César Milstein: October 8, 1927–March 24, 2002

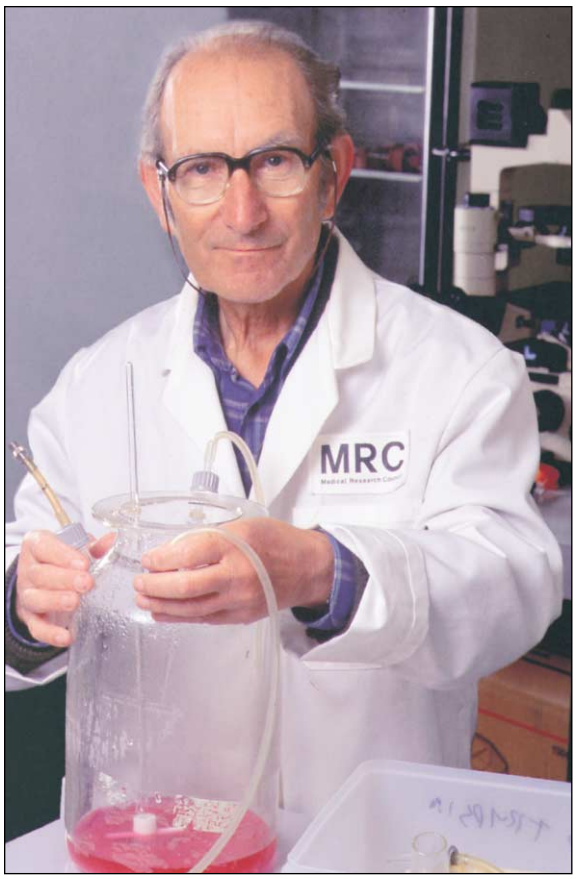
César Milstein died in the early hours of 24th March 2002. He had a long history of cardiovascular illness, to which he finally succumbed. We will always remember his enthusiasm for science and his kind attention to the problems of others. His most famous contribution to biology was the invention of monoclonal antibodies (also called hybridomas, but that was a term he never really liked) for which he was awarded the Nobel Prize for Physiology or Medicine in 1984, along with George Kohler, who worked with César on the key experiments that led to their landmark paper, published in *Nature* in 1975.

César was born in Argentina in 1927 and developed an early interest in immunology through family connections; this was an enthusiasm that was to last throughout his sparkling career. After being a none-too-keen schoolboy, he went to Buenos Aires University to study chemistry. He obtained a BSc and then a Ph.D. studying aldehyde dehydrogenase in the laboratory of Andres Stoppani. He later traveled to Cambridge, England, and worked in the University Department of Biochemistry with Malcolm Dixon, where he obtained a second Ph.D. with studies on the phosphoglucomutase enzyme. It was in the Department of Biochemistry that César met Fred Sanger, who was to be influential in attracting him back to Cambridge after a brief sojourn in his old Buenos Aires Institute as Head of Molecular Biology. César's move to the then-fledgling Laboratory of Molecular Biology with Fred, and a truly extraordinary group of scientists (Brenner, Crick, Huxley, Kendrew, Klug, Perutz), was a wonderful opportunity for him as the MRC support allowed his research to blossom by setting few constraints on what he could do. How well-suited this environment must have been for the young Milstein! The intellectual environment was second to none, and it was there that he developed an interest in antibody structure and diversity, unfettered by the needs of the next grant proposal or the next peer review committee.

An interest in antibody diversity was to stretch from those early times until César's death (he had submitted a paper to PNAS just the week before he died). When he joined the Laboratory of Molecular Biology, he started thinking about potential roles of germline immunoglobulin gene diversity or of somatic diversification of those genes, and he came to the conclusion that the latter mechanism must lie at the heart of antibody diversity. This one fundamental belief was the precursor for the work that was eventually to lead to his Nobel Prize-winning work with Kohler. In his attempts to solve the issue of somatic diversification, Milstein collaborated with George Brownlee on pioneering sequencing work of immunoglobulin light-chain mRNA. This involved labeling mouse myeloma cells with >50 mCi ^{32}P, followed by preparation of purified mRNA for fingerprinting. The days of the "hot preps" were usually Thursdays and would involve César and John Jarvis, his research associate, garbed in full-length lead aprons, spinning 5 liters or more of radioactive myeloma cells to concentrate them into a few milliliters. This would invariably result in a trail of radioactivity across the corridor from the cold room to the hot room where these procedures were carried out. These studies, plus immunoglobulin protein synthesis work, showed that the precursor of the light chain was made with a hydrophobic leader sequence for specifying transfer to the endoplasmic reticulum.

