A Reliable Vaccine

At the **Max Planck Institute of Biochemistry**, researchers are pursuing an idea for producing a completely new class of vaccines with improved results and safety. They have developed a vaccine prototype that cannot multiply independently, but that can effectively encourage host cells to produce a desired antigen.

TEXT **PHILIP WOLFF**

It was a vibrant green glow, and even though Wolfgang Neubert can no longer recall the exact date – it must have been some time in 2004 – he will not forget the sight. The cells infected with the virus in his petri dishes were such a colorful contrast to the pale cell culture that it took just one look for him to know that his idea would take off. Clearly, the pathogens really could be manipulated with a few changes to the genetic makeup, making their host cells produce large amounts of their proteins, marked a vibrant green for experiment purposes, without – and this was important for Neubert – multiplying and infecting other cells. Even the closest neighboring cells around the vibrant dots in the petri dishes remained dark.

AN END TO MULTIPLICATION FOR A VIRUS

This is exactly how the head of the Molecular Virology working group at the Max Planck Institute of Biochemistry in Martinsried thought it should look. A brand new vaccine could result, one that uses its proteins to stimulate a permanent immune response without multiplying itself. What was, at the time, more a thought model than a plan is now really taking shape. Only five years after the promising glow, specialists at a small biotech company in Martinsried are now striving to further advance Neubert's genetically modified virus to a vaccine.

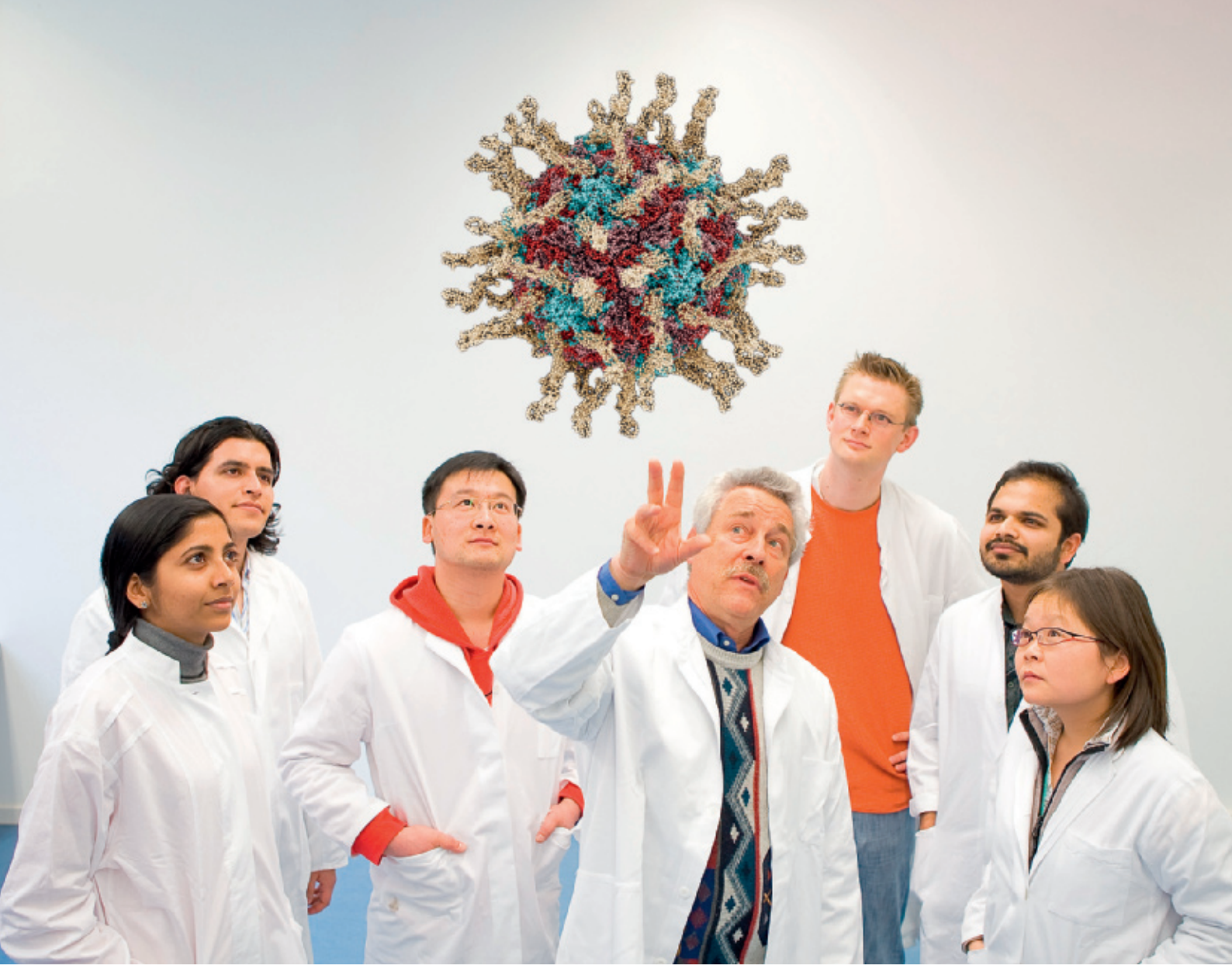
The researchers had modified the virus with a few specifically removed gene sequences from its RNA so that blueprint reading (RNA transcription) and protein production still worked, but the thousand-fold multiplication of the genetic makeup (RNA replication) and its spreading were prevented. Never before had there been such a tricky system – a fact that had bothered Neubert for decades when he explained the state of vaccine development to students. "During lectures, I repeatedly had to explain that, on the one hand, we have non-live vaccines from dead pathogens that are unable to multiply independently or produce proteins and thus do not stimulate immune systems over long periods, so must be administered over and over," he says, "and, on the other hand, we have excellent live vaccines that are beset with all sorts of potential drawbacks."

