Macrophages and nitric oxide: A deadly combination

In the late 1980s, Carl Nathan and colleagues pinpointed nitric oxide (NO) as a molecule that macrophages use to kill tumor cells. This discovery helped verify the role of radicals as signaling molecules in the immune system.

Immunologists in the early 1980s thought immune signaling occurred mainly through macromolecules that bind to specific cell receptors. They would soon be stunned to find that nitric oxide (NO)—a small, chemically reactive gas, not even known to be made by eukaryotes—is used by immune cells to kill tumor cells.

Mixed signals?

The immunology field got a hint that NO might be a host product in 1985, when Michael Marletta and his graduate student Dennis Stuehr at the Massachusetts Institute of Technology found an excess of nitrate in the urine of bacterially infected mice (1). To find its source, the researchers focused on macrophages, immune cells that help defend against infections.

Meanwhile, at the University of Utah, John Hibbs Jr. was also studying macrophages. He wanted to identify the ingredients in a cell culture medium that macrophages needed to kill tumor cells. The key ingredient was the amino acid arginine. When arginine was present, the activated macrophages removed its guanidino group, producing nitrite. An arginine analogue that could not be catabolized prevented tumor cell killing (2). But exposing tumor cells to nitrite alone did not kill them. Hibbs concluded that macrophages must be converting arginine into a reactive nitrogen intermediate (RNI), which then decomposed into nontoxic nitrite.

Getting the message

A contemporary of Hibbs, Carl Nathan at Cornell University Medical College, saw similarities between Hibbs's research and his own. A decade earlier, Nathan had discovered that activated macrophages release hydrogen peroxide and other reactive oxygen intermediates (ROI) (3). He and his colleagues found that ROI contributed to macrophages' abilities to kill tumor cells and pathogens in vitro and in vivo. This conclusion, however, was not accepted by some scientists, who had a hard time imagining how a cell could keep a rein on these seemingly indiscriminate toxic molecules. But when Nathan heard about RNI, he recognized potential parallels with ROI. It struck him that evolution had selected at least two different sets of small, inorganic reactive chemical species for cytotoxic roles. He invited Stuehr to join his lab as a postdoc. They set out to see what sort of RNI was made by activated macrophages, whether and how this product killed tumor cells, and what enzyme produced it.

NO kills

The same year that Stuehr joined Nathan's lab, a team headed by physiologist Salvador Moncada of University College London, UK, published a paper demonstrating that endothelium-derived relaxing factor, which causes blood vessel dilation, was indistinguishable from the radical gas NO (4).

Nathan and Stuehr then performed cell culture studies showing that activated macrophages also make endothelium-derived relaxing factor, a.k.a., NO (5). They later showed that NO killed tumor cells by inhibiting mitochondrial electron transport (6). Both studies were published in 1989 in the *Journal of Experimental Medicine*.

"The work brought together in one clear study the importance of NO in the immune surveillance system," says fellow NO researcher David Wink from the National Cancer Institute. "Two decades after its publication, we are still finding new and remarkable opportunities for therapy and cancer prevention through the conversation about NO and cancer that these papers started."

The findings also reconciled the idea that NO could be used for both a physiological response—such as blood vessel dilation—and a defensive response. "It's a matter of degree," says Nathan. At high levels, NO kills cells. But at low levels, it is able to regulate physiological responses, such as mitochondrial respiration (7).

Carl Nathan

Nathan's lab has since purified and cloned the enzyme that makes NO in activated macrophages, which they called iNOS (8, 9). With John Mudgett and colleagues at Merck, they created an iNOS knockout mouse (10). These mutant mice were sent to over 130 laboratories, where they were used to identify many more functions for iNOS-derived NO, including diverse signaling pathways and the killing of viruses, bacteria, and protozoa (11).

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