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EDITORIAL COMMENTARY

Scylla, Charybdis, and navigating antimicrobial action in the neutrophil phagosome

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> The interaction between phagocytes and bacteria, in its simplest terms, consists of two interdependent phases … the ingestion phase … [and] the second or intracellular phase … Little is known … about the basic bactericidal mechanisms which are operative in the intact white cell.
>
> Zanvil A. Cohn and Stephen I. Morse 1959[1]

1 | WHERE WE WERE

The text above, extracted from the initial paragraph of a paper coauthored by Zanvil Cohn, one of the pioneers in the study of leukocyte biology, highlights two phenomena that stimulated investigations of innate immunity in the middle of the last century, namely phagocytosis and intracellular antimicrobial activity. Although many features of phagocytosis have been characterized since that time, fundamental aspects of the antimicrobial action of neutrophils remain unknown. The report by Ashby et al.[2] provides a refined and nuanced look at the interface between an ingested microbe, *Staphylococcus aureus*, and HOCl generated by the myeloperoxidase (MPO)–H_2O_2–chloride system in neutrophil phagosomes and represents a holistic approach to the analysis of bactericidal mechanisms that recognizes contributions from both phagocyte and its ingested prey.

2 | WHERE WE ARE

The cellular responses that follow engagement with microbes have been extensively characterized. Coincident with phagocytosis, neutrophil granules fuse with the nascent phagosome, thereby releasing their contents into the phagosome, and the NADPH oxidase assembles at the phagosomal membrane and generates oxidants in the phagosomal lumen. The contents of azurophilic and specific granules provide MPO, proteases, and antimicrobial proteins. Simultaneously, the NADPH oxidase converts molecular oxygen to H_2O_2, which is rapidly consumed by reaction with MPO and chloride to generate HOCl and other downstream reactive chlorine species. But now, ~63 years after Cohn and Morse's comment, what do we know about the "basic bactericidal mechanisms" that follow?

In seminal studies of neutrophils from some of the first patients identified with chronic granulomatous disease (CGD), Quie and colleagues[3] linked the newly recognized NADPH oxidase to neutrophil antimicrobial action, and the collected efforts of several investigators demonstrated that defensins and other proteins residing in neutrophil granules exert potent direct antimicrobial activity. Numerous studies have detailed the antimicrobial activities of these systems and agents, alone or in collaboration, against microbes in vitro, and some provide sound biochemical evidence for their action against bacteria phagocytosed by neutrophils. Sometimes the evidence is indirect; for example, the differential killing of *S. aureus* by normal versus CGD neutrophils links oxidants as important contributors to staphylococcal death.[3] In other studies, the presence of biochemical modifications of microbial components provides fingerprints that implicate the action of specific agents. Because the MPO- H_2O_2-Cl system is unique in its capacity to generate HOCl at physiologic pH (reviewed in Ref. [4]), the detection of chlorinated residues in bacteria isolated from human neutrophils provides proof that the MPO-dependent system is active in neutrophil phagosomes and modifies susceptible targets therein. A variety of targets for microbicidal attack have been proposed but whether these potentially lethal changes culminate in microbial death remains unsettled.

3 | WHAT WE ARE MISSING

Despite the many overlapping and complementary activities against a wide spectrum of microorganisms, neutrophils do not always succeed: infections still occur, and in vitro studies demonstrate that a small but significant fraction of ingested bacteria survive within neutrophils. Excluding from discussion those situations where the sheer number of organisms, with or without local tissue damage, overwhelm local host defenses, we need to ask, as do Ashby et al., not only how neutrophils kill but also what mechanisms allow some bacteria (*S. aureus* in Ref. [2]) to survive and to persist in neutrophils.

