What led you to focus specifically on intra-amniotic infection (IAI) by *Ureaplasma*?

Most pregnancies last around 40 weeks. Babies born between 32 and 37 weeks are considered preterm, but those born before 32 weeks are called ‘early preterm’, and these infants are at the highest risk for developing physical or developmental problems that need special medical care and can result in life-long health complications.

It is now recognised that IAIs by maternal genital mycoplasmas are a predominant cause of early preterm birth. *Ureaplasma* spp. (e.g. *U. urealyticum* and *U. parvum*) are consistently associated with histologic chorioamnionitis, preterm birth and adverse perinatal outcomes, such as bronchopulmonary dysplasia (BPD) and/or chronic lung disease and neurodevelopmental disabilities.

Could you highlight the benefits of using rhesus macaque models of preterm birth?

Non-human primates provide unparalleled opportunities for direct comparison with human foetuses/neonates for several reasons. Firstly, rhesus monkeys display the same susceptibility to microorganisms associated with preterm birth as human infants. Second, the hormonal control of parurition and haemochorial placentation with a discrete chorioamnion and amniotic cavity all simulate human pregnancy. Thirdly, unlike rodents, who are born immature in terms of comparable brain maturation, the rhesus neonate has significantly more white matter at birth, making the monkey a far better model for assessing learning, memory and cognitive development. Finally, optimum frequency of neurobehavioral and cognitive testing in neonates and children is not always achievable in humans, and the correlation of important translational measures such as advanced imaging of the foetal and neonatal brain (MRI), neuropathology, together with neurodevelopmental assessments (functional outcomes) would be virtually impossible to achieve.

How has your research contributed to a deeper understanding of how mother and baby mount immune and inflammatory responses to IAI?

White blood cells can be found in substantial numbers within intrauterine tissues and the amniotic fluid of women with IAIs, and play a central role in the pathophysiology of infection-related preterm labour by their production of proinflammatory mediators (e.g. cytokines, prostaglandins and proteolytic enzymes). Yet it remains unclear whether these leukocytes represent a foetal immune response, maternal response, or a combination of the two. Our data, however, indicates that maternal and foetal cells enter the amniotic fluid during IAI, demonstrating that both the mother and baby mount an immune response to *Ureaplasma* IAI.

Interestingly, we have discovered that during azithromycin (AZI) treatment, leukocyte numbers attributed to the foetus decline substantially. This provides further evidence for the beneficial effects of *in utero* treatment of the foetus. A secondary leukocyte surge follows the cessation of antibiotic treatment and the progression to preterm labour onset is of maternal origin.

Can you describe some of your greatest achievements to date?

Over the past year we have made tremendous headway in our ability to keep these very immature and sick neonates alive. Remarkably, our greatest achievement to date has been the survival of our two smallest infants, who where no bigger than the palm of a hand and weighed just 270-300 g (at full term they would weigh 500-600 g). Both infants were born approximately one month early – equivalent to ~30 weeks of human gestation.

One of these infants was exposed to *U. parvum in utero* for 20 days before delivery and was born with mild-to-severe respiratory distress (similar to human babies), eventually developing pneumonia. Mechanical...
ventilation (and surfactant therapy) was required for his survival. Within 12 hours, however, we successfully weaned the infant off the ventilator, treated his pneumonia and he is now six months old and growing steadily.

Most importantly, our antibiotic treatment protocol for *Ureaplasma* IAI given prior to birth has consistently resulted in healthier and more mature infants. Our preliminary data also suggests that neurodevelopment in these infants is somewhat improved compared to infants from infected mothers that did not receive AZI. It’s still early days, but it looks promising and we remain optimistic.

**Where will you be focusing your research efforts next?**

Our future objective will be to determine the extent to which prolonged *Ureaplasma* IAI during pregnancy modulates progressive foetal lung inflammation, abnormal airway development and remodelling, and narrowing with alterations in lung function and physiology, consistent with respiratory illness.

