PEDIPOD AUGUST 2021 TRANSCRIPT

Geoff Marsh
Hello and welcome to Pediapod for August 2021. This month the effects of prenatal inflammation on pulmonary stem cells in an animal model of chorioamnionitis. Chorioamnionitis is an intrauterine infection of the placenta and foetal membranes. It's the leading cause of preterm delivery and is a common risk factor for adverse pulmonary outcomes such as bronchopulmonary dysplasia. There is increasing attention given to the impaired pulmonary stem cells and their role in adverse pulmonary outcomes following pro-inflammatory insults like ventilation and hyperoxia postnatally. However, there's also evidence to suggest that the first negative impacts on pulmonary development occur in utero in the presence of chorioamnionitis. Understanding the effects of inflammation on pulmonary stem and progenitor cells *in utero* is important because although there are several ways that clinicians can aid lung function after birth, there are currently no clinically approved drugs that can restore lung development. In this episode, we meet Niki Reynaert, assistant professor at Maastricht University in the Netherlands, in the department of respiratory medicine, and Tim wolfs, head of the Laboratory of paediatrics, also at Maastricht University. Together, they studied an ovine model of chorioamnionitis in order to study the effects of chronic and acute inflammation on the developing lungs. First off all, I asked Nikki to give me some background on chorioamnionitis.

Niki Reynaert
So chorioamnionitis, technically, is an infection of the foetal membranes but also of the amniotic fluid. It arises from an infection in the urogenital tract of the expectant mother. It can lead to symptoms such as fever, aches, but it typically actually goes unnoticed. That's why it's very difficult to estimate the real prevalence. Numbers you typically find of diagnosed chorioamnionitis range somewhere between 1 to 4% of births, but especially at lower gestational ages it is thought that the prevalence is actually much higher, but as I said, undiagnosed, because it goes without symptoms in the mother. It not only leads to the preterm delivery of the children, the lungs especially are not yet ready to function because they have not developed properly and that actually sets them up to additional need for support in the neonatal intensive care unit, such as ventilation treatment with additional oxygen. But that's not always that good for those vulnerable lungs. So in addition to the chorioamnionitis, which delivers sort of the first hit to the vulnerable lungs, there's also additional treatments in the ICU that cause further damage and that can predispose the child to the development of bronchopulmonary dysplasia, which ultimately sets them up for major problems throughout life such as, for instance, an increased risk to develop asthma in childhood. But also because the lungs never really fully develop, normal lung function at adulthood is not attained, which sets them up to a more quickly deterioration also, to age associated diseases such as COPD.

Geoff Marsh
We know that inflammation comes with a whole host of insults to all sorts of tissues, but tell us about the evidence for the role of stem cells and progenitor cells in chorioamnionitis.

Niki Reynaert
Well, first of all progenitor cells in the lungs are of course essential to driving the development *in utero*. But also in adult lungs, we still have functioning stem cells that when your lungs get injured, even for instance, now during COVID, the lungs have a remarkable capacity to heal, thanks to those stem cells. So because bronchopulmonary dysplasia in particular can be defined as an arrest in the development of the lung tissue, it makes perfect sense that there is some problems, some dysfunction of those progenitor cells through which the lungs never fully developed to their normal capacity. So there has been some clinical evidence that indeed, there is dysfunction of stem cells in the lungs in bronchopulmonary dysplasia, and experimental models, they showed that it's ventilation and hyperoxia that can do that. But we believe that it's not just the postnatal treatments that cause this function of the stem cells predisposing the child to further lung problems, but that they're actually already being impacted by the infection and the inflammatory consequences that occur during the development of chorioamnionitis.

Geoff Marsh
So, Tim, I wonder if you could describe the animal model that you've set up.

Tim Wolfs
We use primarily large animal models, in particular sheep. Development of the lung for humans and sheep are remarkably similar. We inject the amniotic fluid with injections under ultrasonic guidance. We use ureaplasma since if you look in the clinical situation and you culture the amniotic fluid for microorganisms after chorioamnionitis then ureaplasma it's the most cultured microorganism. And next to that we inject the animals with LPS, since LPS is an extremely well characterised, potent inducer of inflammation derived from gram-negative bacteria. And we have injected ureaplasma at the second trimester of pregnancy so in the so-called canalicular phase, and we have given LPS, more acute at the transition of the saccular, and alveolar phase.

Geoff Marsh
So that mimics the chronic and acute inflammation that we see in human chorioamnionitis?

Tim Wolfs
Exactly, yeah.

Geoff Marsh
Once you've done that, can you describe how you are assessing the lung development and the lung health? What are you sampling in your animal model?

Tim Wolfs
We use one lung for immunohistological examination. So that's the right part we normally use. And we use a part for freezing purposes. That means that we can do western blot analysis and RT-PCR. We use the right upper lobe for immunohistochemistry, and the lower lobe for the freezing purposes. We use the left long for ball fluid, so you basically flush with a saline solution and then you can measure all

kinds of cytokines or other proteins as indicators, for instance, for lung injury. And then before we do the sampling, we measure lung function with the so-called PV curve, this is what we do *in vivo*.

Geoff Marsh
Okay, and so let's hear about the results. Nikki, perhaps we'll get back to you. First of all, what was the inflammatory response in the lungs to those two different scenarios of chronic and acute stimuli?

Niki Reynaert
So two and seven days after LPS, you do see clear signs of pulmonary inflammation after infecting the amniotic fluid with LPS. So we see additional neutrophils, macrophages, but also chemoattractants such as interleukin 8 are elevated. If we look 42 days post administering the ureaplasma, there's not that much evidence that there still is ongoing inflammation, but they're still trended to be increased numbers of neutrophils present in the foetal lung tissue.

