Hybridomas: The Making of a Revolution

Scientific prize committees sometimes skimp on their homework. The awards for the hybridoma technique may be a case in point.

The discovery of the hybridoma technique in 1975 was among the most important inventions of the last decade. It promises to afford a powerful weapon against a number of diseases including cancer, and is a potent new tool for the research immunologist. Committees with scientific prizes to bestow have showered their awards on Cesar Milstein, an inventor of the technique. But the original article describing how to make hybridomas had two authors, Milstein and Georges Köhler. Following is an account of how the technique came to be invented.

Some discoveries are made by design, some by lucky accidents, deserved or otherwise, and some en route to a different goal. All three elements can be discerned in the discovery of the hybridoma technique, a means of creating pure and uniform antibody molecules against a chosen target.

When the immune system of the body detects a foreign substance or antigen, it produces cells which make many different kinds of antibody molecule against the invader. The diversity of the immune system in operation makes it hard to study and hard to manipulate. The hybridoma technique is a way of obtaining a line or clone of identical cells which manufacture a single or "monoclonal" antibody. Besides their use in research, monoclonal antibodies have already proved to be excellent diagnostic tools, and the intent is to use them in therapy as well. According to one estimate, the worldwide market for monoclonal antibodies could be half a billion dollars by 1987.

First described in an article in *Nature* of 7 August 1975, the hybridoma technique embodies some two decades worth of knowledge about how to raise antibodies, how to keep cells in culture, and how to make two cells fuse together into one. The catalytic event which brought these lines of inquiry together was the decision by Georges Köhler, then a new Ph.D., aged 28, to do his postgraduate research in the laboratory of Cesar Milstein.

Köhler's aim was to study mutations in the genes that specify antibodies, which are protein molecules known as immunoglobulins. He was interested in the enormous variability of immunoglobulins because for his Ph.D. thesis at the Institute of Immunology in Basel, Switzerland, he had found that the immune system of the mouse can produce a thousand different kinds of antibody molecule against a single antigenic site on an antigen.

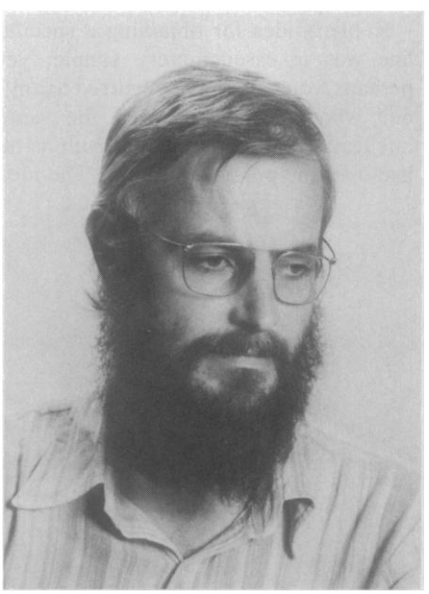
He chose to pursue his mutation project with Milstein at the Laboratory of Molecular Biology in Cambridge, England. Milstein had long been interested in immunoglobulin genetics, and with R. G. H. Cotton had recently made a significant advance in the field. By fusing together rat and mouse cells, they had shown that the constant and variable regions of an immunoglobulin molecule must be spliced together by some mechanism that prevents crosses between species, since no rat-mouse hybrid immunoglobulin molecules were produced.

Köhler's study of mutation in immunoglobulin genes ideally required a clone of cells which could be grown in tissue culture, and which would produce a single kind of antibody, targeted against a known substance. Nothing of the kind existed. The nearest thing were myelomas, tumors of the immune system that are locked into producing a single antibody. A series of myelomas had been induced in mice by Michael Potter of the National Cancer Institute, but the myeloma cells were very hard to establish in culture. Another drawback was that there was no general way of knowing what antigens the myeloma immunoglobulins were directed against. But one of Potter's myelomas, the line known as MOPC 315, had specificity for a known antigen, and Köhler decided he would first try to establish it in culture.

