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Immune 1-2-3 punch against parasites reveals potentially ancient cell death pathway

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The immune system's killer cells deliver a tightly controlled, 3-phase knockout punch that kills intracellular parasites through a novel pathway that an international team led by researchers from the Program in Cellular and Molecular Medicine (PCMM) at Boston Children's Hospital have named "microptosis." This pathway, the investigators report in *Nature Medicine*, is very similar to apoptosis (the controlled cell death program thought to exist only in multicellular organisms), but with subtle differences suggesting that it could be specifically targeted for anti-parasitic or -microbial drug development.

Using a combination of human or specially engineered mouse cells in vitro and in vivo animal models, study senior investigator Judy Lieberman, MD, PhD; study lead investigator Farokh Dotiwala, PhD, with a team lead by the Brazilian parasitologist Ricardo Gazzinelli, DSc, DVM, found that when an immune killer cell, such as a T-cell or natural killer (NK) cell, encounters a cell infected with any of three intracellular parasites (*Trypanosoma cruzi*, *Toxoplasma gondii* or *Leishmania major*), it releases three proteins that together kill both the parasite and the infected cell:

1. perforin, which opens a hole in the membrane of the infected cell
2. granulysin, which enters the cell and attacks the surface of the parasite
3. granzymes, which enter the parasite and trigger a molecular cascade that kills the parasite

The timing of this sequence appears to be tightly regulated so as to remove a parasitic infection without giving it a chance to spread. Lieberman's team noted that in their model system, parasites began to die within 15 to 30 minutes, while the human or mouse cells they infected died some 45 minutes to an hour later. Their experiments also revealed that parasites died only when all three proteins were present.

Lieberman, Dotiwala and their team were particularly struck by how much the granzyme-sparked cascade resembles apoptosis, a controlled form of cellular suicide that helps eliminate damaged or potentially cancerous cells. They found that granzymes interrupt metabolism within the parasite's mitochondria (the cellular power plant). Levels of toxic molecules called reactive oxygen species subsequently skyrocket within the parasite. Shortly afterward, they noted, three things happen: the parasite's DNA starts to condense, their nuclei start to fragment, and their membranes start to bulge or bleb—all features of apoptosis.

These similarities have led the team to dub the process microbe-programmed cell death, or microptosis. Lieberman notes that her laboratory reported a similar reaction to perforin, granulysin and granzyme in intracellular bacteria in a 2014 *Cell* paper. "The conventional wisdom is that programmed cell death only occurs in multicellular organisms," Lieberman said. "But what our work suggests is that you can drive programmed cell death even in microbes. I think it's probably a really ancient pathway."

Parasitic infections such as trypanosomiasis and leishmanaisis remain a largely neglected but very important global health problem. Lieberman believes that the team's findings could open up a new approach to therapy for these conditions.

"While the parasitic enzymes that microptosis acts on are similar to mammalian enzymes," she said, "they are different enough that it should

be relatively easy to develop drugs that target them and leave a patient's cells alone."

More information: Farokh Dotiwala et al. Killer lymphocytes use granulysin, perforin and granzymes to kill intracellular parasites, *Nature Medicine* (2016). DOI: 10.1038/nm.4023

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