Our central hypothesis is that infection with *Ureaplasma* spp. during pregnancy plays a role in the development of childhood asthma and chronic respiratory issues. It is our hope that prenatal treatment with AZI will attenuate the foetal inflammatory response and mitigate lung injury (occurring in utero), thereby decreasing the severity of pulmonary dysfunction and persistence of respiratory disease in the infant.

**UNCOVERING UREAPLASMA**

This is the remit of the multidisciplinary Pregnancy and Perinatal Research Group within the Division of Reproductive Sciences at the Oregon National Primate Research Center (ONPRC), USA, a team led by Assistant Scientist Dr Peta L Grigsby since 2010. Their research focus is on IAs caused by *Ureaplasma* spp. (ie. *U. parvum*, *U. urealyticum*) – a highly prevalent bacterial pathogen responsible for a high proportion of maternal genital tract infections that has been associated with respiratory distress syndrome (RDS) and neurodevelopmental disabilities in babies born prematurely.

Published results from Grigsby and colleagues demonstrate *U. parvum* IAI can be successfully treated with maternal azithromycin (AZI) therapy. Not only does this treatment delay preterm birth but, perhaps more importantly, also reduces the severity of foetal lung and brain injury. In order to fully appreciate the benefits of antenatal antibiotic therapy (using AZI) to treat *Ureaplasma* IAs, the next logical step was to assess the long-term neonatal outcomes, with an emphasis on neurobehavioral and cognitive development.

Initially, pilot studies were conducted, funded by the Collins Medical Trust, to establish a unique special care nursery (SCN) with the appropriate equipment and clinical management plans for moderate to intense critical care levels, extrapolating from current neonatal intensive care unit and veterinary practices. The team was successful in expanding the non-human primate model from the maternal-foetal environment to now include neonatal survival. Recently they have successfully obtained an extramural five-year grant from the National Institute of Child Health and Human Development (NICHD) in order to continue this valuable research.

“No one has attempted to survive preterm rhesus infants exposed to *Ureaplasma* IAs, or in the context of testing new therapeutic approaches (ie. antenatal antibiotics), which makes our animal model truly unique and exciting,” she enthuses.

**THE CENTERS FOR Disease Control and Prevention (CDC) reports that one in eight babies in the US is born prematurely. Preterm births (<37 weeks gestation) are a leading cause of neonatal morbidity and mortality worldwide, putting both mother and child at increased risk of a number of health problems; many premature babies grow up with lasting disabilities, such as learning difficulties, cerebral palsy, chronic lung disease and vision/hearing impairments, while mothers of preterm infants are more likely to suffer from effects including postpartum depression, impaired maternal-infant bonding and recurrent preterm births.**

**At the Oregon National Primate Research Center, USA, the Pregnancy and Perinatal Research Group is conducting groundbreaking investigations into the best ways to treat infants subjected to intra-amniotic infections both before and after birth.**

**PREVENTING PRETERM BIRTHS WITH PRIMATES**

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INTRELLIGENCE
PRIMATE MODEL OF MID-GESTATION UREAPLASMA IN UTERO INFECTION: PREVENTION OF NEUROLOGICAL SEQUELAE

OBJECTIVES

• To assess the therapeutic effect of antenatal maternal antibiotic therapy in preventing or reducing cerebral white matter damage in the neonate (as a consequence of prolonged U. parvum intra-amniotic infection)

• To correlate neurobehavioral outcomes with neuropathologic findings of neonatal brain injury

KEY COLLABORATORS

Dr Robert Schelonka, MD • Dr Cindy McEvoy, MD • Dr Antonio Frias, MD, PhD • Dr Chris Kroenke, PhD • Dr Kamm Prongay, DVM • Dr Heather Sidener, DVM • Brandi Hodge, LATg, CVT • Dr Ken Waites, MD, FAAM • Dr Tom Burbacher, PhD

PARTNERS

Oregon National Primate Research Center (ONPRC) • OHSU • Doernbecher’s Children Hospital • The University of Alabama at Birmingham • Washington National Primate Research Center

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By moving beyond the maternal-foetal environment to perinatal medicine, the researchers have directed their attention to identification of optimum care practices for newborn infants exposed to Ureaplasma spp. in utero, and determination of the safety of treating maternal IAI’s with antibiotics on subsequent postnatal neurobehavioral and cognitive development.

