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# Mammalian Host Defense Peptides

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# Contents

CHAPTER 1

# Overview: Antimicrobial peptides, as seen from a rearview mirror

R. I. Lehrer



Here I sit, having just celebrated my sixty-fifth birthday, wondering why I agreed to write this overview and also why I never learned to type. I will be brief. If these reflections seem uninteresting, remember that nobody is forcing you to read them. The other chapters in this volume will provide an up-to-date and "serious" introduction to the antimicrobial peptides of mammals.

The gene-encoded antimicrobial peptides of mammals are very old, because such peptides also exist in archaea, eubacteria, protists, plants, and invertebrates. Nevertheless their study is relatively new. Consequently it may be helpful to recall the following dialogue. After William Gladstone (1809–98), Chancellor of the Exchequer, witnessed a demonstration of the generation of electricity by Michael Faraday (1791–1867), Gladstone said "It is very interesting, Mr. Faraday, but what practical worth is it?" Faraday replied "One day, sir, you may tax it." To date, mammalian antimicrobial peptides have been tax exempt.

I complete this overview by recounting how the field began and how I got into it and by mentioning some other early investigators. The search for endogenous antimicrobial molecules arose in the middle third of the nineteenth century. Eli Metchnikoff (1845–1916), an insightful Russian émigré who spent his later years at the Pasteur Institute, first recognized the vital role of phagocytes in host defense and also inquired into their microbicidal mechanisms. In those pre-Sigma Catalogue days only trypsin and pepsin preparations were readily available to him. Finding that these did not kill bacteria, Metchnikoff surmised that other leukocyte enzymes might do so. His speculation was proven correct when, over 30 years later, Alexander Fleming described lysozyme. According to the accounts of Lady Fleming, lysozyme's discovery was largely ignored by the medical community of the day because

it was effective only against nonpathogens. When Fleming later described penicillin, this discovery also received little attention, and the industrial development of penicillin had to wait for the exigencies of World War II.

Recognizing the implications of the nascent science of bacteriology, a Scottish surgeon named Joseph Lister (1827–1912) revolutionized surgical practice by using aerosolized phenol (carbolic acid) to prevent infection and by using phenol-soaked lint to dress wounds. No less than the introduction of ether anesthesia in 1846, a generation before, disinfection and antisepsis revolutionized surgical practice. Although Lister knew of Metchnikoff's work, neither knew that phagocytes used disinfectants that were less cytotoxic than phenol. They produced these substances "on demand" through the agencies of two tightly regulated enzyme complexes: nicotinamide-adenine dinucleotide phosphate (NADPH) oxidase and inducible nitric oxide synthase.



Mammalian neutrophils contain myeloperoxidase, an enzyme that converts hydrogen peroxide, a product of NADPH oxidase, into more potent microbicidal oxidants that include hypochlorite and chloramines. During World War I, Henry Drysdale Dakin (1880–1952), an English-born biochemist who once worked at the Lister Institute, joined Alexis Carrel in introducing dilute sodium hypochlorite irrigations to treat wound infections. "Carrel–Dakins solution" was highly effective, and, unlike Lister's phenol, it retained activity in blood. Sodium hypochlorite is also the active ingredient in Clorox, a common household bleach and disinfectant that was "invented" in 1916.

Leukocytes also have much to teach about antimicrobial peptides. The antimicrobial properties of crude leukocyte extracts were noted in the 1940s and 1950s. Although memorable names, such as leukins or phagocytin, were created to describe the phenomenon, precise molecular characterization of the active principle was not yet feasible. The modern era of antimicrobial peptide research began in the mid–1960s when Hussein Zeya and John Spitznagel described highly cationic polypeptides ("lysosomal cationic proteins") in leukocytes from rabbits and guinea pigs. Considering that their most powerful preparative tools were cellulose and free boundary electrophoresis, they had remarkable success in characterizing these peptides. Unfortunately, their progress stopped when most workers in the field became enthralled with an inherited condition called chronic granulomatous disease (CGD).

Indeed, there were many reasons to be interested in CGD. Although the condition was rare, it was serious; most of the affected children sustained frequent infections, and many died by their late teens. The blood neutrophils and monocytes of CGD patients could ingest various bacteria and fungi normally, but showed defective killing of many of them because of deficient production of hydrogen peroxide and related oxidants by their NADPH oxidase.