Since live vaccines are germs that multiply, replication mistakes can occur that can lead to new and possibly resistant pathogens. "A past example of this was the polio vaccine, when vaccinated individuals had to use separate bathrooms so that no virus modified in a person would come into contact with non-vaccinated individuals. And when, at the end of the 1990s, the probability of such vaccine damages in Germany was greater than contracting an illness in the usual way, the switch was made to a non-live vaccine," explains Neubert. Another safety risk of live vaccines is that, although they multiply more slowly than the immune system reacts, everyone has a different immune system – and it can be overworked at times in the very young, the elderly, or in stressed individuals. In these cases, the vaccinated virus can multiply more than intended, resulting in vaccine complications.

NO ARGUMENTS LEFT FOR OPPONENTS OF VACCINATION

The new vaccine from Martinsried should one day offer a solution to both of these problems. The vaccine should not multiply, change, or overburden any immune system, points that would undermine the arguments of vaccine opponents and skeptics whose anti-vaccination stance already led the World Health Organization to reprove Germany, stating that we are not vaccinating sufficiently here and are exposing children to unnecessary risks. At the same time, the innovative vaccine from Martinsried should also remedy the shortcomings of non-live vaccines. "As risk-free as a non-live vaccine, yet as effective as a live vaccine – that is the idea," says Neubert.

The first greenish glowing confirmation for this idea came from proteins that produce the Sendai virus, which is dangerous for rodents but not for people. For many years, researchers working with Neubert studied the replication mechanisms of pathogens



Key learning object: Using the polio virus as an example, Wolfgang Neubert describes the status quo in vaccine development to students and explains the potential disadvantages of live vaccines.

Collage: designergold; photos: Axel Griesch/SPL-Agentur Focus

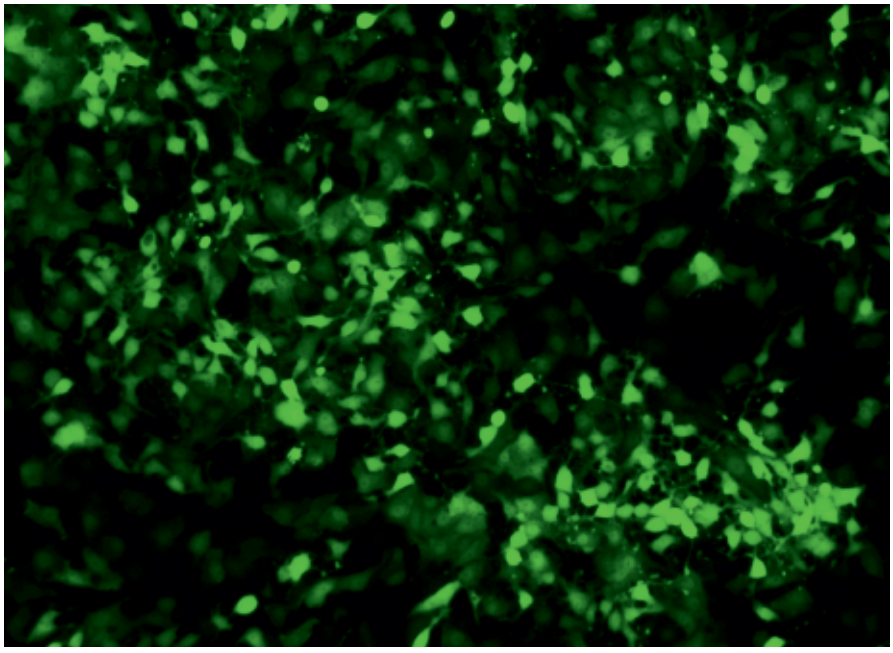
using the Sendai virus and discovered which genes are important for transcription and which are important for the replication of genetic material. They not only removed a gene for replication, but they also multiplied the protein blueprints, causing sparks to fly in the cell cultures. But would the principle also work in an organism? Does the intensity of the glow mean there are enough proteins to stimulate an immune response in living organisms? "That was the second essential question," states Neubert. In conventional live vaccines, each genome multiplies itself ten thousand fold, and all the copies serve as a matrix for protein production. "Naturally, there is more transcription – that is, more protein molecules – than if only a single genome were introduced. I presume that is why my colleagues and I were the only ones to consider the new approach," muses Neubert.

