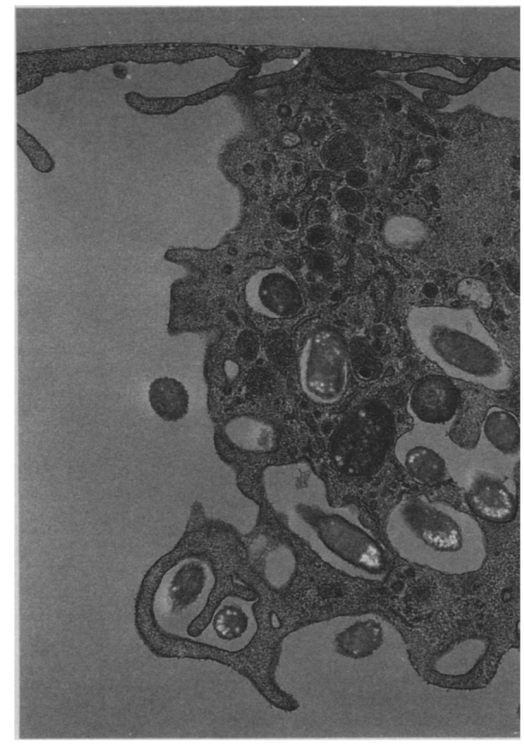
Making the Most of MIPs



Genes governing bacterial stress responses may hold the key to better vaccines

By RICK WEISS

For *Salmonella* bacteria hiding in spoiled foods and contaminated water, the search for a home in a human host resembles an adventure scripted for Indiana Jones.

First there's the flood of human salivary enzymes and the enameled molars that nearly grind you to death. Then, having tumbled into a bath of stomach acids, biliary detergents and pancreatic enzymes, after squeezing between cell walls and wandering blindly through interstitial labyrinths—just when you think you're home free in the bloodstream where nutrients flow like nectar — a behemoth white blood cell, the macrophage, lumbers over and engulfs you.

All is quiet for several minutes in this ominous, intracellular air pocket; then spigots open from every direction, spraying you with powerful acids, hydrogen peroxide and protein-degrading enzymes.

Surely the film ends here.

But no. You are *Salmonella* Jones. And what you've got going for you, in the words of Nancy A. Buchmeier and Fred Heffron, are macrophage-induced proteins, or MIPs.

Stressed *Salmonellae* produce MIPs soon after getting gobbled by a macrophage, Buchmeier and Heffron have discovered. Through some unexplained mechanism, MIPs are probably the key to bacterial survival within these usually deadly immune-system cells, they say.

But scientists may soon gain the ability to subvert the pathogen-protecting proteins for human benefit. Buchmeier and Heffron — molecular biologists at the Research Institute of Scripps Clinic in La Jolla, Calif.—foresee using MIPs to create a new generation of oral vaccines against *Salmonella*-caused diseases such as typhoid fever. And by harnessing the bacterial regulatory genes that control MIP production, they hope to develop potent oral vaccines against a host of unrelated scourges such as cholera and diphtheria, for which only imperfect, intramuscular vaccines exist today.

"The work is exciting," says Samuel Miller, a molecular geneticist at the Massachusetts General Hospital in Boston. "We don't understand *Salmonella* virulence on the molecular level. Hopefully this work will lead to the identification of virulence factors and an understanding of the regulatory systems that control them."

That information, he says, should enable scientists to construct gene-altered versions of *Salmonella* that can trigger, in a single dose, powerful and specific immune responses against a wide variety of diseases.

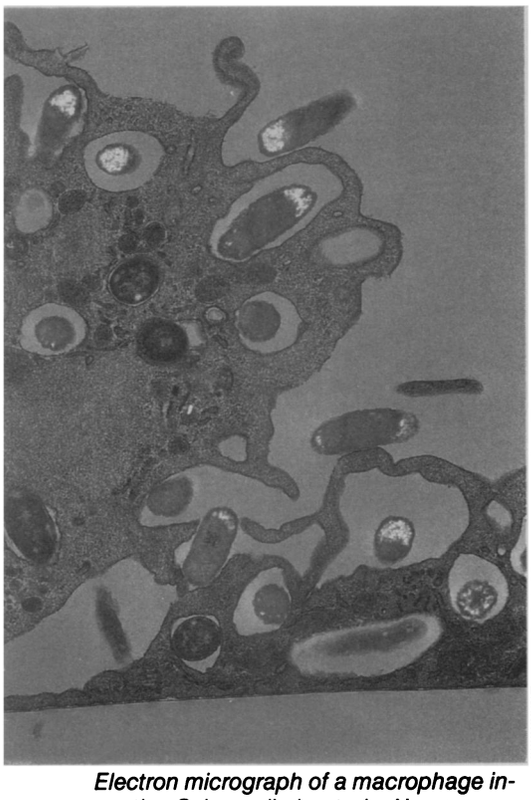
Vaccine design has come a long way since 1796, when Edward Jenner discovered he could prevent smallpox infection in people by inoculating them with the less virulent but related organism that causes cowpox. Nearly 200 years later, the FDA has approved vaccines against dozens of microbes that might otherwise establish themselves within the human body.

Some vaccines consist of killed bacteria or viruses (or, more recently, laboratory-engineered copies of microbial fragments). Others contain live, weakened microbial strains. With all vaccines, the idea is to introduce some characteristic part of the invading organism to the body's white blood cells. This primes the immune system so it can respond more effectively in the event of an actual attack.

But some vaccines — notably those made of living organisms — work better than others.

"People haven't understood why you get much better immunity when you immunize with live bacteria as opposed to with dead ones," Buchmeier says. The discovery of MIPs may solve this mystery, she contends. After all, only a live bacterium can synthesize MIPs in response to being swallowed by a white blood cell. And these proteins may be critical to triggering a strong immune response, Buchmeier says.