Köhler realized after only a few months in Milstein's lab that he could not get the cells to grow. Looking around for another project, he proposed to Milstein the idea of fusing two different mouse myelomas to see what pattern of immunoglobulins would be produced in the hybrid cell. The experiment was similar to the hybrids made by Cotton and Milstein between rat myeloma and mouse myeloma cells. But Cotton had gone back to Australia and Köhler had first to master the cell fusion technique.

The myelomas Köhler chose to work with included another of Potter's lines, known as P3. P3 was one of the few myelomas that had been successfully established in cell culture. Milstein had worked with the line intensively and developed a series of mutant versions of it. Köhler developed a variant of P3 resistant to azaguanine, a necessary property for the cell fusion technique. He fused P3 with another myeloma line, P1, and succeeded in obtaining several hybrid cells which produced the immunoglobulins of both parents.

The experiment, completed after Köhler had been in Cambridge some 6 months, was his first positive result. It gave him confidence with the cell fusion technique, and in particular the knowledge that he could develop lots of hybrid cells from myelomas, even though they were supposed to be poor fusers.



Georges Köhler

At this point, in the fall of 1974, Milstein suggested that, for his next project, Köhler should try to discover what antigen the antibodies produced by P3 were designed to attack. Defining the specificity of P3 cells would have made them an extremely useful test-bed for studying immunoglobulin genetics. But the antigen could be almost anything.

"Cesar wanted me to make a screen to

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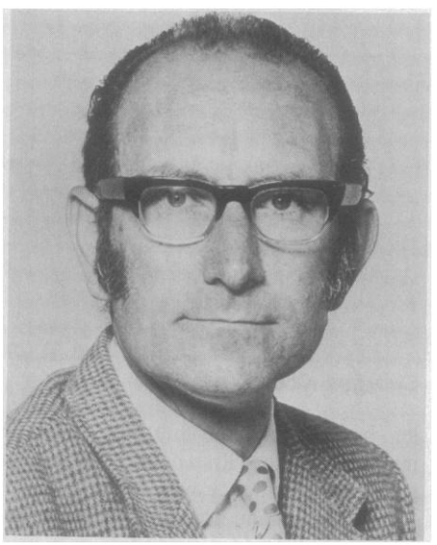
find what the P3 antibody would bind to. I refused to do this seriously, several times. Cesar was fair enough not to insist too strongly," Köhler remarks. Köhler had another project in mind. He was still contemplating other approaches to this original project, in particular that of how to develop a cell that would produce antibody to an antigen of choice.

"I was still thinking of trying to get a line which was specific for a given antigen," says Köhler. "I had the idea in bed, before going to sleep, and then I couldn't sleep at all. I told it to Claudia, my wife, the next morning. Then I went to the lab, and I talked to Cesar, down in the basement where the tissue culture was.

"One of the things about Cesar is that he listens. If you come to him with a crazy idea, instead of dismissing it he will try to find out the good things about it. When I presented this to him, I was very uncertain, and I am grateful that he didn't turn it down immediately. He discussed it seriously, and tried to find out if it was possible at all."

Köhler's idea for obtaining a specific line was in essence very simple, yet perhaps would not have occurred to anyone who had not followed the same intellectual and experimental path as he had over the previous months. The idea



Cesar Milstein

was to fuse a normal lymphocyte with a myeloma, creating a hybrid cell that combined two separate properties of its two parents. The hybrid cell would produce the same single antibody as its lymphocyte parent, and would grow permanently like its cancerous myeloma cell parent.

Lymphocytes are found in the spleen, where they proliferate after an infection.

Nicholas Wade is leaving *Science* where he has been a member of the News and Comment staff since 1971. He is joining the editorial board of the *New York Times*.

Each lymphocyte produces an immunoglobulin that attacks a particular site on the invading antigen. Köhler's idea was to expose a mouse to antigen, then remove its spleen, mix the lymphocytes with myeloma cells, and form as many hybrid cells as possible. With luck, a hybrid might be formed that produced antibody to the injected antigen.

Discussing the idea, Milstein and Köhler could see two reasons why it wouldn't work. One was that lymphocytes were known to be bad fusers. Another was that the likelihood of producing a hybrid with the desired antibody looked extremely thin. The two immunologists calculated that Köhler would have to make and screen about a thousand hybrids to have even a chance of success. Such a task would take at least the rest of his allotted time in Milstein's lab. Köhler said he would try it.