César always tackled scientific problems from many angles, and he was not content with straight molecular biology. Rather, he was also keen to see if cells could discriminate immunoglobulin heavy and light chains of differing origins, and he set about fusing rat and mouse immunoglobulin-producing cell lines. With Dick Cotton, many experiments were done to show how heavy and light chains associate. Later, George Kohler joined César's group as a post-doc. Together they hatched the idea of fusing spleen cells with mouse myeloma tissue culture cells to immortalize the spleen immunoglobulin and, of course, the stroke of brilliance here was that preimmunizing mice with antigen allowed predefined antibody specificities to be captured. This became monoclonal antibody technology. Their paper was published in 1975, and the Nobel Prize was awarded in 1984. César was awarded innumerable additional prizes for this work. He received other accolades such as the Companion of Honor in 1995, and he became the first recipient of the MRC Millennium Medal in 2000. He was a Fellow of the Royal Society of London and of the National Academy of Sciences.

Monoclonal antibody technology was revolutionary because, for the first time, pure antibody species of defined specificity could be made. This had far-reaching implications for biological research and for clinical use (diagnostics and, more recently, therapeutics). Early in the development of monoclonal antibodies, César started to work on monoclonals recognizing surface antigen markers and blood groups. Monoclonal antibod-



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ies are now the basis of many diagnostic assays for proteins in clinical laboratories and have important uses in such areas as cancer imaging. Now their value as therapeutic agents is at last beginning to emerge. For instance, we have recently heard about Herceptin, a monoclonal antibody that binds to a surface receptor and is proving important in management of breast cancers. Therapeutic use of monoclonal antibodies had been César's dream, and he had the gratification of seeing the process put into practice. Discussions about whether monoclonal antibodies should have been patented in 1975 have occupied many people. However, we should remember the immense importance of the invention, which was nurtured from essentially curiosity-driven research. As with almost all inventions, hybridoma technology seems blindingly obvious many years later, but the fact remains that monoclonal antibodies are just beginning to be widely accepted for treatment regimes (and at this time, of course, an original monoclonal antibody patent would be off-patent!).

After hybridomas, César remained true to his early scientific obsession (namely, how somatic mutation arises in immunoglobulin genes) despite his success with monoclonal antibodies and the acclaim this brought him. He mapped out patterns of changes observed during antibody responses using primer-directed cDNA sequencing. This work charted the course of somatic diversification in an immune response. Even when César formally retired in 1995, he remained an active member of the Protein and Nucleic Acid Chemistry Division in the Laboratory of Molecular Biology. In his postretirement years, he continued his interest in the origin of somatic mutation and, in collaboration with Michael Neuberger, he had begun to dissect the enzymatics of the process. Indeed, César's only concession to retirement was not to work on Saturdays any more. César was a prolific writer and published 313 papers in his lifetime, each composed with his characteristic precision.

The way that César confronted the symptoms of his cardiovascular illness was typical of him. His illness was first diagnosed some 20 years ago, and he set about finding all the data he could about it, examining it and deciding how he was going to fight it. He switched from a high-cholesterol diet to low-cholesterol salads and the like, and he immediately took to long walks around Cambridge, before and after work, to stimulate his circulation. He identified a series of local and London restaurants that were able to cope with his strict dietary regime, and of course high tables in the Cambridge colleges were not spared. Occasionally, César gave in to impulse, such as his nearly disastrous white-water rafting in Chile, during which he had a "life-threatening cardiovascular episode," as he casually put it when he returned to work. However, he was generally tenacious in his health regime; one often reads of people fighting their disease, but César truly did.

César's qualities as a researcher go without saying, but I am sure he would not mind my saying that he was not a well-organized scientist. He would not come to the lab with a list of "things to do today." Rather, his style was intellectual, grasping at problems as they arose in the laboratory simply for their sake as scientific problems. He always seemed to have masses of time to discuss and mull over data. His suggestions were not always the most down-to-earth, but therein lay his brilliance; at one time completely outlandish and at another focused and hitting the spot. Those of us who had the privilege to work at the Laboratory of Molecular Biology with César over the years will remember him as a father figure and a friend. He used to describe the Laboratory of Molecular Biology as the "beloved Laboratory," and that was his inspiration. There was another side to César, and it was the one who loved his wife Celia, whom he married in 1953, who loved his home, his garden, his boat, and manifold other special things in life like music, art, cinema, and theater. Of all the things we shall remember about César, his enthusiasm for science was special. At César's funeral, Michael Neuberger told us that César had mentioned in a recent press interview that he wished to be remembered as a nice chap. As Michael said, César's wish has been granted many times over, "he was a very nice chap."

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