Have you ever noticed and wondered how the beautiful lotus flower grows in muddy water and yet rises above the surface to bloom with remarkable beauty? Or, how insects are able to fend off infections in spite of having an ill-defined immune system? Frogs thrive in adverse environment without falling prey to pathogenic microbes. How is this accomplished? Do you realize that your skin is bombarded with a variety of bacteria, fungi and viruses? How does it protect itself from being constantly infected?

The answers lie in the defense mechanisms against invading microbes. A living being is capable of mounting defense against foreign organisms that it may perceive to be potentially harmful. This is known as ‘immunity’. An immune system is a system of biological structures and processes within an organism that protects against disease.

Innate immunity is the first line of host defense mechanism and is so-called because the various components of this type of immune system are inherently present in the body and are ready to respond immediately to invasion by foreign microbes. This system does not confer long-lasting immunity against a pathogen. The innate immune system is the dominant system of host defense in most organisms.

However, if innate immunity fails, the invading organisms may still be detected and attacked by the second line of defense mechanism called the adaptive immunity. This immune system is so called because it adapts its response during an infection to improve its recognition of the pathogen. The adaptive immune system evolved in early vertebrates and allows for a stronger immune response (for example antibody production) as well as immunological memory, where each pathogen is ‘remembered’ by ‘memory cells’. This allows the adaptive immune system to mount faster and stronger attacks each time there is a repeated infection by the same pathogen.

One of the key components of the innate immune system are the ‘antimicrobial proteins’ (AMPs) that have potent, broad-spectrum antimicrobial properties used to fend off a wide range of microbes, including bacteria, fungi, viruses and protozoa. Antimicrobial peptides have been isolated and characterized from organisms representing virtually every kingdom and phylum, ranging from prokaryotes like bacteria and fungi to eukaryotes like plants and animals.

Antimicrobial proteins (AMPs) present in animals, plants and even humans are the body’s warriors fending off attacks from a wide range of microbes including bacteria, fungi, viruses and protozoa.

**Body’s Proteins to Your Defence!**

Vandana Vernekar & Atmaram Bandivdekar
bacteria to multi-cellular organisms in both plant and animal kingdom including humans, thereby suggesting that such polypeptides have served a fundamental role in the successful evolution of complex multi-cellular organisms. Since such proteins are involved in defending the host against infections they are also termed as ‘host defense proteins’.

Now, what sort of molecules are antimicrobial proteins? What are their mechanisms of action? What is the difference between a commonly used antibiotic and an anti-microbial protein?

Antimicrobial proteins (host defense proteins) are a unique and diverse group of molecules, which are divided into subgroups based on their amino acid composition and structure. Proteins that have less than 50 amino acids are designated as ‘anti-microbial peptides’. Most of the anti-microbial proteins are low molecular weight, positively charged polypeptides but some negatively charged peptides and high molecular weight proteins have also been demonstrated to have activities against various bacteria, fungi, protozoa and viruses.

The modes of action of these peptides are varied. Most of them bind to the membranes of the various organisms and disrupt the membranes by the formation of pores. These perforations lead to leakage of cell contents thus killing them. AMPs can also enter the cell and have adverse effects on the inner cytoplasmic components. In addition, they can also stimulate the adaptive immune response mechanisms.

Interesting anecdote of how anti-microbial proteins were first discovered!!

In 1921, Dr. Alexander Fleming observed that respiratory secretions have anti-microbial properties. One day, suffering from a heavy cold, curiosity inspired him to culture his nasal mucus on a petri dish layered with agar jelly containing bacteria. After some weeks, when he looked again at the petri dish, he observed that bacteria growing near the mucus had been inhibited or destroyed. Fleming had found an ‘antibacterial protein’ that occurs naturally in tissues and secretions: mucus, tears and in other body fluids. Since it lysed (dissolved) microbes and had the properties of an enzyme, Fleming called it lysozyme. This important observation alerted Fleming to the power of natural biological antibiotics.

In 1922, he published these findings in the ‘Proceedings of Royal Society of London’ entitled ‘On a remarkable bacteriolytic element found in tissues and secretions’.

It was only after six years, in 1928, that he discovered penicillin, an antibiotic, and the rest is history!!

Antimicrobial Proteins in Species

Amphibians: Amphibians like frogs have been around 300 million years, existing in polluted waters where highly effective defenses against pathogens are a must. The isolation of bombinin and subsequently the magainins from African clawed frogs (Xenopus laevis) led to the investigation and discovery of peptides throughout the amphibian species. In fact, frog skin has been used for medicinal purposes for centuries and is still used today in South American countries!

Insects: Insect antimicrobial peptides include peptides like the cecropins (from cecropia silkmoth, Hyalophora cecropia), a native North American moth and mellitin first described in bees. Fruit flies (Drosophila melanogaster) have at least seven different antimicrobial peptides in their hemolymph, one of them named drosomysin.