For his first experiment he injected a mouse with sheep red blood cells, a highly effective antigen. For the myeloma parent he chose the P3 line he had made azaguanine-resistant. A few days after cell fusion, Köhler could tell just by looking at the bottles that hybrids had been successfully formed. Milstein was away at this time. The critical test, that of seeing if any of the hybrids produced antibody specific for sheep red blood cells, was of course likely on this first occasion to be negative. "I looked at the hybrids growing in the bottles and felt happy with myself for growing them. I was reluctant to test them for their specificity because I thought I probably didn't have specificity yet. So I waited for 7 weeks before testing," says Köhler.

By that time it was around Christmas of 1974. Köhler decided to do a plaque assay, a test in which any hybrids making antibodies specific for sheep red blood cells would form halos in the surrounding medium. Since the assay takes several hours, he started it at 5 p.m. and went home before returning later in the evening. "I asked Claudia to come with me," Köhler remembers, "because it would be so boring to score a negative result. She was trying to calm me down. But she came with me. We went down into the basement of the institute, which has no windows. I looked at the first two plates. I saw these halos. That was fantastic. I shouted, I kissed my wife, I was all happy. The other tests were positive as well. It was the best result I could think of."

Köhler had produced hybridomas, as the hybrid myeloma-lymphocyte cells came to be known. Each hybridoma produces a clone of identical daughter cells, all manufacturing the same immunoglobulin molecule. Monoclonal antibodies are the immunologist's dream because they create simplicity out of complexity. In place of the riot of different antibodies raised naturally to a given antigen, the hybridoma technique makes available a constant pure source of a single antibody. Of such techniques are revolutions made.

"I have many ideas, but often they don't work. In discoveries, the most important thing is to do the experiment," reflects Köhler. The hybridoma technique seemed to have only a marginal chance of working, but the important point was to try it. Further experiments showed that the initial success was not an accident. For reasons that are not completely understood, antibody-producing cells have a much better than average chance of forming hybrids. The next step was to repeat the experiment, and then to make sure that sheep red blood cells were not the only antigen for which the technique worked.

Köhler and Milstein did not hurry to publish their results. The implications of what they had found became clear only step by step, as they realized the scope and generality of the technique. "We had to digest it ourselves," Köhler reflects. The possible practical significance of the technique did not escape their notice. Before publishing their results, Köhler recalls, Milstein got in touch with a British government official to suggest that the technique should be patented. "Cesar said there was no response, so we published," remarks Köhler. As a result, the British government lost whatever chance it had to gain patent protection for the technique, an opportunity that is now being taken up by others.

Köhler and Milstein described their invention in a jointly signed paper submitted to *Nature* on 14 May 1975. The paper was written by Milstein. It describes how to produce hybridomas whose antibodies are specific for sheep red blood cells. Its conclusion: "Such cells can be grown in vitro in massive cultures to provide specific antibody. Such cultures could be valuable for medical and industrial use."

Köhler returned to Basel, to the Insti-

tute of Immunology whose first Ph.D. student he had been. He made an important refinement to the hybridoma technique, that of developing a myeloma cell line that produces no immunoglobulins of its own (previously, hybridomas would manufacture the unwanted immunoglobulins of their myeloma parent as well as the desired product of their lymphocyte parent). He now uses hybridomas for his original project, the study of mutant immunoglobulin-producing cells. "Many people don't understand why I'm not making monoclonal antibodies against all kinds of antigen, but that is something you can find yourself doing all the time," Köhler comments. "I successfully refused in the institute to become a monoclonal antibody maker. If you talk about a career, maybe it was not the right decision."

Köhler could be described as the driving force behind the discovery of hybridomas, but he believes that he could not have made the discovery without Milstein. "I would not have thought about this problem in any other laboratory than Cesar Milstein's and I wouldn't have been encouraged to do the experiment by anyone else but him," says Köhler. It was Milstein who provided the environment for the discovery and who from his own deep knowledge of immunoglobulin genetics and myeloma cells was able to give support and guidance to what Köhler was doing.