Over the next two decades, many laboratories worked to define NADPH oxidase, to ascertain the details of its regulation and structure, and to identify the molecular defects responsible for CGD. During this time, NADPH oxidase was a Holy Grail, and only heretics or skeptics began other quests.

I was also involved in these mainstream issues, but as I tested the neutrophils and monocytes of individuals with CGD or hereditary myeloperoxidase deficiency, I found that they killed many bacteria and fungi with normal or near-normal efficacy. Hence I began to look for other antimicrobial components in leukocytes. By 1974, I had learned how to obtain large numbers of "activated" rabbit alveolar macrophages in considerable purity by using a technique developed by Eva S. Leake and Quentin N. Myrvik. I extracted these macrophages with acid, and subjected the clarified extracts to nondenaturing polyacrylamide gel electrophoresis (PAGE) in pencil-sized tube gels. After the gels were hemisected longitudinally, one half was stained and the other half was sliced at 1-mm intervals with an array of single-edged razor blades. The 60 or so little gel pieces were transferred to test tubes, pulverized in a small volume of distilled water, and the eluted contents were tested against various bacteria and fungi. This simple and direct preparative procedure identified two highly cationic antibacterial and antifungal components. With this preliminary data in hand, I applied for National Institutes of Health funding and six years and three proposals later secured it. Although it is amusing to read the reviewer's comments now, it was less amusing then. Fortunately, I had grants to study postphagocytic ion fluxes in neutrophils and the activation of NADPH oxidase, so the work could continue "on the side."

In the early 1980s, work on insect antimicrobial peptides from Hans Boman's lab in Sweden began to appear. At the same time, the UCLA group (including myself, Judith Delafield, Michael Selsted, Tomas Ganz, and the late Sylvia Harwig) began to isolate and characterize the peptides now called α-defensins. Gradually others began to join the search. I recall that, when I found Bob Hancock's 1989 publication on rabbit NP-1, I sent him a letter (I did not then know him) welcoming him to the "defensin club." A recent Medline keyword search on defensins retrieved well over 1,000 hits. Had I continued to write welcoming letters, I would surely have become an expert typist by now.

By the end of that decade, the first β-defensins had been described in the tracheal epithelial cells and leukocytes of cattle, and Michael Zasloff had captured the imagination of the public with his description of magainins. The first cathelicidin peptides had been recognized, largely through the efforts of Dominico Romeo, Margarita Zanetti, and Renato Gennaro. The first three human β-defensin (HBD) peptides, HBD1, HBD2, and HBD3, were isolated and

described by Harder and Schroeder between 1996 and 2001. More recently, powerful genomics-based search strategies identified 28 "new" β-defensin genes (DEFB) in humans and 43 "new" DEFB genes in mice. Although these numbers are small compared with odorant receptor genes (approximately 900 in humans and 1,500 in the mouse) and some other mammalian multigene families, they are nevertheless impressive. HBD1 is prominently expressed in the human vagina and multiple β-defensin genes are expressed in the human and murine epididymis, suggesting that these peptides play significant roles in reproductive processes.

In any rapidly developing field, surprises can be expected. I end by mentioning two that come from our recent studies. We recently established that several θ-(and α-) defensins are lectins. This property enables them to bind surface glycoproteins and glycolipids involved in cell entry by HIV-1 and herpes simplex viruses. I suspect that the ability to bind sugars could contribute to many other properties, including pathogen recognition and receptor-mediated signaling. At the least, in the words of Linda Loman, "Attention must be paid!"

We have formed somewhat heretical views about the mechanism of action of two exceptionally potent antimicrobial peptides: protegrins and sheep myeloid antimicrobial peptide (SMAP-29). We have evidence that these peptides kill susceptible microbes by inducing a process akin to fresh water drowning – namely, a massive influx of water that overwhelms the microbe's osmoregulatory apparatus. I named this the HOTTER (an acronym for hydro-osmotic transtesseral extrusion and rupture) mechanism. As soon as I get my typing up to speed, I intend to put the supporting data into a manuscript.

The principal risk in "naming names" comes from leaving some out. Although I expect no complaints from Metchnikoff or Lister, if I did not mention you in the view from my rearview mirror, then perhaps you were and are in front of me. Please excuse the lack of references. I will learn how to use my citation manager after mastering typing. By the time a second edition comes around, I should have it perfected.