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Tick Saliva: New Target for Lyme Disease Vaccine

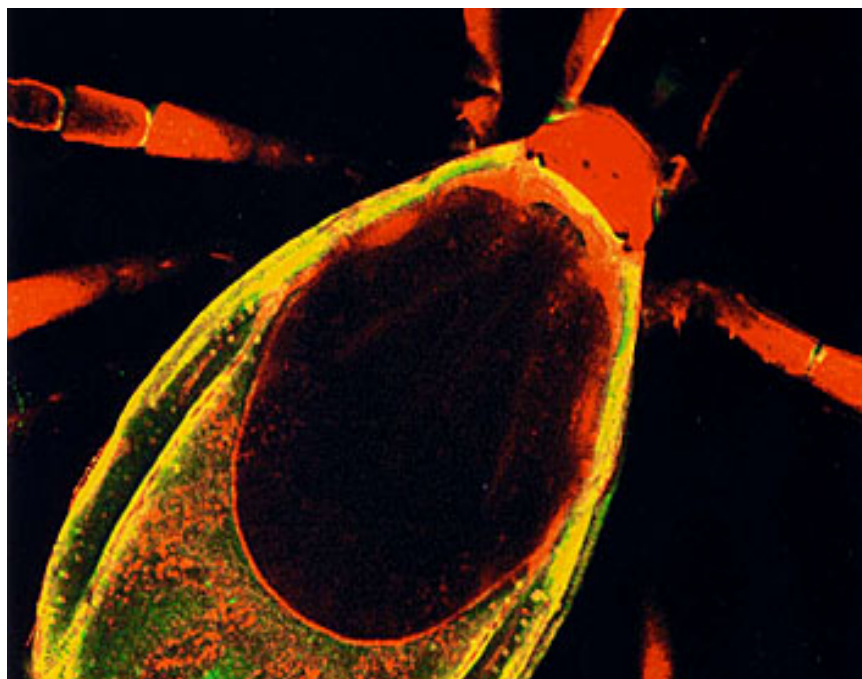


Image Title: - Utpal Pal, Ruth Montgomery, and Erol Fikrig

A protein found in the saliva of ticks may prove to be an attractive target for a new type of Lyme disease vaccine. In studies in mice, Howard Hughes Medical Institute researchers at Yale University produced an antiserum against a protein in tick saliva that significantly reduced the likelihood that mice could be infected with the tick-borne bacterium that causes Lyme disease.

Lyme disease first manifests in humans as a rash that may pass unnoticed. As the infection worsens, symptoms may include fever and chills, joint swelling, numbness, weakness, and even heart problems. The findings suggest a new way forward for Lyme disease vaccine development.

Vaccines have traditionally targeted unique proteins found on the surface of pathogens. In the new studies, published in the November 19, 2009, issue of *Cell Host & Microbe*, the researchers show that it is possible to target molecules carried by a disease vector – not the pathogen itself. This could be an effective strategy to prevent Lyme disease, as well as malaria, dengue fever, and other diseases carried by arthropods such as ticks and mosquitoes, said senior author Erol Fikrig, a Howard Hughes Medical Institute investigator at Yale University.

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When the bacterium that causes Lyme disease is transmitted to a mammal via a tick's bite, the bacterium wraps itself in a protein cloak that makes it invisible to the host's immune system. That cloak is made from a protein found in tick saliva, which the Lyme bacterium, *Borrelia burgdorferi*, causes the tick to produce in excess. In *Cell Host & Microbe*, Fikrig and his colleagues describe a way to turn this cloak of invisibility into a vulnerability.

Fikrig, who is chief of infectious diseases at the Yale School of Medicine, said vaccine development — even as far back as Louis Pasteur's discoveries in the 1880s — has historically relied on using a weakened form of the pathogen, or a component of it, to evoke an immune response that would protect against later encounters with the same microbe.

"For vector-borne diseases, where the bacteria are transmitted by a tick or a mosquito, we wanted to know: Is it possible there is something that is not pathogen-based that can be targeted?" Fikrig said.

"The tick isn't just a syringe," Fikrig said. Tick saliva contains a variety of unsavory ingredients that help the insect's five- or six-day blood meal proceed unnoticed by the host, and the presence of the pathogen actually changes the composition of the tick's saliva. For example, the saliva contains anesthetics that keep the bite from stinging and blood thinners to prevent clotting.

