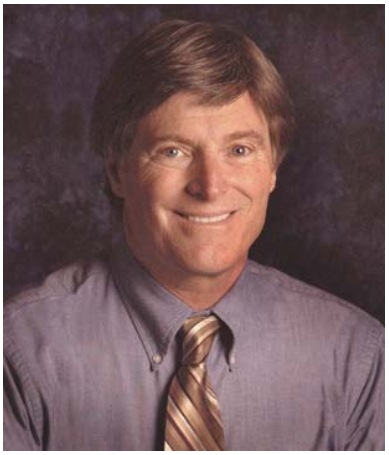
Antimicrobial Peptides…

Small, But Potent Killers



Jay Hardy, CLS, SM (ASCP)

Jay Hardy is the founder and CEO of Hardy Diagnostics. He began his career in microbiology as a Medical Technologist in Santa Barbara, California.

In 1980, he began manufacturing culture media for the local hospitals. Today, Hardy Diagnostics is the third largest media manufacturer in the U.S.

To ensure rapid and reliable turn around time, Hardy Diagnostics maintains six distribution centers, and produces over 2,700 products used in clinical and industrial microbiology laboratories throughout the world.

A new approach is needed, as we explore novel types of antimicrobials for which the pathogens that currently plague us have not yet developed resistance.

Perhaps scientists have overlooked one of nature's most potent molecules in the arsenal against invasive organisms.

Consider that the cornea of the eye of an animal is almost always free of signs of infection. The insect flourishes without lymphocytes or antibodies. A plant seed germinates successfully in the midst of soil microbes. How is this accomplished?

What are AMPs?

Both animals and plants possess potent, broad-spectrum antimicrobial peptides (AMPs), which they use to fend off a wide range of microbes, including bacteria, fungi, viruses and protozoa.

The molecular structure of the human antimicrobial peptide, Definsin. The red portion denotes positively charged amino acids. Green denotes hydrophobic amino acids. This characteristic is key to its destructive capabilities.

What sorts of molecules are they? How are they employed by animals in their defense? As our need for new antibiotics becomes more pressing, could we design anti-infective drugs based on the design principles these molecules teach us?

Antimicrobial peptides are a vital component of the innate immune response and are found among all classes of plant and animal life.

Powerful Agents of Healing

AMPs consist of small proteins with potent broad-spectrum antimicrobial activity. AMPs alter the host immune response through receptor-dependent interactions and have been shown to be important in such diverse functions as

angiogenesis, wound healing, and chemotaxis. It appears that AMPs work hand in hand with other defense mechanisms in the human body such as vitamin D, as we shall see below.

AMPs have been demonstrated to kill Gram negative and Gram positive bacteria (including strains that are resistant to conventional antibiotics), mycobacteria (including *Mycobacterium tuberculosis*), enveloped viruses, fungi and even transformed or cancerous cells. Unlike the majority of conventional antibiotics it appears as though antimicrobial peptides may also have the ability to enhance immunity by functioning as immunomodulators. Although some organisms, such as the human gut commensals, *Lactobacillus* spp. and *Fusobacterium nucleatum*, appear to be resistant to AMPs, this resistance cannot be transferred as we have seen with other antimicrobials.

A Protein Designed for Invasion

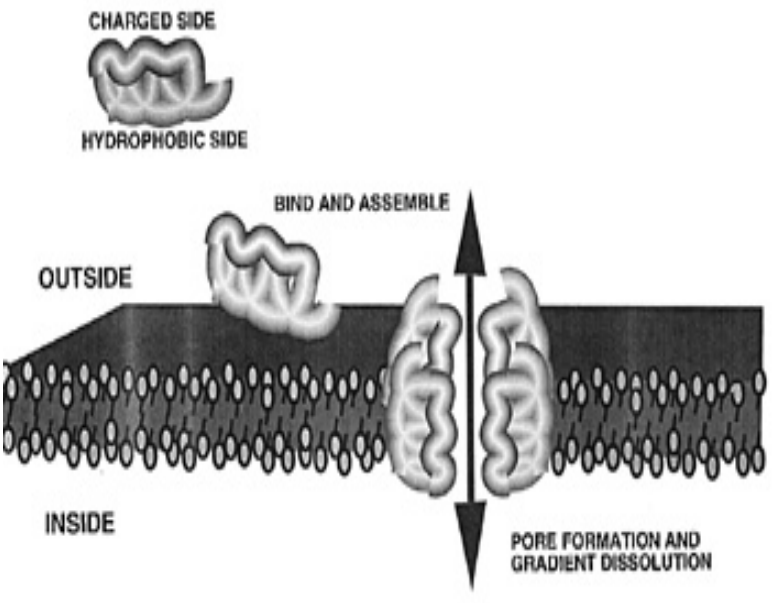
These AMPs are usually positively charged and have both a hydrophobic and hydrophilic side that enables the molecule to be soluble in aqueous environments yet have the capability to the enter lipid-rich membranes of various microbes. Once inside a target membrane, the peptide kills target cells through diverse mechanisms.

