Scientists identify new strategy to fight deadly infection in cystic fibrosis

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New research suggests that lowering excessive levels of a protein in immune system cells could be a strategy to clear an infection that is deadly to patients with cystic fibrosis (CF).

Researchers determined that normalizing levels of the protein, called p62, in cells from mice carrying the most common mutation that causes CF will jump-start a natural cellular process that clears away the offending bacteria.

The scientists had previously determined that in cells from mice and humans carrying the CF mutation, the bacteria that cause this infection interfere with an important survival process in immune system cells; they also attributed this interference to elevated levels of p62.

The survival process, called autophagy, allows a cell to digest parts of itself to produce energy when it is experiencing starvation. In many infections, autophagy also helps digest pathogens and clear them away.

The bacterium, Burkholderia cenocepacia, causes a severe and persistent lung infection in patients with CF and is resistant to nearly all known antibiotics. Various types of chronic lung infection are responsible for about 85 percent of deaths in CF patients.

"Autophagy also controls inflammation, so when you decrease p62 levels in a CF mouse model and that improves autophagy, you are controlling inflammation produced by Burkholderia cenocepacia. And that's what

we are trying to do for patients – save them from inflammation," said Amal Amer, associate professor of microbial infection and immunity and internal medicine at Ohio State University and senior author of the study.

While relatively rare, B. cenocepacia infection is highly transmissible in patients with cystic fibrosis. By causing either severe sepsis or massive inflammation that damages lung tissue, the infection amounts to a death sentence for CF patients.

To lower p62 levels, the researchers introduced a small interfering RNA molecule, or siRNA, to silence a specific gene and reduce the protein's activation. Amal plans to next test this protein-lowering technique in mice that are models for cystic fibrosis. Designing a similar strategy in humans would require many years of additional study, she noted.

The study is published in the current issue of the *Journal of Biological Chemistry*.

The cells that can use autophagy to clear infection are macrophages, which are first responders in the immune system that consume offending pathogens.

In previous work, Amer and former Ohio State doctoral student Basant Abdulrahman showed that in macrophages isolated from both mice and humans that carried the most common CF mutation, the bacterium would invade the macrophage and thrive instead of being digested and cleared away as it was in cells without the mutation.

The research group showed that rapamycin, an existing drug known to stimulate autophagy, helped control B. cenocepacia infection in mice that serve as a model for cystic fibrosis.

"Rapamycin worked well as a proof of concept, but it has so many side effects that it's hard to imagine giving it to small children with CF for an extended period of time. That's why we looked for another method," said Amal, also an investigator in Ohio State's Center for Microbial Interface Biology (CMIB).

For this study, the researchers conducted experiments in macrophage cells derived from mice carrying the CF mutation and compared them to macrophages from normal, healthy mice.

The researchers observed in macrophages with the mutation that when p62 is elevated, other cell components clump together, causing disruption to the autophagy process.

"p62 is a sticky protein, so high levels of it lead to the formation of aggregates. Once we get rid of that sticky protein – the glue – these protein aggregates will be able to go where they are supposed to go and allow the autophagy process to work properly," Amer said.

Abdulrahman observed that in cells with the CF mutation, a key molecule gets caught up in those clumps. This molecule, beclin1, has a critical autophagy job, essentially escorting foreign particles to the cell parts that digest them and clear them away.

"Our hypothesis was that if we downregulate p62, this will release beclin1 from the aggregates. Once it's available, we will have active autophagic machinery that is able to control the infection," said Abdulrahman, first author on the paper.

In contrast, lowering p62 in macrophages from normal mice allowed the B. cenocepacia bacteria to grow. This confirmed that p62 actually controls the infection in cells from healthy animals but has the opposite effect when the CF mutation is present, she said.