In 2005, Fikrig and his colleagues found that tick saliva also harbors Salp15, a protein that shields the tick from mammalian immune cells known as T-cells. The Lyme disease bacterium drives the tick to overproduce Salp15, so that it can use that protein to remain invisible to the host's immune system.

“For us, a central question was, if the spirochete requires the tick protein for infection, and it’s coated with it, can we actually target this protein?” Fikrig said. To test the efficacy of Salp15 as an immunizing agent, researchers injected a few mice with an antiserum against Salp15. Other mice, to be used as controls, were injected with an inactive serum. The following day, both groups of mice were injected with *B. burgdorferi* coated by the cloaking Salp15 protein.

When the mice were examined a week later, all of the control mice showed signs of Lyme disease, but only half of the mice treated with the Salp15-antiserum were sick. When infection did occur in Salp15-injected mice, the antiserum was still protective: It helped to reduce the total amount of *Borrelia burgdorferi* bacteria in the body compared to control mice. After three weeks, 40 percent of the mice given Salp15 antiserum remained Lyme-free. Those that showed infection had lower levels of the Lyme bacteria in their hearts and joints than control mice.

One value of creating a vaccine against a protein produced by the vector, Fikrig said, is that it might enhance the effectiveness of a traditional pathogen-based vaccine. “Take a disease like dengue virus or malaria, for which there is no highly efficacious vaccine,” he said. The vaccines that do exist follow the traditional model -- that is, they are based on components of the pathogen that infects the host. Fikrig suspects that including a vector protein like Salp15 in a vaccine that also targets a pathogen component could boost the vaccine’s ability to prevent disease. “Let’s say the vector targets are 50 percent efficacious and some pathogen components 40 to 50 percent. Combine the two and you might have a very good vaccine.”

To test that idea, Fikrig’s team combined the Salp15 antiserum with an existing pathogen-based vaccine against Lyme disease. That vaccine, which Fikrig helped develop in the early 1990s, promotes immunity to a protein on the surface of the *Borrelia burgdorferi* pathogen called outer-surface protein A, or OspA. The vaccine was on the U.S. market from 1998 to 2002, when the manufacturer, GlaxoSmithKline, withdrew it, blaming poor sales. In an article that Fikrig wrote earlier this year for the journal *Future Microbiology*, he noted that concerns about potential side effects may have been a factor in the drug company’s decision to withdraw the Lyme disease vaccine.

In another set of experiments reported in *Cell Host & Microbe*, Fikrig and his colleagues combined low-dose Salp15 antiserum with a lower-than-effective dose of OspA monoclonal antibody and administered the mix to one group of mice. Other mice received either protective doses or low doses of just one of the two vaccine components. Each mouse was then exposed to 10 ticks carrying the Lyme-disease bacteria.

Mice treated with a combination of low-dose OspA and Salp15 fared better than mice treated with either agent alone at low dose. Only 25 percent of mice treated with both agents showed signs of Lyme infection. In addition, only 20 percent developed signs of arthritis, and their inflammation was low

compared to the inflammation of controls. These mice also showed the lowest spirochete burden.

In contrast, 90 percent of mice treated with low-dose OspA alone and 100 percent of untreated mice were infected with *Borrelia burgdorferi*.

These results eased one of the researchers' concerns about combining the two approaches, Fikrig said. Since Salp15 normally mutes the body's immune response to the spirochete invader, there was concern that it might blunt the immune response to OspA, and fail to produce any combined benefit. But their experiments ruled out that possibility. "We immunized with both together and found out that wasn't the case," he said.

In additional tests, mice were inoculated with a form of Salp15 that the researchers produced in the lab. These mice developed antibodies that protected them against infection when they were exposed to Lyme-carrying ticks. Three weeks after tick exposure, 40 percent of the inoculated mice showed no signs of infection, while 95 to 100 percent of controls were infected. Further research suggested that Salp15 probably protected the mice from infection by flagging the cloaked *Borrelia burgdorferi* spirochetes, which led immune cells to ingest the invaders, Fikrig said. Mice inoculated with both Salp15 and OspA fared even better— 80 percent remained uninfected. The combined impact of the two vaccines suggests other areas of research, and Fikrig says his group has already begun investigating whether a similar vaccine strategy might be effective in preventing malaria.