing lungs and for this reason it is strongly associated with postnatal adverse lung outcomes. However, the specific contributors and mechanisms involved in the adverse development of the lungs during prenatal inflammation need to be identified.
Clinically, lungs of children with postnatal lung morbidities seem to have difficulties to recover from adverse lung development and to resume healthy growth, which frequently leads to the development of long-term lung morbidities. Additionally, pre-clinical studies in animals have shown that prenatal stress affects prenatal lung development by altering developmental signaling pathways. Both, clinical and pre-clinical investigations point towards disturbances in lung development already *in utero*, which are primarily mediated by endogenous stem/progenitor cells.

Endogenous epithelial stem/progenitor cells

Endogenous epithelial stem/progenitor cells have a fundamental role in the developing lungs, as they form the different epithelial layers in the proximal (upper) and distal (lower) airways, and in the alveoli. Generally, they are responsible for homeostasis and repair of the lungs after injury, prenatally and throughout life. Due to the crucial role of endogenous stem/progenitor cells during prenatal lung development, we hypothesized that prenatal inflammation negatively affects endogenous epithelial stem/progenitor populations in the immature lungs, and thereby potentially contributes to postnatal injury. To test this hypothesis and sub-questions we used pre-clinical ovine models for prenatal infection (leading to inflammation) and perinatal stress (pre- and postnatal stress combined).
In chapter 2 preterm ovine lungs were exposed to different prenatal stressors and stress environments, including chronic versus acute inflammation, caused by bacterial vs endotoxin exposure. Chronic and acute inflammatory exposure *in utero* resulted in a reduction in epithelial stem/progenitor population numbers and function in proximal, distal airways and alveoli, while pulmonary function was improved. The impact on stem/progenitor cells varied according to lung location (proximal, distal airways, alveoli) where the stem/progenitor cells reside, trigger type, timepoint and duration of exposure

(chronic vs acute). Hereby, acute inflammation caused by endotoxin exposure decreased distal stem/progenitor population numbers most prominently.

Clinical inflammation rarely occurs as a single event, but frequently consists of series of multiple negative events during fetal development. Moreover, intrauterine infections, such as chorioamnionitis, usually have a polymicrobial character, including amongst others microbes, viruses and fungi. We therefore expanded our pre-clinical model from chapter 2 to mimic the clinical prenatal setting more closely; i.e. we combined the single prenatal pro-inflammatory triggers to a consecutive exposure model in preterm ovine lungs. We showed in chapter 3 that sequential exposure, starting with chronic inflammation, led to protection as well as sensitization of the preterm lungs towards a second acute inflammatory trigger. In particular, while vascular development was more prominently reduced after consecutive exposure, alveolar development, including surfactant synthesis, was enhanced and associated with improved lung mechanic properties. Taken together, also in the presence of multiple inflammatory insults, the timing and duration of exposure are important determinants that render the preterm lungs partially more susceptible (vasculature) and partially more tolerant (alveoli) towards various inflammatory events.

In order to follow up on prenatal changes and to understand the interplay of pre- and postnatal insults on the developing lung, we established a pre-clinical model in which pre- and postnatal stressors are combined. In this model, which we present in chapter 4, preterm lambs were exposed to endotoxin prenatally, delivered prematurely and ventilated postnatally for 3 consecutive days. Hereby we found that prenatal inflammation significantly impaired postnatal lung function, which was not detected in animals with postnatal exposure only. In these pre- and postnatally exposed animals no changes in endogenous stem/progenitor cells were observed.

In chapter 5 we not only further discuss the results and main findings of the different chapters in a broader perspective, but we also depict preliminary data showing the presence of endogenous epithelial stem/progenitor populations in neonatal human lungs from preterm infants with chronic lung diseases (CLD) like bronchopulmonary dysplasia (BPD) and respiratory distress syndrome (RDS) and in lungs from preterm infants with pulmonary-unrelated diseases (Non-BPD). We revealed no difference in endogenous stem/progenitor cell numbers between BPD/RDS and non-BPD/RDS patients, while reductions in endogenous stem/progenitor population numbers were found in our pre-clinical studies (chapter 2, 3), and have been reported in literature. These differences in study outcomes for endogenous stem/progenitor cells between human and sheep resulted most likely from variations in the models, including gestational ages, pre- and postnatal stressors, treatment regimens and species. Importantly, the patient numbers in the groups of the human cohort need to be expanded to increase the power of the results.

In aggregate, the findings presented in this thesis emphasize the impact of prenatal inflammation on the development of the lungs. We deliver proof that important developmental factors can be disturbed in the prenatal phase and that these might potentially contribute to postnatal adverse lung development. The obtained insights in disturbed fetal development will further facilitate and contribute to the development of new therapeutic interventions for infants with CLDs, such as BPD, for which no efficient long-term cure is available.

Cell-based therapy

Unfortunately, the current treatment strategies for infants with severe CLD, such as BPD, are still not capable to reduce the incidence of these diseases, resulting in long-term respiratory problems. While therapies ease disease symptoms to improve quality of life, these beneficial effects seem to be transient and cannot cure BPD or prevent long-lasting pulmonary function impairment that frequently is the long-term consequence in adults with a history of childhood BPD.

Importantly, the likelihood to develop BPD is increased when prenatal inflammatory stress is followed by a secondary inflammatory insult after birth, such as mechanical ventilation. Therefore, we hypothesized that an efficient treatment should have a strong anti-inflammatory and regenerative character, considering the frequent pro-inflammatory nature of perinatal stress and the enduring inflammatory situation in BPD patients. In this context, we administered multipotent adult progenitor cells (MAPC), a mesenchymal stem cell (MSC) subtype with strong immunomodulatory and regenerative properties, intravenously at the interface of prenatal inflammation and postnatal mechanical ventilation - two representative clinical inflammatory events as described in chapter 4.

We demonstrated that MAPC treatment, following prenatal inflammation, reduced pulmonary edema and increased regeneration in alveoli, thereby preventing functional impairment of the lungs and leading to improved postnatal lung outcomes. These findings underscore that MAPC therapy is a promising therapy for preterm infants to prevent pulmonary disorders following perinatal stress.

Conclusively, this thesis reveals that early negative exposure, in the form of prenatal inflammation, has a great impact on fetal lung development, in particular by reducing endogenous epithelial stem/progenitor population numbers and function. In parallel, treatment after induction of chorioamnionitis as initial inflammatory event, but before mechanical ventilation as secondary inflammatory hit, with a potent immune modulator and regeneration enhancing mediator, like MAPC, can reduce postnatal injury and prevent adverse pulmonary outcomes. Taken together, timing of negative exposures early in fetal development and subsequent treatment strategies, are fundamental factors that can

influence the development of the preterm lungs negatively and positively, respectively. These findings are clinically relevant for the in-depth understanding of CLD in neonates and highly promising to treat or even prevent CLD in the first place, and to reduce long-term consequences for a better future of preterm neonates.