PERSPECTIVES: IMMUNOLOGY

Defensins and Host Defense

Tomas Ganz

The production of antimicrobial peptides and proteins is an important means of host defense in eukaryotes (*1*). The larger antimicrobial proteins are often lytic enzymes, nutrient-binding proteins or proteins containing sites that target specific microbial macromolecules. The smaller antimicrobial peptides (defined here as peptides containing fewer than 100 amino acids) act, at least in part, by disrupting the structure or function of microbial cell membranes. In the last 20 years, hundreds of antimicrobial peptides have been found in plants and in the cells and body fluids of multicellular animals, from mollusks to humans. Some antimicrobial peptides are produced constitutively; others are induced in response to infection or inflammation. Studies of the regulation of antimicrobial peptide synthesis in *Drosophila* have been particularly fruitful, providing new directions for the analysis of mammalian host defense (*2*). Although antimicrobial peptides display a variety of shapes and amino acid compositions, many of those found in vertebrates are defensins, 3- to 6-kD β-sheet peptides that contain three disulfides and are encoded by related genes (*3*). Structurally and functionally similar defensin-like peptides also abound in insects, other invertebrates, and plants. Now, two reports in this issue (*4, 5*) and a third published 2 weeks ago (*6*) provide new insights into the biology of vertebrate defensins.

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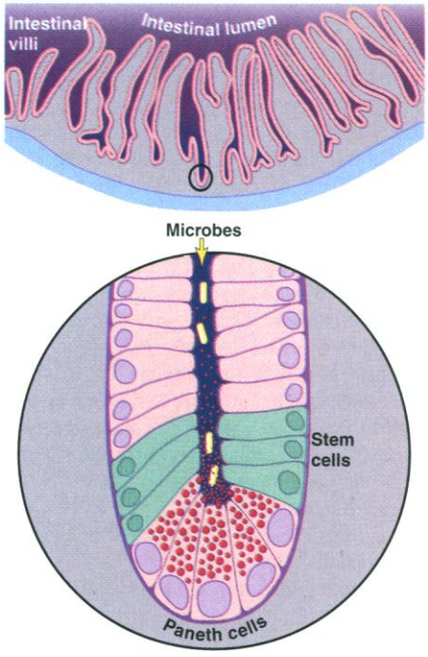
Like most antimicrobial peptides, defensins are cationic (polar) molecules with spatially separated hydrophobic and charged regions. This arrangement allows them to insert themselves into phospholipid membranes so that their hydrophobic regions are buried within the oily membrane interior and their cationic regions interact with anionic phospholipid head groups and water. In the membrane, some defensins assemble into multimeric pores. Defensins and other antimicrobial peptides preferentially disrupt bacterial membranes that are rich in negatively charged phospholipids. Conversely, the lower anionic phospholipid content of the cell membranes of higher animals may provide relative protection from collateral damage.

The author is in the Department of Medicine, UCLA School of Medicine, CHS 37-055, Los Angeles, CA 90095, USA. E-mail: tganz@mednet.ucla.edu

Depending on the specific pattern of their cysteine spacing and disulfide connections, vertebrate defensins fall into two structural classes, α and β. The molecular shapes of the two classes are similar, and their genes reside in the same gene cluster, indicating a common evolutionary origin. In vitro, defensins (at micromolar concentrations) have a broad spectrum of antimicrobial activity against bacteria, fungi, and even some enveloped viruses. In mammals and birds, defensins are among the most abundant polypeptides secreted by phagocytic white cells involved in host defense against bacteria and fungi (*7*). During phagocytosis, ingested microbes are exposed to very high concentrations of defensins. The function of defensin-rich Paneth cells (*8*)—specialized secretory cells in the small intestine—is less certain. In addition to defensins, Paneth cells also contain lysozyme and secretory phospholipase A2, antimicrobial enzymes that clearly mark them as host defense cells (see figure on this page). The location of Paneth cells in intestinal crypts (narrow pits that harbor stem cells for the continual regeneration of the intestinal surface) suggests that Paneth cell secretions protect stem cells from pathogenic microbes. In many other epithelia, defensins are produced constitutively or induced in response to infection or inflammation.



