# Study identifies new mechanism for antibacterial immunity

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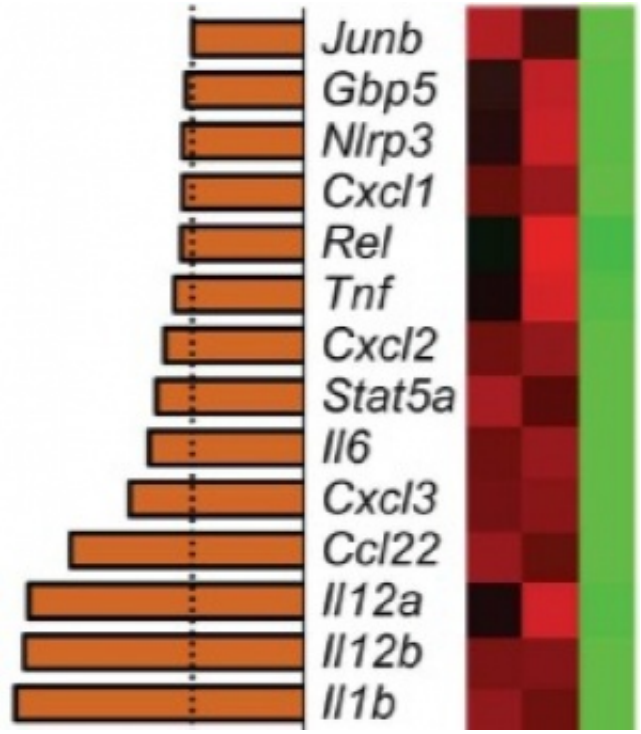


Many genes involved in cytokine signaling had altered expression in caspase-8-deficient mice. Credit: University of Pennsylvania

The innate immune system serves as a first-line defense, responding to infections almost immediately after a pathogen makes its way into the body. This response is carried out in two major ways: the cell can amplify the message that the body has been invaded, triggering an inflammatory response to recruit other cells to help fight off the

pathogen, or the cell can undergo programmed cell death in order to stop the spread of infection and perhaps even release signaling molecules that alert neighboring cells to the presence of an invader.

While most scientists had believed those two responses to be distinct, mediated by different pathways, a new line of study led by University of Pennsylvania researchers suggests that is not the case. The findings, reported in *PLOS Pathogens*, instead show that the enzyme caspase-8, which the group had previously shown to play a significant role in triggering cell death upon infection with the bacteria *Yersinia*, also regulates the production of inflammatory cytokines, the signaling molecules that help carry out a robust immune response.

"The fundamental conclusion of the paper is that the activity of this enzyme is necessary for it to regulate these inflammatory cytokines," said Igor Brodsky, the paper's senior author and an assistant professor in the Department of Pathobiology in Penn's School of Veterinary Medicine. "In the absence of caspase-8, or when its enzymatic activity is lacking, innate immune cells have a general defect in their ability to respond appropriately to microbial products."

Because there are people with mutations in caspase-8 who have severe immunodeficiency, the findings help shed light on one potential reason for their susceptibility to infection: an inability to marshal a robust inflammatory response due to caspase-8 not effectively "turning on" the appropriate genes.

The study was coauthored by Penn Vet's Naomi H. Philip, Alexandra DeLaney, Lance W. Peterson, Melanie Sandos-Marrero, Jennifer T. Grier, Yan Sun, Meghan A. Wynosky-Dolfi, Erin E. Zwack, Baofeng Hu, Carolina López and Daniel Beiting; Jorge Henao-Mejia of Penn's Perelman School of Medicine; Tayla M. Olsen and Andrew Oberst of the University of Washington; Anthony Rongvaux of the Fred

Hutchinson Cancer Research Center; and Scott D. Pope of the Yale University School of Medicine.

The study built off another one led by Brodsky, published in *PNAS* in 2014, which showed for the first time that caspase-8 was essential in inducing programmed cell death in response to *Yersinia*, which is responsible for plague and food poisoning.

Following up on that finding, they performed a classic immunological experiment by creating what are known as bone marrow chimeras, mice that are irradiated, killing off all of their own bone marrow cells, and then given a bone marrow transplant from other types of mice, enabling an animal to simultaneously possess cells with different genetic profiles.

They found that cells from mice lacking caspase-8 produced lower levels of cytokines than wild-type cells. Surprisingly, they also found that a mixture of wild type bone marrow cells together with those that lacked caspase-8 was not able to restore the ability to produce cytokines to the cells lacking caspase-8.

"This experiment didn't turn out the way we expected," Brodsky said. "According to our earlier study, if the only function of caspase-8 was to regulate cell death, we would have expected the presence of wild type cells to rescue the ability of the caspase 8 knock out cells to make inflammatory mediators, but that wasn't the case."

This told the researchers that, beyond functioning to induce cell death, caspase-8 plays a role within cells to produce cytokines.

Diving deeper into the details of this response, the researchers examined how levels of specific cytokines were altered in mice that lacked caspase-8, finding reductions in cytokines associated with several different toll-like receptors, which are expressed on innate immune cells

and respond broadly to molecules associated with pathogens.

When they exposed wild type and caspase-8-deficient immune cells to LPS, a molecule found in all gram-negative bacteria, and then analyzed the RNA transcripts that were activated, the researchers found that levels of more than 500 genes were differentially expressed in the caspase-8-deficient cells, including many involved in cytokine and chemokine signaling.

"That was really interesting to us because it could be one explanation for why people with defects in caspase-8 have immunodeficiencies and are more susceptible to several bacterial and viral infections of the respiratory and nasal tract," Brodsky said. "It could either be caspase-8's role in cell death, or it could be its important role in gene expression."

Because of caspase-8's diverse roles, Brodsky and colleagues wanted to see what form of caspase-8 was necessary for its stimulation of cytokine production. It was previously believed that the unprocessed form of caspase-8 was inactive and that it had to undergo processing in order to cleave its target proteins. To see if this was the case for its enzymatic activity, the team used a CRISPR-Cas9 strategy to generate mice that had a version of caspase-8 that could not be processed.

To their surprise, though these mice had deficiencies in programmed cell death, they could still produce cytokines. Further experiments showed that a "partner" protein called cFLIP works together with the unprocessed form of caspase-8 to optimally produce cytokines in response to bacteria.

In future studies, Brodsky and his colleagues hope to investigate whether caspase-8's role in stimulating cytokine production is general to many types of pathogens; in their current work, they found that it did not hold in an infection with Sendai virus. They would also like to identify the

downstream targets that are cleaved to mediate the caspase-8-induced to cytokine production.

**More information:** Naomi H. Philip et al, Activity of Uncleaved Caspase-8 Controls Anti-bacterial Immune Defense and TLR-Induced Cytokine Production Independent of Cell Death, *PLOS Pathogens* (2016). DOI: 10.1371/journal.ppat.1005910

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