Geoff Marsh
And you mentioned at the beginning that there was this suspicion wasn't there that the effect of chorioamnionitis might be working through an effect on progenitor cells and stem cells, what happened when you looked at those in the animal model?

Niki Reynaert
So although not a lot of people have ever been very interested in looking at the upper airway stem cells because it's generally assumed the upper airway is already fully developed at the time of infection or postnatal hits. Not unexpectedly acute inflammation after LPS administration, we did see that there was a diminished number of these basal cell populations. Now with the chronic infection 42 days after administering the ureaplasma, there were no more indications that these basal cells were affected. But it doesn't exclude the possibility that sooner after the administration of the UP, that these stem cells would have been affected. But at this protracted time point, it was only the LPS that acutely diminished the basal cell numbers.

Geoff Marsh
So that's the upper airways, the so called proximal progenitor cells and stem cell population, but you also looked at the distal progenitor cells, the alveolar stem cells. So what happened there?

Niki Reynaert
So we looked at populations both in the small airways so the bronchioles, but also in the alveolar region. And we saw that both the acute and chronic inflammatory models, they showed that there were less of these TTF positive cells, so less club cells in the bronchioles and also less alveolar type 2 cells in the alveoli, and associated with this diminished number of cells, we also saw that the transcription factor that is responsible for deriving these progenitor cell functions, SOX9, the RNA levels of that transcription factor were also lower in both the acute and the chronic injury models. So less proliferation, but does that also mean that they're functioning less is one other thing that we wanted to address. So for that, we assess the expression of marker for the type 1 cells because the type 2 alveolar epithelial cells, they are the stem cells to give rise to type 1 cells, the flat cells that are

responsible for facilitating gas exchange. So in addition to there being less type 2 cells and less proliferation, there was also an indication that both the chronic and the acute inflammatory models actually diminished the expression of type 1 cell markers. So we see less proliferation, we see less numbers, but also less of them giving rise to type 1 cells.

Geoff Marsh
So what affect did that then have on surfactant synthesis?

Niki Reynaert
Yeah, so if we have less type 2 cells giving rise to less type 1 cells, you could also hypothesise that they must then be producing less surfactant, which would then result in a more prone to collapse situation for the alveoli. But when we looked at the surfactant proteins that are responsible for lowering the surface tension, we actually did not see signs that surfactant proteins B and C would actually be expressed lower, there's actually tendencies, that the expression of these surfactant proteins was actually higher, especially in the LPS situations. And this corresponded nicely with also an improved lung function that we see after the acute LPS administration, which has been observed previously, as well. So what we actually believe is that when the cells have to make a decision between their two main functions: producing the surfactant to keep the lungs open, or producing more type 1 cells, which would benefit them on the longer term, they actually choose to produce the surfactant at the cost of the development of long at a cellular level.

Geoff Marsh
Does that mean that there is an opportunity for targeting progenitor cells and stem cells to prevent some of those long term effects?

Tim Wolfs
Yeah, I think that's a very important question. I'm really happy to say that Niki and I got an important grant from the Dutch Lung Foundation, particularly to address this question. What we are now doing is studying the more long term consequences of important *in utero* hits like intra-amniotic infection, in combination with adjacent hits postnatally like mechanical ventilation in the context of preterm delivery. So we are basically mimicking the clinical situation of multiple hits together with preterm delivery. And we do this in the presence of a potential pharmacological intervention and we choose at this moment for exogenous stem cell therapy. First of all, because bone marrow derived stem cells that we use do have strong immunomodulatory capacities, which is important in the context of the inflammatory hits that a child is exposed to. And secondly, an important argument to use stem cells is their regenerative capacities. Since we hope, we postulate that we can use their stem cells to compensate for the delay in development that is induced by inflammatory hits in the perinatal phase.

Geoff Marsh
In terms of those clinical applications, one of the stark results from this study is just the fact that a lot of these effects happen *in utero*. Could you envisage a way of compensating for this lack of stem or progenitor cells *in utero* as a therapy? Or does this have to happen postnatally?

Tim Wolfs
Theoretically, it's possible. You can, for instance puncture the umbilical cord, and during pregnancy administer drugs, including stem cells, intravenously. I have to say, from a feasibility point of view, that I consider the chance really low that this is going to happen in real practice. On top of that, at this moment, it's really difficult to establish that mothers are suffering from an intra-amniotic infection. Interesting in this context is that we have shown a proof of concept study that you can identify an intra-amniotic infection from exhaled breath, so using volatile organic compounds. But nevertheless, suppose that we can establish an intra-amniotic infection *in utero*, then I still consider the chance low that we can give stem cells. What is more feasible is that you give interventions to the mother that cross the placenta, that do reach the foetus. I think that is something that is absolutely realistic in the future. And then you can think about the extracellular vesicles, for instance, from stem cells which also have been proven to be effective in the context of inflammation-related adverse outcomes of foetuses, neonates and adults. It might be really relevant to mention that in the longitudinal study study I just pitched, that we also look at the pharmacological regime. So that we play around with the moment when we admitted the stem cells. So we do this in the experimental setting *in utero,* postnatally, and a combination of the two. Yeah, I think that this will deliver important answers on questions which are at this moment unknown- is there synergy between repetitive injections of stem cell therapy? Can you create same potential protective effects if you give the stem cells directly after birth or is the *in utero* administration better? These are very relevant questions that we will tackle in the research programme we are at this moment enrolling.

Geoff Marsh
That was Niki Reynaert and Tim Wolfs from Maastricht University in the Netherlands. And that's it for this episode. Please join us again in a month for your next instalment of Pediapod. I'm Geoff Marsh. Thank you for listening.