In the scope of a European cooperation project, biologists Maria Grazia Cusi from the University of Siena and Patricia Johnson from the National University of Ireland answered the second crucial question. They administered Neubert's vaccine viruses to mice, "in the form of a spray, because we wanted to simulate the pathogen's natural path of infection via the mucous membranes to see if an antibody barrier could already be built up there," explains Neubert. Otherwise, even if there are enough antibodies in the bloodstream, some pathogens can multiply freely in the mucous membranes and destroy them, or make the vaccinated individual an unknown carrier of the virus. The amount of proteins that Neubert's modified Sendai virus produced should serve to prevent these risks.

The news that it actually worked in mice reached Wolfgang Neubert by e-mail in 2006. Diagrams showed how strong the immune response was in the blood and the lung wash fluid of the lab animals. "That was when it became clear that we should continue developing the procedure," states Neubert. The technology transfer arm of the Max Planck Society, Max Planck

top | Cells previously infected with Sendai viruses glow green in the petri dish: The cells produce large quantities of viral proteins that stimulate the immune system. The clever thing about it is that the viruses are unable to multiply and attack other cells.

bottom | The biotech stronghold of Martinsried has recently seen an arrival from Switzerland. AmVac AG opened a subsidiary here in February 2009: Head of Research Marian Wiegand, Michel Klein, Melinda-Kinga Karpati, Wolfgang Schmidt and Wolfgang Neubert (from left to right).



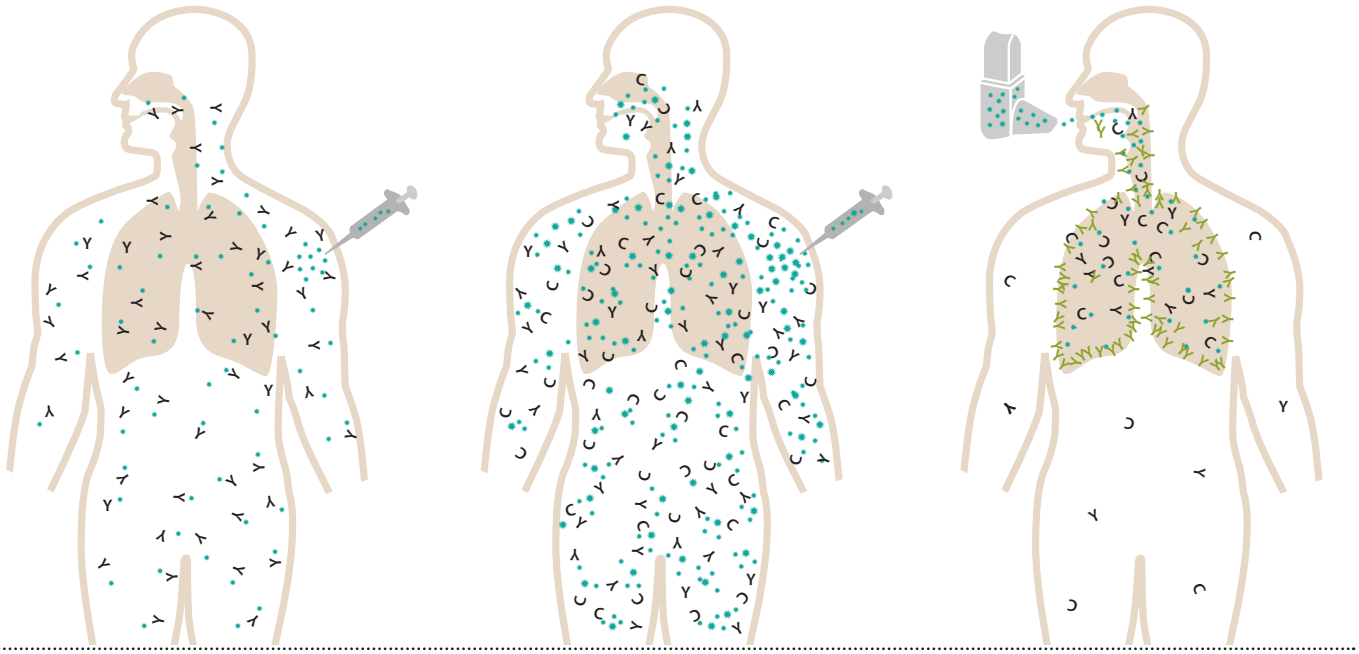
Innovation, identified a suitable lawyer, and the process was patented the same year. "We looked around right away to see which partner could advance the development to a product," says Neubert.

