

Liaison in a Test Tube

In the mid-1970s, Georges Köhler, later Director at the **Max Planck Institute of Immunobiology** in Freiburg, succeeded in fusing together a short-lived immune cell and a rapidly dividing cancer cell. The result was an immortal cell chimera with the ability to produce identical (“monoclonal”) antibodies, ushering in a revolution in biology and medical science. In 1984, Köhler was awarded the Nobel Prize along with César Milstein and Niels Kaj Jerne. The researcher, who died young, would have celebrated his 70th birthday this year.

TEXT **ELKE MAIER**

Pregnant or not? The frog can tell: if urine from a woman is injected into a female African clawed frog and the hormones in the urine cause the frog to lay eggs within a day, the answer is yes. Of course, it's easier and faster to test for pregnancy using a test strip: the result appears in the form of a colored line within minutes. The basis of the test is monoclonal antibodies that bind to the pregnancy hormone human chorionic gonadotropin, triggering the color reaction.

Since it became possible to produce them in the laboratory, monoclonal antibodies have revolutionized far more than just pregnancy testing. As universal molecular biology tools, they are now an indispensable part of biological and medical science: They help identify individual molecules in mixtures, detect cancer cells in the body, and diagnose diseases. They also play a role in organ transplantations and cancer therapy.

The method of creating monoclonal antibodies wasn't the result of biomedical research with a view to achieving marketable results. Its discoverers were passionate basic researchers whose aim was to improve our understanding of how the immune system works. The younger of the two, Georges Köhler, was just 28 years old at the time of the breakthrough.

Georges Jean Franz Köhler was born in Munich on April 17, 1946. After studying biology in Freiburg, he took up doctoral studies at the Institute of Immunology in Basel, a think tank funded by the pharmaceutical company Hoffmann-La-Roche. His supervisor, Fritz Melchers, didn't realize at the time that he was dealing with a future Nobel Prize laureate and wondered about his doctoral student's lax work ethic: “Georges liked to be out in nature. He never worked on weekends. But he often had a lot of crazy ideas,” says Melchers today.

What interested Köhler most was the kaleidoscopic variety of antibodies. These Y-shaped molecules form our immune system's first line of defense. They have the task of binding to invaders, such as viruses, bacteria, toxins or even cancer cells, and eliminating them from the body. Every antibody has its very own “foe”: recesses at the ends of its two arms fit precisely into a specific foreign structure, known as an antigen, like a key in a lock. When an antibody encounters its counterpart – whether a soluble protein, the surface of a bacterium or a cancer cell – it binds firmly to it.

Antibodies are produced by B lymphocytes, white blood cells that are able to recognize foreign structures and proliferate. In order for the body to be armed against a wide range of antigens, an armada of several million different antibodies patrols the bloodstream. Each B lymphocyte produces just one very explicit type of antibody.

How this immense number of different antibodies comes about has long been a mystery: Are the various plans encoded in the DNA, or are they produced in B lymphocytes as a result of random mutations? Researchers had been stumped by this question. To answer it, they needed immune cells that could be grown in the laboratory and that also produced antibodies with known specificity against a particular target molecule. The problem was that normal lymphocytes quickly perish outside the body.

Despite this obstacle, Georges Köhler was determined to tackle the antibody problem. He hoped to obtain the tools for doing so from immunologist César Milstein, who was conducting

Casting a critical eye: Georges Köhler examining his latest experiments in the laboratory. As a basic researcher, his aim was to unravel the secrets of the immune system. Conveniently, he discovered monoclonal antibodies as a byproduct of his research efforts.



research at the MRC Laboratory of Molecular Biology in Cambridge. Milstein was regarded as a brilliant experimenter and specialist in the field of cell fusion. In the spring of 1974, Georges Köhler set off for England together with his wife Claudia and their three children.

Milstein's laboratory had succeeded in cultivating myeloma cells obtained from mice. These degenerate immune cells behaved exactly like cancer cells in that they were able to propagate indefinitely. At the same time, they produced antibodies, albeit of unknown specificity. B lymphocytes, however, produced a specific type of antibody, but died in cultures. A cell was sought that could do both: proliferate in the laboratory and also produce specific antibodies.

