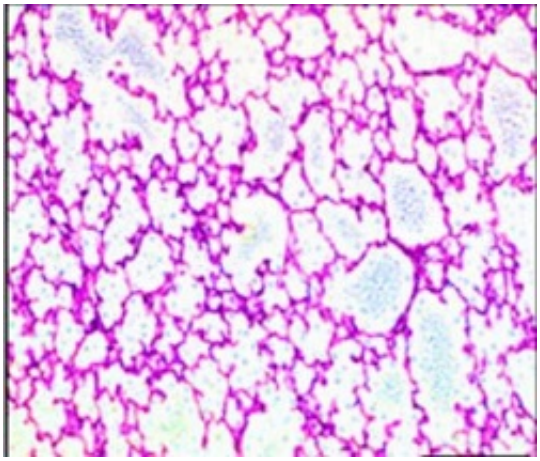
Researchers identify the molecular roots of lung damage in preemies with GI disease

June 29 2016



Healthy lung tissue in a mouse. Credit: Copyright 2016. The American Association of Immunologists, Inc.

Johns Hopkins researchers report they have figured out a root cause of the lung damage that occurs in up to 10 percent of premature infants who develop necrotizing enterocolitis, a disorder that damages and kills the lining of the intestine. The finding, they say, led them to identify and successfully test a potential treatment for the lung damage in a mouse, which may one day be offered to human infants.

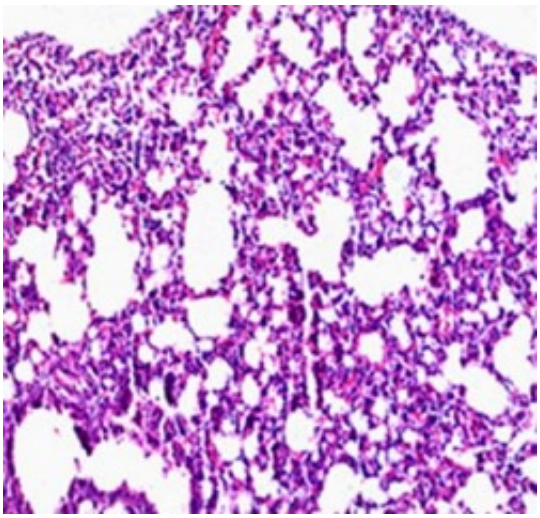
A summary of the research was published online June 15 in the *Journal of Immunology*.

Necrotizing enterocolitis is marked by severe inflammation of the large and small intestine and bowel, striking an estimated 5 to 10 percent of babies born prematurely. The disease kills intestinal tissue and can damage other organs, leading to lifelong growth impairment and other disability. Some 40 percent of infants with necrotizing enterocolitis develop lung damage, and nearly 50 percent of patients who develop necrotizing enterocolitis will die, according to the study's authors. Some that survive may not be able to breathe on their own for many months; others remain permanently disabled.

In a bid to alter the grim numbers, a team led by David Hackam, M.D., Ph.D., surgeon-in-chief for the Johns Hopkins Children's Center and professor of surgery at the Johns Hopkins University School of Medicine, built on an earlier discovery showing that one of the main drivers of necrotizing enterocolitis is a receptor on the surface of intestinal cells called Toll-like receptor 4, or TLR4. This receptor acts like a switch, which detects bacteria in the intestine and then releases chemicals to summon the immune system to attack. When the switch is turned on, necrotizing enterocolitis develops.

To see if this same bacterial receptor was important for related damage in the lungs, the researchers in the new study genetically engineered mice that lacked this receptor in their intestinal lining.

When they introduced gut bacteria from a mouse with the rodent form of necrotizing enterocolitis into the intestines of the mice without the TLR4 receptor, those mice didn't develop lung damage.



Lung tissue from a mouse with necrotizing enterocolitis. Credit: Copyright 2016. The American Association of Immunologists, Inc.