Plants: Antibacterial peptides have also been described in many plant species. The first antibacterial peptide isolated from a plant species was a purothionin from the wheat plant (Triticum aestivum). Defensins are known to be present in plants besides proteins like cyclotides, snakins and thaumatin. Whereas most antimicrobial proteins from animal species have antibacterial activity, plant anti-microbial proteins have a high antifungal activity, reflecting the relative importance of fungal as opposed to bacterial pathogens in the plant world. Peptides isolated from roots, seeds, flowers, stems, and leaves have demonstrated activities towards plant pathogens, as well as against bacteria pathogenic to humans.

Birds: β-defensins, the ubiquitous molecules found in other living beings, have also been identified from blood and epithelial cells of...
Some host defense proteins present in the human body

Skin: Catelicidins (LL37)
Respiratory tract: Collectins (SP-D, SP-A), Lysosyme
Gastro-intestinal tract: Mucins, lactoferrin, histatins
Genito-urinary tract: Lipocalin, cystatins, seminogelins
Granules of immune cells: Defensins and Lysosyme

### Difference between antibiotics and anti-microbial proteins (AMPs)

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Anti-microbial proteins (AMPs)</th>
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<tbody>
<tr>
<td>Antibiotics are molecules containing two more drastically modified amino acids usually elaborated in bacteria and fungi</td>
<td>Anti-microbial proteins are polypeptides containing more than ten amino acids, produced by all species of life (including bacteria)</td>
</tr>
<tr>
<td>Do not enhance immunity</td>
<td>Enhance immunity by stimulating the adaptive immune system (second line of defense mechanism)</td>
</tr>
<tr>
<td>Human body can become resistant to antibiotics</td>
<td>Human body does not easily become resistant to AMPs because of their multi-faceted actions</td>
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### One of the key components of the innate immune system are the ‘antimicrobial proteins’

for their anti-HIV activity. Several proteins including defensins, lactoferrin, catelicidin (LL-37), histatins, thrombospondin have demonstrated activities against HIV in in vitro studies.

### Therapeutic Potential

Although this is a relatively recent field of research, some progress in clinical applications as well as in the field of agricultural research has been made. For instance, introduction of antimicrobial genes in tobacco and potatoes has been successful in transferring some benefit against disease.

**Nisin** (isolated from bacteria) is being considered for stomach ulcers and protegrin (first described in pig) for oral infections. Clinical trials of protegrin peptides have also been initiated in chronic lung infections like cystic fibrosis. Magainin (from frog skin) derivative has been found to be effective against polymicrobial diabetic foot ulcers. A patented AMP (MBI-594) is being tested for acne.

Studies both in the laboratory and in the clinic have confirmed that the body is less resistant to anti-microbial peptides than conventional antibiotics. Natural anti-microbial proteins will help in creating new lines of treatment against microbial pathogens because these have been proven to be effective over thousands of years in both the animal and plant kingdom.

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Chicken, turkey, and ostrich and the stomach contents of King penguin (spheniscins).

Mammals: Perhaps the most researched mammalian peptides are the defensins. Defensins have been categorized into two groups, beta-defensins and alpha-defensins. Besides these, a number of host defense proteins like lysozyme, lactoferrin, bactenecins, indolicidins, and cathelicidins are known to be present within the granules of neutrophils, in mucosal or skin secretions of mammals.

Humans: Important components of innate immunity in humans (as in other mammals) are anatomical barriers, intact normal flora and anti-microbial proteins. Physical barriers such as blinking of the eyes and the movement of cilia present in the mucous membranes help in sweeping away harmful microorganisms. Normal bacterial flora present on the skin and in the digestive tract do not allow colonization by non-indigenous and pathogenic bacteria.

In humans, host defense proteins are present at sites that routinely encounter pathogens; for example, the skin, the mucosal surfaces, body fluids, the different systems of the body and the immune cells.

The skin, which is constantly bombarded with microbes, is host to a number of AMPs. Mucosal surfaces in the eyes, nose and mouth secrete protective molecules that subsequently find their way in body fluids like tears, nasal fluids, and saliva. The respiratory system, the digestive system, and the urogenital tract contain proteins that can combat pathogenic bacteria, fungi and viruses. Blood has a number of immune cells like neutrophils, macrophages etc, the granules of which contain potent anti-microbial molecules.

Lysozyme, an antimicrobial protein, first described by Sir Alexander Fleming in nasal fluids is distributed in the body. Molecules like defensins have multiple functions in host defense. Catelicidins, lactoferrin, and interleukins are also present throughout the body.

Since the advent of HIV/AIDS some proteins have and are being studied