THE SCIENTIST ADVISES, A PHARMA FIRM DEVELOPS

Not exactly an easy task. Large pharmaceutical companies prefer to purchase perfected procedures, "and some do so only to file them away so that existing products will not have to be renegotiated." For this reason, we were hoping to identify a – preferably young and innovative – company with existing expertise in new vaccine processes. AmVac, a biopharmaceutical company from Switzerland that specializes in developing therapeutic vaccines that work via mucous membranes, met our criteria. "The technology from Martinsried was simply a good match," states Marian Wiegand, research head at AmVac Research GmbH, which moved into the complex in Martinsried. Max Planck Innovation and AmVac signed a license agreement in late 2007.

Since then, Wiegand has been fostering the development with Wolfgang Neubert advising him. The goal now is to test the method on people very soon. In order to do so, the RNA of a human pathogen must be built into the genetic material of the Sendai virus. Like a military launcher system that allows various warheads to be placed on it, Neubert's vaccine virus should someday introduce a variety of pathogens into people, ranging from malaria to influenza. However, researchers must first demonstrate that it works. To do this, they chose the respiratory syncytial vi-

Photos: Jürgen Sauer (bottom), AmVac (top)



Unlike the conventional non-live and live vaccines (left and center) the new vaccine against the RS virus (right) is inhaled. The antibodies in the mucous membranes provide an initial barrier against infection and long-term immunity through antibodies in the blood (Y) and specific immune cells (C).

rus (RSV), which causes respiratory diseases that are particularly dangerous for small children. "The virus uptake is through the airways, where it multiplies in the airway cells and destroys them. I can't prevent this by administering an RSV vaccine by injection because that merely results in antibodies in the blood circulation," explains Neubert.

MAKING SURE THAT NO MONEY GOES DOWN THE DRAIN

The questions that partner labs are currently investigating in mice experiments include: How many transcription segments of RNA are required for the RS vaccine virus to produce enough proteins for the desired immune response? Once researchers start pilot tests on humans, the vaccine cannot be altered – and the same goes for the production processes. After all, because the virus is unable to multiply independently, its production requires special genetically prepared cells that are provided by the virus' removed replication genes. This production method, now an indispensable process in recent vaccine technology, must – unlike in test labs – meet stringent cleanliness requirements and offer stable, consistent quality. "In the next two years, we have to define the production and the product exactly, because anything that deviates in the clinical phase goes down the drain together with a lot of money," stresses Neubert.

In two years, researchers hope to start an initial test phase that is scheduled to last roughly 24 months and involve as many as one hundred test persons to determine if the vaccine causes harm. If it proves inoccuous, the first use study will follow, in which researchers can adjust only the dosages and the vaccine intervals. If the next large efficacy study involving thousands of test subjects is also concluded sucessfully, meaning that the vaccinated individuals compared to a non-vaccinated control group remain healthy and immune during the winter RS virus season, a vaccine licensing process can be intiated. "If that all goes smoothly, the vaccine will be available in seven and a half years," says Wiegand.

Then the product merely needs a marketing name. It is currently referred to as the "PΔ 2 - 77 RSV" – a non marketable, intimidating formula whose figures represent the number and position of the amino acids that Neubert once cut out of the virus' RNA. Back when there was little more than a green light for the idea.

GLOSSARY

Viruses
A virus is essentially a nucleic acid (RNA or DNA) containing the information to control the host cell's metabolism. Since viruses are parasites and do not have their own metabolism, they can replicate only within the host cell.

Airway cells
An airway cell infected by a virus can generate up to thousand viruses within a few hours. The upper airway cell perishes during the replication and leaves the bronchial tree bare, unprotected and susceptible to superinfection.

Illustration: Christoph Schneider, based on material from AmVac