Until recently, most investigations of antimicrobial activity have approached ingested microbes as if they were passive and inert targets of attack. However, to survive threats in their environment and from competing microorganisms, microbes must sense potential danger, including that presented in phagosomes, and mount responses to protect themselves against threats to their viability. For example, the transcription factor OxyR in Gram-negative organisms, and PerR in Gram-positive organisms, responds to H_2O_2 exposure by promoting expression of several genes, including those encoding antioxidants. Transcription of genes controlled by OxyR increases in *Escherichia coli* fed to normal neutrophils but not after ingestion by neutrophils from patients with CGD, which are unable to generate oxidants.[5] Furthermore, *E. coli* mutants deficient in OxyR are more susceptible to killing by normal neutrophils, suggesting that the capacity to counteract metabolic stress triggered by expression of OxyR in response to oxidants dictates in part the fate of ingested *E. coli*. Transcription factors HypT and RclR increase expression in response to HOCl, and RclR expression in *E. coli* increases after phagocytosis by human neutrophils,[6] demonstrating that HOCl (and other reactive chlorine species) produced in neutrophils reacts intracellularly within ingested organisms.

Taken together, evidence indicates that ingested microbes can disarm H_2O_2 via antioxidants such as catalase, glutaredoxin, thioredoxin, or periredoxin, respond specifically to reactive chlorine species, or target specifically a critical agent, as staphylococcal inhibitor of MPO does with human MPO.[7] MPO-dependent oxidation of methionine residues in bacterial targets contributes to antimicrobial action,[8] which *S. aureus* within neutrophil phagosomes counters by up-regulating expression of *msrA1*, a gene encoding methionine sulfoxide reductase and catalyzing the repair of oxidized methionine residues in proteins.[9] These few examples demonstrate that the interface between host and microbe within neutrophil phagosomes is dynamic and reciprocal. As if this complexity were not enough to vex investigators, consider that there is heterogeneity among individual phagosomes within a single neutrophil[10] and that the growth phase and metabolic state of organisms during an infection vary as well.

4 | WHERE WE NEED TO BE

To advance our understanding of antimicrobial action of neutrophils, contemporary studies must include an integrated view of the interface between the intraphagosomal responses of neutrophils and phagocytosed microbes and embrace two opposing perspectives, one from each member of the pair of antagonists. State-of-the-art investigations must recognize that the collaborative efforts of granule contents and locally generated oxidants to retard or kill ingested prey are countered by microbial actions to survive.

With this recognition in mind, Ashby et al. examine the contribution of bacillithiol, a prominent thiol in *S. aureus*, to microbial defense against the MPO-dependent generation of HOCl and other reactive chlorine species.[2] Oxidation of bacillithiol reflects HOCl production by neutrophils and parallels the loss of staphylococcal viability. In a simpler system, the bacillothiol deletion mutant, unable to combat HOCl-induced damage, would be more susceptible to neutrophil killing. However, the deletion of bacillithiol had minimal effect on the fate of ingested staphylococci, testimony to the redundancy and resilience of microbial defenses against attack. It is likely that compensating antioxidant systems were up-regulated in the bacillithiol deletion mutant and served to protect vulnerable sites against HOCl-mediated modification. Redundancies in systems in both host and microbe compound the challenges for those committed to explicating how neutrophils kill and how ingested microbes successful thwart those mechanisms.

In Greek mythology, Scylla and Charibdys were two sea monsters that guarded the strait of Messina. Personifications of rocky reefs and whirlpools, respectively, in the strait, they were dangerous threats to sailors who attempt to pass. Homer's Ulysses successfully navigated the strait but at considerable cost. In opting to avoid Charibdys and sail instead close to Scylla, he lost six sailors but brought the ship and the rest of the crew through the strait. By *choosing the lesser of two evils*, which is the principle dramatized by the myth, Ulysses reached his immediate goal, Thrinacia, the island of the sun.

The contemporary investigator exploring the fate of microbes in neutrophils cannot follow Ulysses' example—to study *either* neutrophil killing alone *or* microbial responses to toxic agents in vitro—but needs instead to face the challenge of elucidating the tactics employed by *both* host and microbe to achieve a comprehensive and integrated understanding of the "basic bactericidal mechanisms," our island of the sun. However, in my opinion, the interactions between neutrophils and ingested microbes are too dynamic and the variables too many to allow us to formulate a unified theory of "basic bactericidal mechanisms," although it should be possible to understand determinants of the fate of individual species in specified experimental settings.

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