Cathelicidins and defensins are major groups of epidermal AMPs. Decreased levels of these peptides have been noted for patients with atopic dermatitis and Kostmann's syndrome, a congenital neutropenia. In addition to important antimicrobial properties, growing evidence indicates that AMPs alter the host immune response through receptor-dependent interactions.

Methods of Mass Destruction

Antimicrobial peptides have exploited a fundamental difference in the design of the membranes of microbes and multicellular animals. Bacterial membranes are organized in such a way that the outer lipid layer is negatively charged. In contrast, the outer layer of the cell membranes of plants and animals is composed principally of lipids with no net charge. Because AMPs are positively charged, they are attracted to the negative charge of the microbial membrane. This accounts for the fact that AMPs do not attack the host tissue membranes that are rich in neutral cholesterol.

How do antimicrobial peptides actually kill microbes? Many hypotheses have been presented, which include the disruption of the microbial membrane functions and the fatal “punching” of holes in the membrane.



However, AMPs are not an indestructible super hero, in that some organisms such as Morganella and Serratia do not have a sufficient negatively charged lipid membrane to be vulnerable to their attack.

Is Vitamin D an Antibiotic?

Recent research shows that vitamin D up-regulates ability to fight infections by helping produce over 200 antimicrobial peptides, the most important of which is cathelicidin.

The sunshine vitamin plays an important role in the stimulation of AMPs, which are essential to the fight against disease.

Dr. Philip Liu and colleagues at UCLA, showed that vitamin D might be, in effect, a potent antibiotic. Vitamin D increases the body's production of naturally occurring AMPs, which are produced in numerous cells in the human body where they directly and rapidly destroy the cell walls of viruses and bacteria, including tuberculosis. Furthermore, Liu showed that adding vitamin D to African American serum (African Americans have higher rates of TB) dramatically increased production of these naturally occurring antibiotics.

Could it be that large doses of vitamin D could induce an increased production of AMPs to reach therapeutic levels? Research is currently being conducted to explore this synergistic partnership of vitamin D and AMPs.

Possible Pharmaceutical Applications

The growing problem of resistance to conventional antibiotics and the need for new antibiotics has stimulated interest in the development of antimicrobial peptides as human therapeutics. Most pharmaceutical effort has been devoted to the development of topically applied agents, such as the AMP known as the magainin analogue pexiganan. Currently the strategy is to develop topical agents largely because of the relative safety of topical therapy and the uncertainty surrounding the long-term toxicology of any new class of drug administered systemically.

The broad antimicrobial spectrum of antimicrobial peptides positions them for consideration as 'chemical condoms' to limit the spread of sexually transmitted diseases, including *Neisseria gonnorhoeae*, Chlamydia, human immunodeficiency virus (HIV), and Herpes simplex virus (HSV).

In addition, it has been found that AMPs can enhance the potency of existing antibiotics *in vivo*, probably by facilitating access of antibiotics into the bacterial cell, a phenomenon previously recognized for the cationic peptide component of polymyxin.

Conclusion

The fact remains that most animals, including insects and creatures like the octopus and starfish, rely heavily on antimicrobial peptides for defense against microbes, and do so quite effectively without the help of lymphocytes, a thymus, or antibodies.

These mechanisms can be effectively exploited to develop new therapeutic modalities in order to keep one step ahead of increasingly resistant microbes.

Even though AMPs have been studied for 20 years, there is still much to discover as this exciting new area of microbiology unfolds.

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