Small proteins in the cornea protect against bacterial infection

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When it comes to germ-busting power, the eyes have it, according to a discovery by University of California, Berkeley, researchers that could lead to new, inexpensive antimicrobial drugs.

A team of UC Berkeley vision scientists has found that small fragments of keratin protein in the eye play a key role in warding off pathogens. The researchers also put synthetic versions of these keratin fragments to the test against an array of nasty pathogens. These synthetic molecules effectively zapped bacteria that can lead to flesh-eating disease and strep throat (*Streptococcus pyogenes*), diarrhea (*Escherichia coli*), staph infections (*Staphylococcus aureus*) and cystic fibrosis lung infections (*Pseudomonas aeruginosa*).

The findings, to be published in the October issue of the *Journal of Clinical Investigation*, could lead to a powerful new weapon in the battle against disease-causing invaders. These keratin fragments are relatively easy to manufacture, making them good candidates for low-cost therapeutics, the study authors said.

"What's really exciting is that the keratins in our study are already in the body, so we know that they are not toxic, and that they are biocompatible," said the study's principal investigator, Suzanne Fleiszig, a professor at UC Berkeley's School of Optometry who specializes in infectious diseases and microbiology. "The problem with small, naturally occurring, antimicrobial molecules identified in previous research is that they were either toxic or easily inactivated by concentrations of salt that are normally found in our bodies."

These new small proteins in the study were derived from cytokeratin 6A, one of the filament proteins that connect to form a mesh throughout the cytoplasm of epithelial cells.

"We used to think that cytokeratins were primarily structural proteins, but our study shows that these fragments of keratin also have microbe-fighting capabilities," said study lead author Connie Tam, an assistant research scientist in Fleiszig's lab. "Cytokeratin 6A can be found in the epithelial cells of the human cornea as well as in skin, hair and nails. These are all areas of the body that are constantly exposed to microbes, so it makes sense that they would be part of the body's defense."

In a commentary published alongside the study, Michael Zasloff, professor of surgery and pediatrics at Georgetown University's School of Medicine, said these "keratin-derived antimicrobial peptides appear to be exciting new biocompatible candidates for development as human anti-infective therapeutics."

The researchers in Fleiszig's lab came upon cytokeratin 6A in their efforts to solve the mystery behind the eye's remarkable resilience to infection. They noticed that the surface of the eye, unlike other surfaces of the body, did not have bacteria living on it, and that corneal tissue could handily wipe out a barrage of pathogens in lab culture experiments.

"It is very difficult to infect the cornea of a healthy eye," said Fleiszig. "We've even used tissue paper to damage the eye's surface cells and then plastered them with bacteria, and still had trouble getting bacteria to enter the cornea. So we proposed that maybe there were antimicrobial factors that are unique to the eye."

In the hunt for this mystery compound, the researchers cultured human corneal epithelial cells and exposed them to the *P. aeruginosa* bacteria. They used mass spectrometry to sort out which peptides were most active in fighting off the bacteria. Cytokeratin 6A-derived peptides emerged the winners, and surprisingly, peptide fragments as short as 10 amino acids were effective.

To confirm that they got the right protein, the researchers used gene-silencing techniques to reduce the expression of cytokeratin 6A in the cornea of mice. With a key defense disabled, the amount of bacteria that adhered to the corneas increased fivefold.

Tests showed that cytokeratin 6A-derived fragments could quickly kill bacteria in water and in a saline solution, showing that the salt contained in human tears would not dilute the protein's effectiveness. Other experiments indicated that cytokeratin 6A fragments prevented the bacteria from attacking epithelial cells, and that the proteins cause bacterial membranes to leak, killing the pathogen within minutes.

The researchers noted that further research could reveal numerous different keratin fragments in the body's innate defense system.

"Keratins may represent a novel class of antimicrobials with the potential to be designed to selectively kill specific pathogens," said Tam.

More information: Cytokeratins mediate epithelial innate defense through their antimicrobial properties, *Journal of Clinical Investigation*, 2012. doi:10.1172/JCI64416

Commentary: Defending the cornea with antibacterial fragments of keratin, doi:10.1172/JCI65380

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