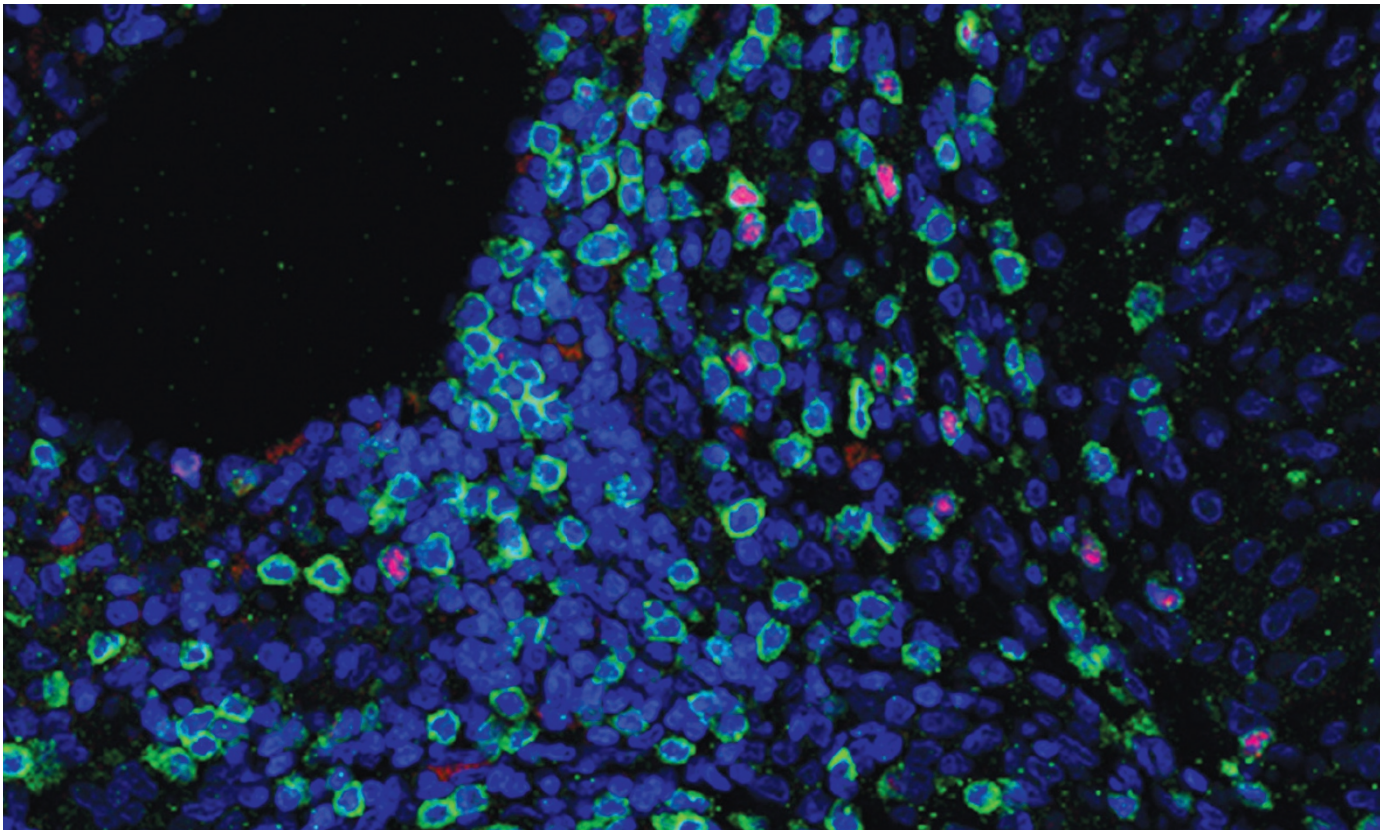

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T cells (green) and nuclei (blue) in a tuberculous granuloma stained for regulatory T cells are important regulators of macrophage activation.

VACCINES

An age-old problem

Researchers are on the hunt for a better alternative to the BCG vaccine.

BY SARAH DEWEERDT

It is one of the most widely administered vaccines in the world. In use for nearly a century, it has been given to over a billion people. And yet, it is still not good enough.

The bacillus Calmette–Guérin vaccine, or BCG, is a live but weakened bacterium that is about 80% effective at protecting young healthy children from severe forms of tuberculosis (TB) — particularly tuberculous meningitis, which affects the brain, and miliary TB, a systemic infection of the body.