THE POWER OF PRIMATES

Rhesus monkeys are ideal models of preterm birth, being in many ways biologically similar to humans in terms of pregnancy and birth and also, crucially, susceptible to the same microorganisms associated with preterm birth. “We use a pregnant rhesus model and mobile catheter system,” Grigsby explains. “This allows us to study maternal and foetal interactions in a relatively inaccessible intra-uterine compartment, and to take matched samples of maternal and foetal plasma and amniotic fluid at any time. Such longitudinal studies are not possible in humans.” By establishing IAI’s in pregnant rhesus monkeys, and then treating them with AZI, the team is also able to gain unprecedented insights into the progress of such infections and treatments (effectiveness & safety).

Of course, the use of rhesus monkeys does not come without its limitations, and there are a number of ethical and financial constraints. “We work exceptionally hard to balance our research goals with optimal care and consideration for the health and wellbeing of each animal,” outlines Grigsby.

There are also practical limitations: “Our preterm rhesus monkeys are about three times smaller than the smallest human babies that are born preterm, which raises many challenges, especially regarding equipment,” she explains. “We have become quite creative in adapting our equipment for the tiny monkeys. One such example is that instead of using regular-sized cotton buds, which are far too big, for collecting nasal swabs from baby monkeys, we use dental proxabrush go-between cleaners.”

A UNIQUE NURSERY

The researchers are also investigating the protective effects of maternal AZI treatment on neonates exposed to IAI’s and to identify the best practices for preterm neonate care within the ONPRC’s SCN.

“Our SCN provides dedicated housing and intensive care 24/7, and is staffed by trained personnel with highly specialised skills in biomedical research, neonatal intensive care nursing and clinical/surgical veterinary care.” Grigsby, who spends a substantial time working at the Center, continues: “In many ways it is analogous to a human neonatal intensive care unit”. The SCN is equipped with everything that a preterm rhesus monkey might need, from ventilators to incubators. Her neonatal group has developed critical care management plans designed to promote positive health outcomes. She conducts Newborn Health Assessments 15 and 45 minutes after birth, and evaluates heart rate, respiration, rectal temperature, muscle tone, activity and skin colour. “Furthermore, since prematurely born infants are expected to have mild-moderate RDS, infants can be supported with supplemental oxygen and/or assisted ventilation as indicated by clinical symptoms,” Grigsby elaborates. Meanwhile, in infants with clinical signs of respiratory failure, surfactant is administered just as it is in human babies to help increase lung compliance, oxygen exchange and to prevent collapse of the lung.

Once an infant is stable it is transferred out of the SCN. Its health and development are then tracked, in collaboration with the Behavioural Sciences Unit at ONPRC. The researchers have employed the Primate Neonatal Neurobehavioral Assessment (PNNA) – a series of tests designed to measure characteristics critical to early development and is modelled after similar human tests.

They have suggested that prolonged Ureaplasma spp. infection can lead to neonatal brain inflammation and white matter damage, the team hypothesises that maternal antenatal antibiotic therapy may prevent or limit this; so far, there has been compelling initial evidence in support of this, with the first U. parvum preterm infant survivor to have been treated with antibiotics in utero showing no signs of cognitive or neurological delays.

NOT JUST MONKEYING ABOUT

Looking back at what has already been achieved – their surviving preterm rhesus monkeys serve as a testament to the success – Grigsby is hopeful that the group will continue to make the same strides in medical understanding well into the future. “This project has essentially merged two clinical worlds – clinical neonatology and veterinary medicine,” she notes, asserting that the group’s interdisciplinarity is its greatest asset. “We’ve been working with some of the top neonatologists in the US, perinatologists specialising in maternal-foetal medicine, as well as clinical veterinarians and behaviourists.”

Ultimately, Grigsby and her group hope to continue collaborations with professionals from all disciplines – including animal and human health researchers and professionals from a variety of fields – to ensure that their findings can be translated into the clinic and fulfil their lifesaving potential. “It has been crucial to collaborate with clinicians to ensure we are designing experiments and applying our results in real world situations,” she underlines.