New options for NEC

By Tracey Baas, Senior Editor

A team from the **University of Pittsburgh School of Medicine** has mouse data showing that amniotic fluid can decrease the severity of necrotizing enterocolitis, a GI disease primarily seen in premature infants.¹ An important next step will be exploring in greater detail the precise factors in amniotic fluid that underlie the beneficial effect and turning those factors into medicines.

The effects of necrotizing enterocolitis (NEC) range from mucosal injury to necrosis and perforation. It is the leading cause of death from GI disease in premature infants and is thought to develop due to an abnormal response to the microbial flora that colonizes the GI tract.

Standard care has not changed much over the past 30 years and involves intensive antibiotic regimens, food withdrawal, fluid control, peritoneal drainage and potentially surgery and resection of the affected intestine. In cases in which the infant requires surgery, the survival rate is about 50%.²

The mechanistic details of how NEC develops remain unclear, although recent studies have suggested activation of toll-like receptor 4 (TLR4) within the intestinal epithelium leads to mucosal injury by way of increased enterocyte apoptosis and decreased intestinal healing.³ TLR4 is the receptor for lipopolysaccharide (LPS).

Because the premature intestine is bathed in amniotic fluid that is lost at early delivery, the Pittsburgh team hypothesized that the fluid might exert a restraining influence on TLR4 signaling. If so, they wanted to identify the specific factors within the fluid providing protection to the intestinal epithelium.

In fetal mice, injection of amniotic fluid plus LPS into the GI tract decreased activation of TLR4 and proinflammatory cytokine expression compared with injection of LPS alone. In fetal mice injected with amniotic fluid and LPS, the epidermal growth factor receptor (EGFR) inhibitor Erbitux cetuximab reversed the inhibitory effects of the amniotic fluid, suggesting amniotic fluid inhibits TLR4 signaling through EGFR.

In a newborn mouse model of NEC, daily enteral delivery of amniotic fluid significantly decreased NEC severity compared with no treatment ($p < 0.05$). Protection was lost if mice were pretreated with Erbitux or received EGF-depleted amniotic fluid. EGFR is found on the cells in the intestine. EGF is found in the liquids that bathe the intestine, such as amniotic fluid.

Next, the researchers wanted to see whether decreased intestinal

EGFR levels in the intestinal epithelium would attenuate the protective anti-TLR4 effects of amniotic fluid and put newborns at risk for NEC.

Newborn mice with NEC had lower intestinal EGFR expression than healthy newborn mice. EGFR levels were restored with daily enteral delivery of amniotic fluid. Moreover, premature human infants with NEC showed lower intestinal EGFR expression than fetuses at a comparable gestational age and premature infants whose NEC had resolved.

These results suggest a lack of amniotic fluid-mediated EGFR signaling could predispose premature infants to NEC. Results were published in the *Proceedings of the National Academy of Sciences*.

Baby steps

The team's next steps include clinical trials of the amniotic fluid delivery strategy to prevent NEC in premature infants. The group thinks amniotic fluid itself is a viable treatment option but also is interested in teasing out factors that could be purified to complement EGF to prevent NEC, either by inhibiting TLR4 signaling or modulating other pathways.

"We have not made much progress for NEC, a disease that strikes often with little forewarning in the second to fourth week of life or beyond," said Barbara Warner, professor of pediatrics at the **Washington University in St. Louis School of Medicine**. "The holy grail is really prevention. It is actually intriguing to ponder use or reuse of amniotic fluid for prevention of NEC rather than administration of exogenous substances."

"If further studies would indicate a possible therapeutic role of amniotic fluid for preterm infants, then the immediate challenge is to be able to collect enough amniotic fluid at elective caesarean sections to make this treatment feasible," said Per Torp Sangild, professor of human nutrition at the **University of Copenhagen**. "In addition, if we were to install a donor amniotic fluid bank, it would also be important to exclude any detrimental immunological or other negative side effects of providing amniotic fluid from different mothers."

"There is actually a large amount of amniotic fluid that is discarded with every delivery. It could be collected readily at the time of delivery by simple aspiration, providing enough volume for daily delivery to the preterm infant," noted David Hackam, team leader of the Pittsburgh researchers, associate dean for medical student research, professor of surgery and associate professor of cell biology and physiology at the University of Pittsburgh School of Medicine and co-director of the Fetal Diagnosis and Treatment Center at the **Children's Hospital of Pittsburgh of UPMC**. "A donor amniotic fluid bank could be a further possibility."