But BCG offers little protection to adolescents and young adults from the form of the disease that spreads widely and causes most deaths: pulmonary TB. Moreover, BCG is a replicating bacterium; in children infected with HIV the vaccine itself can cause disease, known as BCG-osis.

Many experts say that eradicating TB will not be possible without an improved vaccine that protects against pulmonary TB. Because of drug resistance and the complexity of TB treatment regimens, "It is very clear that by far the most sustainable intervention would be if you have a vaccine that prevents disease," says Willem Hanekom, director of the South African Tuberculosis Vaccine Initiative (SATVI) in Cape Town.

At first glance, the effort to develop an effective vaccine against pulmonary TB seems poised to be richly rewarded. More than US$95 million went towards TB vaccine development in 2011 alone, and over a dozen candidates are in clinical trials. Just over ten years ago there were none.

The vaccine candidates are not only numerous but varied, using different mechanisms and dosing strategies. But this multitude of possibilities also reflects the field's biggest problem: a lack of knowledge about *Mycobacterium tuberculosis* and how it interacts with the human immune system. "We don't know enough to exclude anything at the moment," says Helen McShane, a vaccinologist at the University of Oxford, UK.

These challenges were highlighted by the failure of one promising vaccine candidate, MVA85A, to show efficacy against TB in a clinical trial in early 2013.

MVA85A is designed to stimulate immunity to 85A, one of several thousand molecules, or antigens, produced by *M. tuberculosis*. The vaccine delivers the antigen into cells — where it can trigger an immune response — via a genetically engineered virus, so MVA85A is known as a viral vector vaccine.

The research team conducting the trial, including Hanekom and McShane, gave MVA85A injections to 1,399 South African infants who had been vaccinated with BCG earlier in infancy[1]. Several vaccine candidates are designed to work this way, known as a prime–boost strategy. It is thought that priming the immune system with BCG and then administering a booster dose of a new vaccine will continue to protect children against the most dangerous forms of TB while adding protection against pulmonary TB.

But, after about two years, children who had received the MVA85A vaccine had the same rate of TB infection and disease as children who had not[1]. "That it showed no efficacy is surprising, because this vaccine was vetted in many ways," says Daniel Zak, a principal scientist at the Seattle Biomedical Research Institute in Washington.

Mouse and non-human primate studies had shown that MVA85A provides increased protection against TB over BCG alone; it is not clear

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why these results did not translate to humans.

"Tuberculosis is a complex pathogen," says McShane. "It hides inside cells most of the time, which means the immune response required to clear it is predominantly a T-cell response." T cells are involved in a process called cellular immunity, in which, among other things, they induce infected cells to 'commit suicide'. By contrast, most of the successful vaccines in use today are based on humoral immunity, which mainly involves the production and secretion of antibodies into the extracellular fluid. Scientists have a better understanding of humoral immunity and the vaccines that stimulate it, but on its own it does not clear bacteria from cells.

TRIED AND TESTED

Despite the lack of efficacy shown in the MVA85A trial, researchers say it was a success in other ways. It was the first efficacy trial of a TB vaccine in more than four decades. It demonstrated that a TB vaccine can be tested in infants according to modern-day ethical standards, and that researchers can develop the infrastructure and analytical methods needed to carry out a large clinical trial in a resource-poor country[2]. "In a way it's become a template for how to do such a study," says Hanekom.

With so many vaccine candidates waiting in the wings, it is a template that could get a lot of use. The next vaccine candidate likely to start efficacy trials is known as M72. It is an example of what scientists call a subunit or protein-adjuvant vaccine: a cocktail of two antigens, 32A and 39A, plus an adjuvant, a chemical that enhances immune responses. As with MVA85A, scientists envision using M72 as a booster vaccine in individuals who have already received BCG.

Unlike the MVA85A trial, which was confined to infants, a team including SATVI researchers plan to test M72 efficacy in young adults, because they are more affected by pulmonary TB. So far, the team has shown that the vaccine is safe and induces a good T-cell response when administered to adults in South Africa either without TB or with latent infection[3]. An upcoming study will test whether the vaccine reduces the risk of developing active disease over the course of three years. It will involve around 7,000 participants in three African countries, Hanekom says, and is likely to begin by the end of 2013.

This is the first protein-adjuvant vaccine candidate to be tested for efficacy, notes Jelle Thole, executive director of the Tuberculosis Vaccine Initiative in Lelystad, the Netherlands, which supports and coordinates TB vaccine research throughout Europe. It was chosen largely because it happens to be the vaccine in this category that was the most developed. But in the future, Thole adds, vaccine candidates within each category should be tested against each other, with the one that does best in animal studies and at inducing immune responses in healthy volunteers moving forward first to efficacy trials.