The solution occurred to him in bed one night, Georges Köhler later recounted: What if a lymphocyte could be fused with a myeloma cell? Would it then be possible to transfer the frenzied reproductive behavior of the tumor cell to the immune cell? Köhler imagined cobbling together an immortal, antibody-producing chimeric cell. "I was suddenly wide awake and couldn't get back to sleep. I thought about it all night long. The

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Monoclonal antibodies did not originate from targeted medical research but from the dreams of a young biologist. Georges Köhler [...] was searching for a simple experimental method for his immunological research.

next morning at breakfast, I told Claudia about my nocturnal thoughts," he reported. At the institute, he immediately sought out Milstein in the basement among the cell cultures to discuss his idea with him.

In the fall of 1974, Georges Köhler set to work. As a test antigen he used red blood cells from sheep, which he injected into mice. Once the immune response had run its course in the mice, he removed their spleens. He homogenized the spleen tissue to access the B lymphocytes that are particularly prevalent there, and then mixed these with myeloma cells. To help the liaison along, Köhler reached into the immunologist bag of tricks and added a special virus to act as a molecular matchmaker. Now it was a matter of waiting to see if the two cell types would accept the forced marriage and produce the desired type of antibody.

For seven weeks, Köhler kept the cells in a nutrient solution, where they multiplied without restraint. Only then did he dare to conduct the ultimate test: would the hybridomas – as the hybrid cells were called – produce antibodies against the test antigen? To find out, he transferred the cells to Petri dishes with medium containing red blood cells from sheep. If the desired antibodies were present, they would bind to the blood cells and break them down. Bright halos, called plaques, would then appear around the cell colonies.

Köhler started his experiment at around 5 p.m. It would take four or five hours to obtain a result – enough time to enjoy a re-



A toast: Georges Köhler (middle) and his colleagues at the Institute drink to the Nobel Prize.

laxing dinner at home. Then he returned to the institute. He took his wife along for moral support in case the experiment failed. Together, the two entered the windowless lab in the institute's basement. Köhler picked up the first two Petri dishes. The plaques stood out clearly against the dark background.

"I cheered, I kissed my wife, I was ecstatic. It was the best result I could imagine," he later recalled. Georges Köhler had created cellular factories that produce identical antibodies as if on an assembly line. Because they are all derived from the same cell line, they are called monoclonal.

On August 7, 1975, Köhler and Milstein published their method in the prestigious journal *NATURE*. "Such cultures could be valuable for medical and industrial use," they wrote at the end of their article. That, as it turned out, was a monumental understatement.

A short time later, the hybridoma technique triumphantly entered laboratories. It soon became very clear that it was much more than a useful research tool for immunologists. It enabled scientists to fashion tailor-made antibodies against any antigen – in virtually unlimited quantities.

Monoclonal antibodies can be added, for example, to a complex mixture to target individual molecules. They can be tagged with bright dyes and then released to track down bacteria, viruses or cancer cells. They can be used as transport vehicles for delivering drugs directly to specific tumors. They help prevent post-transplantation tissue rejection. The possibilities are endless.

For the pharmaceutical industry, monoclonal antibodies have become a billion-dollar business. Before their publication appeared, Milstein had offered the method to the British government for patenting. He received no reply. Evidently, the government officials somehow missed the landmark moment of molecular biology.

After his discovery of the century, Georges Köhler was inundated with offers. He rejected them all. Instead of succumbing to the temptation to become "the highest-paid custom tailor of monoclonal antibodies," he remained loyal to basic research. In 1984, he became Director at the Max Planck Institute of Immunobiology in Freiburg. A short time later, a call came from Stockholm: Georges Köhler and César Milstein had been awarded the Nobel Prize in Medicine, together with Danish immunologist Niels Kaj Jerne. In the following years, Köhler worked to unravel the mysteries of the immune system. On March 1, 1995, he died of heart failure. He was only 48 years old.