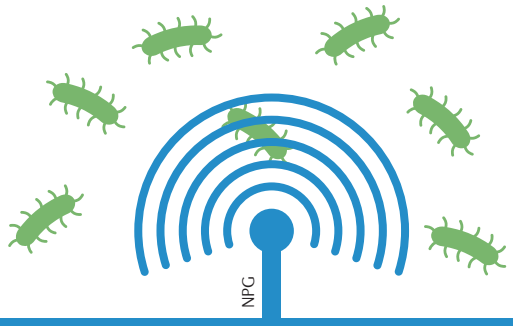

PATTERN RECOGNITION RECEPTORS

Sensing tinkering toxins

The formation of inflammasomes is triggered following the detection of pathogenic microorganisms by pattern recognition receptors (PRRs). Pyrin — a relatively unexplored PRR — has a role in immunity and auto-inflammatory disease but its physiological function has been unclear. Now, Feng Shao and colleagues show that pyrin triggers inflammasome activation by sensing bacterial modification and inactivation of RHO GTPases.

Clostridium difficile, which is an important cause of hospital-acquired diarrhoeal infections, secretes two protein toxins — namely, TcdA and TcdB. These toxins inactivate members of the RHO and RAS GTPase families by monoglucosylating a threonine residue that is important for GTP binding. Previous studies have indicated that TcdA and TcdB can activate the inflammasome. Indeed, Shao and co-workers found that recombinant TcdB triggered caspase 1 activation, interleukin-1β production and pyroptosis in mouse bone marrow-derived macrophages, and they showed that this was dependent on the glucosyltransferase activity of TcdB. Hence, the RHO-glucosylating activity of TcdB triggers inflammasome activation in mouse macrophages.

Inflammasome sensor molecules connect to caspase 1 by interacting with the pyrin domain of the common adaptor protein ASC, and bone marrow-derived macrophages from *Asc*$^{-/-}$ mice were resistant to inflammasome activation in response to TcdB stimulation. Next, the authors investigated which inflammasome sensor molecules interact with ASC to detect TcdB. They tested ten pyrin domain-containing proteins and showed that only pyrin (which is encoded by *Mefv*) senses TcdB. Small interfering RNA-mediated knockdown of pyrin in bone marrow-derived macrophages inhibited TcdB-induced caspase 1 activation, and inflammasome activation in macrophages from *Mefv*$^{-/-}$ mice was abolished in response to TcdB. Thus, pyrin mediates TcdB-induced inflammasome activation.

As RHO family GTPases are the targets of TcdB and other bacterial effector proteins, the authors examined whether pyrin could sense pathogen-induced modification of these signalling molecules. Three bacterial RHO-adenylating effector proteins with a Fic domain — which is a conserved domain in post-translational modification enzymes — were investigated for their ability to activate the inflammasome. All three Fic domain-containing proteins, but not their catalytically inactive mutants, induced caspase 1 inflammasome activation. This activation required ASC and was disrupted in bone marrow-derived macrophages from *Mefv*$^{-/-}$ mice. These results further support that pyrin senses bacterial modification of RHO GTPases.

TcdB targets the RHO subfamily, RAC and CDC42, whereas the closely related *Clostridium sordellii* lethal toxin TcsL and *Clostridium botulinum* C3 toxin target RAC and CDC42, or the RHOA/B/C subfamily, respectively. The fact that C3 toxin, but not TcsL, could also activate the pyrin inflammasome suggests that pyrin detects modification of the RHO subfamily. Notably, TcdB, the three Fic domain-containing proteins and the C3 toxin all modify the GTPase switch I region but with different chemical groups.

Burkholderia cenocepacia, which causes fatal lung infection in immunocompromised individuals, is known to require the bacterial type VI secretion system (T6SS) to inactivate RHO. In this study, mass spectrometric analysis showed that RHOA recovered from wild-type infected mouse dendritic cells was deamidated at an aspargine residue in the switch I region, whereas this modification was barely detected in uninfected cells or cells infected with a T6SS-deficient mutant. Furthermore, the rate of replication of *B. cenocepacia* was similar in *Mefv*$^{-/-}$ and *Asc*$^{-/-}$ macrophages, and was much higher than in wild-type cells. Wild-type mice infected with *B. cenocepacia* developed more severe lung inflammation than *Mefv*$^{-/-}$ and *Asc*$^{-/-}$ mice. Thus, the pyrin inflammasome is important for immune defence against *B. cenocepacia* by sensing bacterial T6SS-induced modification of RHO GTPases.

Together, these data show that pyrin is a specific immune sensor for different bacterial modifications of RHO GTPases. Although most mammalian PRRs recognize microbial products, detection of pathogen activity has previously been described for PRRs in plant innate immunity.

Elisabeth Kugelberg

> “the pyrin inflammasome is important for immune defence against *B. cenocepacia*”

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