"Ideally, the next step would be to identify the specific components of amniotic fluid—presumably soluble proteins—that explain the Pittsburgh group's experimental findings," said Daniel DiGiulio, clinical instructor of infectious diseases at the **Stanford University School of Medicine**.

"Ideally, the next step would be to identify the specific components of amniotic fluid—presumably soluble proteins—that explain the Pittsburgh group's experimental findings."

**—Daniel DiGiulio,
Stanford University
School of Medicine**

“A slurry made of amniotic fluid proteins and given enterally to infants born prematurely could be used as an attempt to mimic the protective effects of amniotic fluid,” said Peter Giannone, associate professor of pediatrics and director of neonatal-prenatal medicine at **The Ohio State University Wexner Medical Center** and director of neonatology at **Nationwide Children’s Hospital**.

Sangild said that “future studies in other animal models are a natural next step to further characterize the effects of amniotic fluid, now elegantly demonstrated by Hackam and co-workers in a mouse model of NEC. Preterm pigs spontaneously develop this devastating disease and appear to be another good model to test the protective effects of amniotic fluid.” Preterm pigs would be easier to handle than 10-day-old mice in order to establish the delicate feeding and enteral delivery protocols and compare the effects of amniotic fluid to mother’s milk, he said. “Maternal milk contains a significant amount of EGF-like activity.”

He also said “it would be important to acknowledge that effects may potentially differ among species—mice, pigs and humans. My group has observed that TLR expression or signaling did not significantly correlate with NEC sensitivity in pigs. Although the role of the TLR4 system and EGFR is convincing in the mouse model, I think it will be important to further differentiate between early initiating events of NEC and the later pathology effects in both the mouse and pig NEC model.”

The changes in the TLR4 system and EGFR might be a consequence of the disease rather than an initiating cause, said Sangild. “If TLR4 and EGFR are shown to be factors that lead to NEC, attempts could be made to influence these systems by means other than amniotic fluid.”

Hackam agreed. “My team already has used computer-assisted drug design to produce TLR4 antagonists to dampen intestinal inflammation. We have tested the lead compounds in the newborn mouse model of

NEC and in human intestinal tissues *ex vivo*. Results will be published in the near future,” he said. Hackam also has ongoing projects to use the compounds to dampen inflammation in arthritis and sepsis.

TLR4 inhibitors in development include **Eisai Co. Ltd.**’s eritoran, which is in Phase III testing to treat sepsis; **Takeda Pharmaceutical Co. Ltd.**’s TAK-242, also in Phase III trials for sepsis; and **VBL Therapeutics Ltd.**’s VB-201, which is in Phase I testing for rheumatoid arthritis (RA) and Phase II trials for psoriasis and inflammatory disease.

A patent application for the work has been filed by the University of Pittsburgh School of Medicine and is available for licensing.

Baas, T. *SciBX* 5(30); doi:10.1038/scibx.2012.776

Published online Aug. 2, 2012

REFERENCES

1. Good, M. *et al. Proc. Natl. Acad. Sci. USA*; published online June 25, 2012; doi:10.1073/pnas.1200856109
Contact: David J. Hackam, Children’s Hospital of Pittsburgh of UPMC, Pittsburgh, Pa.
e-mail: david.hackam@chp.edu
2. Afrazi, A. *et al. Pediatr. Res.* **69**, 183–188 (2011)

COMPANIES AND INSTITUTIONS MENTIONED

Children’s Hospital of Pittsburgh of UPMC, Pittsburgh, Pa.
Eisai Co. Ltd. (Tokyo:4523; Osaka:4523), Tokyo, Japan
Nationwide Children’s Hospital, Columbus, Ohio
The Ohio State University Wexner Medical Center, Columbus, Ohio
Stanford University School of Medicine, Stanford, Calif.
Takeda Pharmaceutical Co. Ltd. (Tokyo:4502), Osaka, Japan
University of Copenhagen, Copenhagen, Denmark
University of Pittsburgh School of Medicine, Pittsburgh, Pa.
VBL Therapeutics Ltd., Or Yehuda, Israel
Washington University in St. Louis School of Medicine, St. Louis, Mo.