The investigators then created mice without the bacterial receptor in the cells lining the lungs. When they added gut bacteria from mice with necrotizing enterocolitis into those mouse intestines, the animals also failed to show lung damage.

"What this told us," Hackam says, "is that having the TLR4 receptor in both the intestine and the lungs is required for lung damage from necrotizing enterocolitis."

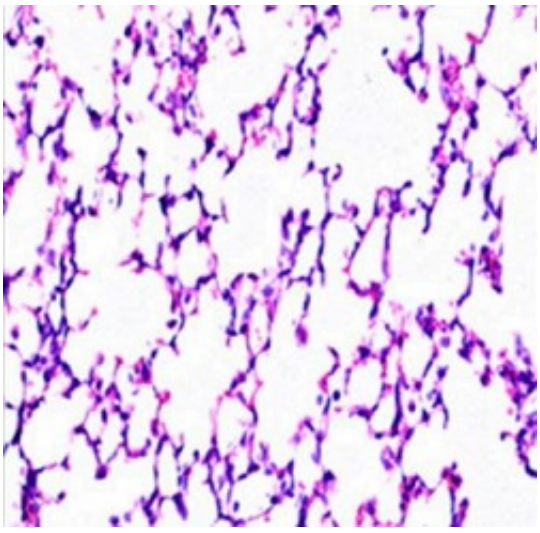
In an attempt to understand how the intestine and the lung may be linked in this disease, the researchers were aware of evidence that dying intestinal cells in preemies with necrotizing enterocolitis release the inflammation-promoting molecule known as HMGB1, which binds to the TLR4 receptor. To see if HMGB1 promoted similar inflammation in the lungs, the researchers put HMGB1 directly on the lungs of healthy newborn mice. Further study showed these mice turned on genes associated with inflammation and caused an influx of infection-fighting

white blood cells into the lungs.

By engineering mice without HMGB1 in the cells lining their intestines and adding gut bacteria from a mouse with necrotizing enterocolitis, the scientists found these mice had less lung damage than healthy mice given the gut bacteria. In addition, by taking HMGB1 out of commission in the lungs using a special antibody, the researchers were able to prevent the necrotizing enterocolitis-associated lung damage.

The researchers say other experiments showed that inflammation-promoting molecules like HMGB1 need to be released first from the intestines, which then leads to lung damage in mice with necrotizing enterocolitis.

Because the main culprit behind the initiation of lung damage in mice was the TLR4 receptor, Hackham says, the researchers wanted to see if blocking this receptor from working in the lungs could stop lung inflammation and lung damage.



Lung tissue from a mouse with necrotizing enterocolitis after C34 treatment. Credit: Copyright 2016. The American Association of Immunologists, Inc.

In a study published last year, Hackam's team reported the creation of a set of compounds that stuck to the TLR4 receptor in the same spots that detect bacteria, preventing the receptor from putting the immune system on alert.

For the new study, the researchers tested one of those compounds, called C34, by aerosolizing it and having the preemie mice with necrotizing enterocolitis breathe it in daily through a tiny syringe for four days. The treated mice had less inflammation in the lung tissue, and their lung cells also turned on less inflammation-promoting chemicals.

"The compound C34 that is a naturally occurring sugar similar to one found in breast milk, which we believe may be why breast milk has been shown to be protective against necrotizing enterocolitis," says Hackam. "The breast milk sugar may also bind to the TLR4 receptor in a similar way that C34 does and provide some protection against inflammation.

"What we've seen here is affirmation of the fact that we can't begin to come up with treatments to help patients without first finding out what the molecular causes of those diseases are," Hackam says.

The researchers plan to continue animal studies and prepare to eventually test the sugarlike compound in humans.

More information: Pulmonary Epithelial TLR4 Activation Leads to Lung Injury in Neonatal Necrotizing Enterocolitis Published online before print June 15, 2016, DOI: 10.4049/jimmunol.1600618 , The *Journal of Immunology*, June 15, 2016. www.jimmunol.org/content/early … nol.1600618.abstract