Milstein declines to discuss the intellectual history of the discovery from his own perspective or to comment on a draft of this article, but he has given his views in an article entitled "Monoclonal antibodies," in the October 1980 issue of *Scientific American*. The result of the experiment performed with Cotton, Milstein writes, "suggested to Köhler and me a possible answer to our need for an antibody-producing cell in the mutation experiment. It occurred to us that it might be possible to fuse a normal lymphocyte or plasma cell with a myeloma cell and thus to immortalise the expression of the plasma cell's specific-antibody secretion." Milstein's account is somewhat unclear, however, on the details of who actually developed the idea of hybridomas: "I cannot think that if my research aim five or six years ago had been the production of monoclonal antibodies, I would ever have stumbled on the idea of attempting simultaneously to derive mutant antibody-secreting cells in one corner of the laboratory and to fuse two myeloma cells in another corner. Yet that was the combination that led to the initial production of monoclonal antibodies against sheep red blood cells." The combination that led whom?—Milstein does not say.

Now that the importance of hybridomas has become so obvious, prize committees around the world have rushed to associate their awards with the discovery. The preponderance of the awards have gone to Milstein alone, not to Milstein and Köhler. Köhler does not object, since he assumes that the committees had in mind Milstein's distinguished career as an immunologist, rather than the invention of the hybridoma technique specifically.

This is true in some cases, such as the $100,000 Wolf prize for medicine, given to Milstein and two other immunologists in 1980 for general contributions to immunology. But other prize-giving committees have cited the hybridoma technique specifically, attributing its invention to Milstein. The press handout for the $100,000 General Motors Sloan prize, which Milstein shared in 1981 with a virologist, talks of "hybridoma technology, which Dr. Cesar Milstein developed, and for which he is receiving the Sloan prize."

There might be reasons for crediting the hybridoma technique to Milstein alone, but since the original article describing the technique was signed jointly by Köhler and Milstein, award committees that publicly differentiate between the two authors should perhaps bear the burden of proof for doing so. But Joseph G. Fortner, president of the General Motors Cancer Research Foundation which awards the Sloan prize, declines to explain the basis for the allocation of credit.

The $22,000 Horwitz prize of Columbia University was given in 1980 to Milstein alone, with an emphasis on the hybridoma technique. The press release, noting the Horwitz prize committee's frequent anticipation of the Nobel Prize selectors, talks of "Milstein's hybridomas" and describes the discovery as one "which Dr. Milstein made with an associate, George (sic) Köhler." Asked the reason for the separation of the two authors, a member of the prize committee said the decision was made by inference from the scientific literature, but he noted the phenomenon whereby students coming to a distinguished laboratory often make discoveries that should properly be attributed to the lab chief. "Milstein had previously done work on rat-mouse hybridomas, from which this was a logical consequence. Köhler just happened to be in the lab when this came up," says this member. However, the rat-mouse hybrid cells studied by Milstein and Cotton were not hybridomas: they were hybrids between two myelomas, whereas a hybridoma is the fusion of a myeloma with a lymphocyte.

The $15,000 Gairdner Foundation award was given jointly to both Milstein and Köhler in 1981. The Foundation itself made no decision about the allocation of credit, since it selected a nomination whose author cited hybridomas as a joint discovery.

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It is customary to say that recipients are "honored" by a prize, but with discoveries of the magnitude of hybridomas the flow of prestige is more nearly the other way around: it is the prize-giving institutions that absorb the credit reflected from the discovery. Perhaps that is why some prize-givers seem happy to associate themselves with important new discoveries without apparently probing very deeply into the historical and intellectual background.

Maybe those with prizes to award would manage to make a more significant contribution to science if they sought primarily to honor a discovery, not the discoverers per se. If the prize committees were to publish a scholarly account of how the discovery came to be made, those cited in the account would receive due credit, and the public would better understand how often an important discovery stands at the apex of a rich and diverse set of findings, contributed by many different researchers over a long period of time.

It is commonly assumed among immunologists that the invention of the hybridoma technique will eventually be the subject of a Nobel Prize. But no number of prizes can add to the distinction of so notable a discovery.—NICHOLAS WADE