PEGGY GREB (K9885-1)

Dairy scientist Max Paape and cell biologist Yan Wang use fast protein liquid chromatography to purify bovine recombinant CD14.

An Udder Solution for Bossie's Woes

It's almost impossible to protect dairy cows from *E. coli* and other coliform bacteria. These gram-negative bacteria lurk in bedding and other damp areas—even in the cleanest dairy barns—waiting for a nice, warm udder to infiltrate. And they make life miserable for 3 million U.S. dairy cows that show visible signs of acute infection . . . not to mention costing an estimated $1.4 billion in annual losses for Bossie's owners from incapacitated cows and milk that can't be sold.

Coliform bacteria account for about 40 to 50 percent of mastitis cases in the United States, and 80 percent of these cows will become visibly sick, says Agricultural Research Service dairy scientist Max Paape. Of the 3 million cows infected annually, the bacteria put about 300,000—or one-tenth—out of commission entirely. And many die from shock induced by the bacterial toxin, or endotoxin.

"Standard therapies haven't been successful in relieving symptoms and reducing mortality from acute coliform mastitis," Paape says. "Vaccines have had limited success in reducing clinical symptoms, but they don't eliminate the coliform organisms."

That's about to change. Late last year, ARS filed a patent application on a recombinant gene that promises both effective treatment for infected cows and prevention in future cows bioengineered with the gene. It codes for a protein—

soluble CD14—that's naturally suspended in cows' milk and blood plasma.

The protein binds to the endotoxin and neutralizes it. That prevents the cow's immune system from overreacting to the toxin, a reaction that bungles the infection-fighting process and could put the animal into shock, explains Paape. He is at ARS' Immunology and Disease Resistance Laboratory in Beltsville, Maryland.

CD14 also sensitizes the lining of a cow's mammary glands to very low levels of endotoxin—produced by just a few bacteria. Once sensitized, these mammary cells start an attack against the infiltrating bacteria before they can get a hoofhold and pour out enough endotoxin to make the cow sick, explains cell biologist Yan Wang, who conducted this research with Paape for her doctoral dissertation. She is now at the National Institute of Allergy and Infectious Diseases in Rockville, Maryland.

Paape and Wang are co-inventors of the recombinant gene and its applications, along with colleague Dante Zarlenga, a molecular biologist who specializes in cloning and designing genes.

A Bet That Paid Off

Paape had already found the CD14 protein embedded in the membranes of white blood cells in cows. And the soluble kind was known to increase during coliform infections in humans and laboratory animals, says Wang. So she and Paape predicted that they'd find the soluble protein in cows' milk. They also bet it could temper the animals' acute reaction to coliform endotoxin while initiating an appropriate response to the infiltrating bacteria. They bet right.

They enlisted Zarlenga's expertise to clone the gene for soluble CD14.

He used as a template the gene for the type of CD14 that stays bound to membranes. Because the gene for the membrane-bound form is longer than the one for the soluble version, he and Wang clipped off the extra bases on one end before inserting the recombinant CD14 gene into bacterial cells.

Then they transferred the gene to insect cells in order to produce enough of the protein to test.

Tissue culture studies showed that the recombinant CD14 protein binds to endotoxin, effectively neutralizing it. The researchers expect it will do the same when injected into a sick cow's blood, but they don't have enough of the protein yet for such a study.

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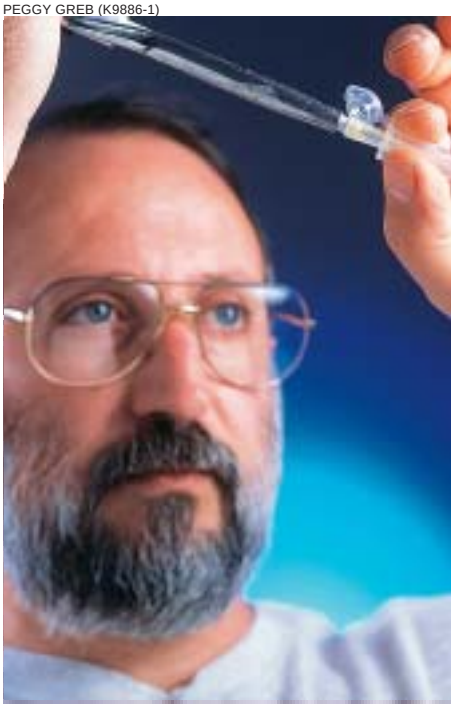

Molecular biologist Dante Zarlenga performs a step in the process of cloning the gene that codes for CD14, a protein that can neutralize toxins created by mastitis-causing bacteria.

"For the first time, veterinarians will have a product to prevent acute endotoxin shock in dairy cows," says Paape. Wang emphasizes that CD14 "is a protein found naturally in cows, so any side effects should be minimal."

Prevention Preferred Over Cure

CD14 works to prevent infection, too. Paape and Wang incubated the protein with endotoxin in a culture dish to form a complex. When they injected the protein-endotoxin complex into cows' teats, it stimulated the mammary cells to launch an appropriate response—one that brings in the white blood cells that gobble up coliform bacteria—without calling in the cavalry.

"It needs more testing in cows," says Wang, "but I think it's very promising for both treatment and prevention."

While it's not feasible for dairy producers to inject CD14 into their cows' teats regularly, the gene for CD14 can be designed and inserted into tomorrow's dairy cows so that it produces the protein only in their mammary cells. And that's not science fiction.

ARS colleagues in the Gene Evaluation and Mapping Laboratory at Beltsville have already produced a cow with engineered immunity. "Annie" is a clone of a Jersey cow whose mammary cells produce a protein that promises to prevent infections from *Staphylococcus*.

Two of those colleagues, ARS physiologist Robert Wall and support scientist Juli Foster-Fry, are constructing a designer CD14 gene and will insert it into mice. If tests show that it works, Wall plans to insert it into cows. Ultimately, his laboratory wants to bioengineer a cow that is protected against all mastitis-causing organisms. And that's when you'll see Bossie smile!—By **Judy McBride,** formerly with ARS.

This research is part of Animal Health, an ARS National Program (#103) described on the World Wide Web at http://www.nps.ars.usda.gov.

Max J. Paape and Dante Zarlenga are with the USDA-ARS Immunology and Disease Resistance Laboratory, 10300 Baltimore Ave., Bldg. 1040, Room 105, Beltsville, MD 20705-2350; phone (301) 504-8302, fax (301) 504-5306, e-mail mpaape@anri.barc.usda.gov, dzarlenga@anri.barc.usda.gov. ◆