A vaccine of a third type, based on a whole, live bacterium, may also be nearing efficacy trials. "This is one of the few candidates that is trying to be better than BCG," says Stefan Kaufmann, director of the department of immunology at the Max Planck Institute for Infection Biology in Berlin.

Kaufmann is one of the developers of the vaccine, called VPM 1002. It is based on BCG, which in turn is derived from *M. bovis*, which causes TB in cows. He notes that other types of vaccine contain only one or a few antigens to stimulate an immune response. His team chose to work with a whole live bacterium because "we thought that the whole antigen repertoire that is present in BCG might be a better choice," he says.

If it works, Kaufmann hopes that VPM 1002 will one day replace BCG as the standard immunization given to infants. It could also be combined with one of the booster vaccines now in development.

To create VPM 1002, Kaufman's team inserted a gene called listeriolysin into BCG and deleted another gene, encoding a subunit of urease[4]. These genetic changes make BCG more visible to the immune system. For example, BCG mainly stimulates CD4 T cells whereas VPM 1002 is better at also stimulating CD8 T cells. Kaufmann argues that a broader immune response is more likely to result in improved protection from the different forms of the disease.

"We don't know enough to exclude anything at the moment."

VPM 1002 has bested BCG in mouse studies, and its safety has been assessed in newborns in South Africa. The results of that study have not yet been published, but "everything went very well," Kaufmann says. The next step is to secure approval — and funding — to test the vaccine's efficacy.

GOING ROGUE

Meanwhile, some scientists are beginning to rethink what a successful TB vaccine might look like. For example, the Seattle-based Bill & Melinda Gates Foundation, which has contributed hundreds of millions of dollars to TB vaccine research, has issued a call to develop vaccines that rely on mechanisms other than T cells, recruiting parts of the immune system such as natural killer cells or antibodies that are not big players in the body's normal immune response to TB. That is likely to be a difficult exercise, but Zak thinks it might be worth trying, as "there's something that's missing" from the immune response induced by T-cell vaccines so far.

McShane, on the other hand, is not giving up on T-cell-inducing vaccines, or indeed on MVA85A. She is studying whether MVA85A will work if delivered directly to the airways as an inhaled vaccine. "We need to generate more potent vaccines. Delivering vaccines to the lung appears to be one way to do that," she says. Her lab is also investigating whether combining MVA85A with another molecule designed to boost the immune response will improve efficacy.

Others are reconsidering not just how but when the vaccine should be delivered. For example, some investigators are asking whether a booster dose of BCG itself could improve protection against pulmonary TB. And an effective vaccine may require even more frequent dosing. "I can imagine a scenario where you need to take a puff of your TB vaccine on your birthday," says Kevin Urdahl, a principal investigator at the Seattle Biomedical Research Institute.

How to prioritize clinical trials and translate basic research more effectively into vaccine development is also generating interest.

"The big issue in vaccination right now is that we don't have a good correlate of protection," says Thole. In other words, there is no test or measurement that can define whether or not a person is protected from TB. "So we don't know what kind of immunity a vaccine needs to induce."

Researchers know that an effective TB vaccine must induce TB-specific T cells, but this does not seem to be the whole story. Take the MVA85A trial, in which researchers observed a T-cell response to the vaccine despite its lack of efficacy. Ideally, additional correlates of protection would be identified by comparing immune responses in individuals who are protected by a vaccine to those in individuals who are not protected.

The negative results of the MVA85A trial mean that researchers cannot make this comparison, so this strategy remains elusive. Still, McShane hopes to mine the trial data to see if there are any differences in the immune responses between study participants who became infected with TB and those who did not.

Others are looking not for correlates of protection but for what they call correlates of risk. By following the natural history of TB from the time of infection, "we can comprehensively define the immune responses in people that progress to [active] disease and those who do not progress", says Zak, who is conducting such studies in collaboration with SATVI.

Researchers could then conduct clinical trials of new vaccine candidates in people at high risk of progressing to active disease (see 'Latency: A sleeping giant' page S14), boosting the statistical power of smaller, more rapid studies.

The need for a better clinical trial strategy is acute, some researchers say. "My fear is that if we push the clinical trials too quickly, and spend a lot more money getting results [from vaccines] that don't provide much protection, then we'll have lost our chance and the resources will run out," says Urdahl. "There's more basic research to do on the front end to make sure that we're putting the right candidates into the pipeline." ■

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