The Scripps scientists' discovery of MIPs was a natural outgrowth of studies relating to stress responses in bacteria. "We've known that bacteria can synthesize proteins in response to environmental changes, such as heat," says Buch-



Buchmeier

Electron micrograph of a macrophage ingesting Salmonella *bacteria. Numerous rod-shaped* Salmonellae *are located in vacuoles within the macrophage.*

meier. However, she adds, these studies have never focused on bacterial responses to immune-cell attack.

Moreover, scientists know little about the genes that code for stress-induced proteins, or what triggers these genes to turn on when they do.

"There are many levels of defenses that bacteria encounter in the course of infection," explains MIT's Miller. "Each is a different environment, and to survive each environment, the bacteria have to express different genes."

In particular, adds Dale Spriggs of the National Institute of Allergy and Infectious Diseases (NIAID) in Bethesda, Md., the inside of a macrophage represents one of the more extreme environments that bacteria happen upon. "Clearly it's an alien, hostile environment – one that has evolved with the express purpose of killing these kinds of organisms," he says.

Buchmeier and Heffron reasoned that bacteria, especially those such as *Salmonella* that have better-than-average survival rates in the body, might secrete some kind of protective factor after ingestion by a macrophage. But they had no idea which proteins, if any, these bacteria might produce under such conditions. In the May 11 SCIENCE, they report solving at least part of that mystery, with the first description of proteins synthesized by *Salmonella* in response to the antagonistic environment encountered inside macrophages.

The Scripps researchers identified many of the proteins synthesized by *Salmonella typhimurium* within cultured macrophages and compared these with *Salmonella* proteins synthesized under identical culture conditions but without macrophages. They used a drug that blocks macrophage protein synthesis to ensure that their analysis was limited to *Salmonella* proteins.

Of the 405 bacterial proteins they analyzed, 12 were uniquely expressed in the macrophage environment – including two proteins whose levels were already known to increase in response to heat.

The researchers found another 22 proteins produced by bacteria in both environments but synthesized at least four times faster in the macrophage cultures. In their report, Buchmeier and Heffron hypothesize that these 22 "may be essential under a variety of conditions, but are needed at higher concentrations during macrophage infection."

To test their theory that some MIPs are crucial to *Salmonella* virulence, the team analyzed the protein profiles of two mutant *Salmonella* strains that do not survive well inside macrophages and do not cause disease. Sure enough, one lacked six MIPs; the other failed to produce nine MIPs. One missing MIP was common to both mutants.

While those experiments suggest that some of these proteins protect the bacteria, their production within the macrophage – the immune cell that serves as a kind of "point man" in the antibody-making process – also triggers an enhanced immune response. In the end, it appears, the balance between bacterial virulence and the resulting immune response determines whether a patient will get better.

Finally, by comparing the MIP profiles of various *Salmonella* strains under different conditions, the researchers determined that at least three different regulatory genes control the production of several MIPs each. Somehow, these genes recognize environmental changes outside the bacteria, and under appropriate conditions tell various MIP genes to initiate MIP manufacture.

"This is the first description of specific changes in protein synthesis following infection of macrophages," Buchmeier says. Determining whether these MIPs actually confer virulence will require further experiments. But already, the Scripps scientists have determined that the two most prevalent antibodies made in response to *Salmonella* infection are directed against two MIPs. The researchers suspect some MIPs may play stress-related roles unrelated to virulence, yet may stimulate a strong immune reaction. If so, they suggest, scientists might make an excellent vaccine against *Salmonella* by engineering a bacterial strain lacking critical disease-causing genes but containing genes that code for antibody-triggering MIPs.

Moreover, since *Salmonellae* entering the body via the digestive tract can survive until ingested by macrophages, such a vaccine would be effective when taken orally. Indeed, a new typhoid vaccine approved by the FDA in January is an oral vaccine, made from a live, chemically weakened strain of *S. typhi,* which causes typhoid fever in humans. But scientists say that strain remains poorly understood, and a specifically engineered strain might provide better protection.

Public health officials like oral vaccines because they are painless and easy to administer, improving the chances that many individuals will get vaccinated. And in the Third World, where the most serious *Salmonella* infections commonly occur and sterile needles are scarce, oral vaccines are safer than those requiring syringes.

Vaccine designers may apply MIP studies to other diseases, too, but first scientists must get a better handle on precisely which regulatory genes tell MIP genes to turn on. This should enable genetic engineers to splice into *Salmonellae* some genes coding for other disease antigens, and place them under the control of these regulatory genes. When exposed to macrophages, such an engineered *Salmonella* would turn on its protein-making machinery, but the resulting proteins would be, for example, cholera antigens that trick the body into making cholera antibodies.

"There's a lot of activity now in this area, trying to make more specific mutations [in *Salmonella*] both to improve the current typhoid vaccine and to use as a Trojan horse to carry other antigens," says Spriggs of NIAID. "The nice thing about using *Salmonella* is that, given orally, it gets into the immune system and stimulates a good, solid immunity there."

The discovery of MIPs provides an unprecedented view of the mechanisms involved in bacterial virulence and should shed some new light on the molecular nature of protective immunity, Spriggs and others say. Still, Heffron notes, much more research must follow for scientists to identify exactly which MIPs are most useful in triggering a protective response without causing disease.

"Virulence is a complicated process, requiring the interaction of many proteins," Heffron says. But by teasing apart the roles of individual MIPs and other factors, he and others look forward to getting the body's own macrophages to trigger production of key antigenic proteins.

By feeding these key antigens to the body's antibody-boosting machinery, Heffron predicts, "you're going to get a whopping immune response." □