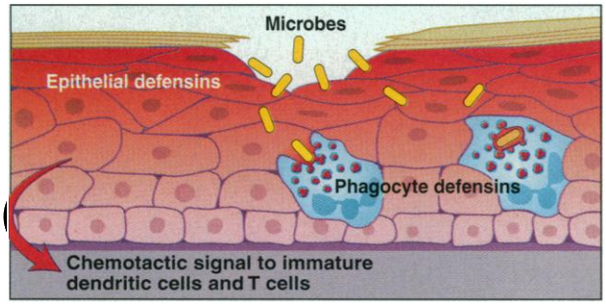
Defense of the realm. A model of defensin activity in intestinal crypts. Paneth cells release defensins (red) and other antimicrobial substances into the crypt in response to microbial penetration. The critically important intestinal stem cells (green) are exposed to the highest concentrations of defensins and may be protected against infection. Defensins may act as signaling molecules in the gut lumen where their concentration is much lower.

The expression of defensins in tissues varies markedly among animal species. Rats but not mice have defensins in their white cells, but both species produce Paneth cell defensins in the gut and epithelial β-defensins in other epithelia. The peculiar defensin distribution in mice and the many closely related defensin genes have complicated attempts to study their function by engineering defensin-deficient mice.

Wilson *et al.* (*6*) report an alternative approach to ablating defensin genes: they instead disrupted the gene for matrilysin, which is required for activation of Paneth cell defensins. These peptides are initially synthesized as inactive prodefensins with an NH_2-terminal anionic propiece that is cleaved off by matrilysin (a tissue metalloproteinase) to generate the much smaller, mature defensin peptide (*9*). Matrilysin is expressed in Paneth cell granules together with perhaps more than 20 different α-defensins (cryptdins). Disruption of the matrilysin gene prevents the normal posttranslational proteolytic activation of intestinal α-prodefensins. The investigators found that, relative to normal mice, matrilysin-deficient mice were less able to kill exogenous *Escherichia coli* bacteria in the gut and were more susceptible to death after infection with orally administered *Salmonella typhimurium* (*6*). The observed host defense defects are certainly consistent with the proposed antimicrobial role of defensins in the small intestine. However, it remains to be seen whether the micromolar concentrations of defensins required for in vitro antimicrobial activity are generated when the Paneth cells release the contents of their granules into the gut. Possibly additional polypeptides that depend on activation by matrilysin contribute to host defense, and sublethal concentrations of defensins may act synergistically with other substances in the intestinal environment. Moreover, by acting as signaling molecules, some defensins could increase resistance to microbial infection by activating other host defenses.

In their report on page 525, Yang *et al.* (*4*) investigated the chemoattractant activity of two recently identified human β-defensins, HBD-1 and HBD-2 (*10–12*). HBD-1 is constitutively expressed in kidney tubules and to a lesser extent in the

pancreas and other epithelial sites; HBD-2 is induced in skin and other epithelia during inflammation (see figure on this page). At submicromolar concentrations, these defensins attracted both immature dendritic cells and memory T cells, which initiate a primary and recall immune response, respectively. The effect was evidently mediated by the CCR6 chemokine receptor because β-defensins effectively competed with the receptor's ligand, MIP-3α. If the same mechanism functions in vivo, the release of these two defensins from injured epithelial cells would recruit dendritic cells and memory T cells to infected tissues, thereby promoting the development of adaptive (antibody and T cell–mediated) immunity. Like the β-defensins, human neutrophil α-defensins also attract T cells. In addition, some defensins also block the adrenocorticotrophin receptor and could inhibit the production of the immunosuppressive adrenal steroid hormones during acute infection (*13*). The ability of some defensins to act as signaling molecules could explain their biological effects when their concentrations are too low to be directly microbicidal.



