Killing controversy

How do neutrophils kill pathogens? A 1967 paper by Seymour Klebanoff provided a neat answer. But far from being resolved, the question still provokes vehement arguments.

Neutrophils are short-lived phagocytes. As the primary microbe-killing cells of the innate immune system, they contain small vesicle packets, or granules, of deadly toxins. These granules fuse with phagosomes containing engulfed bacteria and deliver their fatal payload. But which of the granules' contents are doing the killing, and how?

Peroxide power

Prior to 1967, it was known that bacterial phagocytosis results in rapid oxygen consumption by the cell (1), and that this "respiratory burst" produces large amounts of hydrogen peroxide (H_2O_2) (2). This H_2O_2 is a potential substrate for the enzyme myeloperoxidase (MPO), which catalyzes oxidation of substances by the decomposition of H_2O_2, and is particularly abundant in neutrophils (3). This possible link led Klebanoff to investigate the microbicidal effects of MPO.

Klebanoff mixed live bacteria with MPO and low levels of H_2O_2 (higher levels would kill bacteria directly), but saw no effect on viability. Reasoning that MPO might act indirectly, converting a harmless substance into something toxic, Klebanoff added iodide to the mix. The cellular halides chloride and iodide, when oxidized, become the potent germicides hypochlorous and iodine. MPO quickly oxidized iodide to iodine and the bacteria were killed.

Using traceable iodide, Klebanoff then showed that bacteria-containing neutrophils converted the iodide to iodine, whereas resting (nonphagocytosing) neutrophils or those treated with an MPO inhibitor, did not. This indicated that bacterial phagocytosis and the resulting MPO activity lead to and are required for iodine incorporation. These results, published in the *Journal of Experimental Medicine*, suggested that neutrophils use MPO-catalyzed iodination as a bactericidal mechanism (4). The report was quickly followed with another paper showing that chloride is equally bactericidal (5). Importantly, normal cellular levels of chloride provide sufficient substrate for MPO to kill ingested microorganisms, proving the system's physiological relevance.

Klebanoff's findings neatly tied together the respiratory burst, the formation of H_2O_2, and the presence of MPO.

...Or protease power?

So what's the controversy? "It sounded great, but it's wrong," says Anthony Segal (University College, London, UK). This provocative stance is based on a number of observations. Segal found that only a small amount of the oxygen consumed in the respiratory burst is used for iodination and that the majority of proteins that get iodinated belong to the host not the bacteria (6, 7). Iodination fallout affecting host proteins is to be expected, but Segal argues, "if the object of the exercise is iodinate bacteria, then you would see it—it would be gross." Additionally, Segal notes that one in a thousand people are MPO deficient but don't succumb to infections.

Segal instead believes that proteases, also found in the granules, are the neutrophils' bacteria-killing machines. His team made mice that lacked two of the proteases, cathepsin G and elastase, and showed that their neutrophils could no longer kill bacteria, even though iodination appeared normal (8). He goes so far as to suggest that the MPO system is not involved in killing inside the phagosome at all but merely disposes of H_2O_2, which is itself just a byproduct of the respiratory burst.

In addition to H_2O_2, superoxide (O_2^-) is also produced during the respiratory burst. In Segal's model, superoxide is important for readily mopping up free protons in the phagosome, thus raising the pH to levels at which the proteases work best. H_2O_2, on the other hand, just needs to be got rid of. Thus, far from iodination (or chlorination) being a bactericidal mechanism, Segal believes it is instead a readout of MPO's clean-up job.

Seymour Klebanoff

Although people lacking MPO are healthy, MPO-deficient neutrophils in culture are slow to kill microbes, suggesting MPO is an early killing mechanism and that later-acting mechanisms such as the proteases are able to compensate in in vivo (9). This is precisely Klebanoff's view. He believes that, "in normal cells the MPO system is probably the predominant killing mechanism, but there are others." He agreed that proteases do contribute but qualifies this, adding, "especially when the MPO is not functioning."

Since there is good evidence that both mechanisms, MPO and proteases, are microbicidal, "it's not either, or," says Klebanoff. "It's and." However, the final verdict as to whether MPO or protease is the predominant killing mechanism, or whether it's somewhere in the middle, is still not in.

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