Inflamed defenders. A model of defensin activity in an infected epithelium. Epithelial cells synthesize antimicrobial defensins (red) both constitutively and in response to infectious and inflammatory stimuli. Other defensins are introduced by the influx of phagocytic cells that use them to kill ingested microbes. Released defensins attract dendritic cells and memory T cells, setting the stage for the adaptive phase of the immune response.

Comparison of defensin genes across vertebrate species indicates that they are rapidly evolving. As variations in defensin sequences and patterns of tissue expression bring out distinct aspects of defensin biology in each species, studies of defensins in diverse animals have often provided crucial insights. The article by Tang *et al.* on page 498 (*5*) reports on studies of white cell defensins in the rhesus monkey. Several of the defensins isolated from rhesus white cells resembled their human counterparts, but one was unique and initially refractory to conventional analysis. The puzzle was solved brilliantly when the investigators determined that they were dealing with a cyclic peptide generated by head-to-tail peptide splicing of the products of two similarly truncated α-defensin genes! Although otherwise similar to α-defensins with their six cysteines, these novel defensin genes contained a "premature" stop codon in the segment after the third cysteine. This resulted in generation of two abbreviated defensin molecules that each donated 9 amino acids to the final, 18–amino acid cyclic product: a θ-defensin stabilized by three parallel disulfide bonds. This remarkable feat of posttranslational processing could be reconstituted by transfecting HL-60 human leukemia cells with the two defensin cDNAs. This elegant model should provide the requisite tools that will allow the processing and splicing mechanism to be worked out. The new θ-defensin molecule resembles porcine white cell protegrins, hairpin-shaped peptides with 16- to 18-amino acid residues and two disulfide bonds (*14, 15*). Like protegrins, θ-defensins retain full activity at salt concentrations present in blood, a feature that makes them interesting candidates for development as antibiotics.

Despite advances in prevention, diagnosis, and treatment, infectious diseases continue to challenge us. We are facing the limitations of vaccine-based immunization strategies and the increasing resistance of microbes to existing antibiotics. The renaissance of research into innate host defense mechanisms that do not depend on specific recognition of individual antigens offers the promise that some of the many substances that mediate the innate resistance of plants and animals to infections may prove useful as templates for new antibiotics or immunostimulants.

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PERSPECTIVES: DEVICE PHYSICS

In Search of Low-*k* Dielectrics

Robert D. Miller

Within the next few years, high-performance chips containing as many as 0.5 billion transistors on a single chip will be produced. These advanced chips may contain up to 10,000 m of on-chip wiring connecting the individual devices with each other and with the outside world. However, such increased device and wiring densities cannot be achieved with currently used materials. The search is now on for materials that can replace silicon dioxide as the insulator in these future devices. But despite a bewildering number of candidate materials under investigation, a clear winner has yet to emerge.

In a typical microchip, layers of copper interconnect wiring are separated by a dielectric insulator, traditionally silicon dioxide (see the figure). Both the resistance of the metal and the capacitance of the insulator increase markedly as the wiring dimensions and pitch decrease, resulting in crosstalk and capacitative coupling between the metal interconnect lines and thus increased signal delays (*1*). Several semiconductor manufacturers have demonstrated recently that the traditional Al(Cu) wiring can be replaced by pure copper, which has a resistivity only 60% of that of aluminum (*2*). Performance gain is then limited by the intra- and interlayer capacitance, dictated primarily by the dielectric constant of the insulator. This has fueled the frantic search for new dielectric insulators with lower dielectric constants k than silicon dioxide ($k = 3.9$ to 4.2) (*3*).

Any replacement low-k material must meet the current integration requirements, such as thermal stability in excess of 400°C, good mechanical properties, low ion content, breakdown fields in excess of 2

The author is at IBM Research Division, Almaden Research Center, San Jose, CA 95120–6099, USA. E-mail: rdmiller@almaden.ibm.com

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Stable URL:
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