Obituary

César Milstein (1927–2002)

César Milstein began to study antibody diversity at a time when almost nothing was known about its molecular and genetic basis. As a logical step in that work, with Georges Köhler he devised the hybridoma technique for fusing cancer cells with antibody-producing cells to produce homogeneous (monoclonal) antibodies. This invention revolutionized wide areas of biomedicine, and Milstein and Köhler, together with Niels Jerne, were awarded the 1984 Nobel Prize in Medicine or Physiology.

Milstein was born into a Jewish family in Bahía Blanca, Argentina, and graduated from the University of Buenos Aires with a thesis on aldehyde dehydrogenase. In 1958, he won a scholarship to work at the University of Cambridge in Britain, where he did a second PhD, again in enzymology. There he met Fred Sanger, who had just won his first Nobel prize for determining the structure of insulin. Meeting Sanger was critical for Milstein's career. He returned to Argentina in 1961, but soon became dissatisfied with the political climate and, in 1963, took up Sanger's invitation to join the Medical Research Council's Laboratory of Molecular Biology (LMB), which had already attracted other top scientists from abroad such as Max Perutz, Aaron Klug and Sydney Brenner. It was on Sanger's advice that Milstein then began to study antibody structure and diversity: this was to be his life's work, with LMB in Cambridge his scientific home.

Milstein's hope was that the structural comparison of different antibodies would reveal the secret of antibody diversity. But things turned out to be more complicated. Soon after his arrival in Cambridge, it was discovered that antibody molecules contained variable (*V*) and constant (*C*) regions and the 'two genes, one polypeptide' hypothesis was proposed. According to this idea, separate genes encode *V* and *C* regions, and there are thousands of *V* genes in the germ line — the set of genes inherited by offspring. This was shockingly unorthodox, and other ideas were put forward. Among them, in 1966, was Brenner and Milstein's proposal of a mechanism that introduces 'somatic' (non-germline) mutations selectively into *V* segments of antibody genes. This proposal proved inadequate to explain primary antibody diversity. But it later became a leading model for the diversification of antibodies in immune responses, the main subject of Milstein's research for the latter part of his life.

MRC LMB

Innovator in immunology

By the beginning of the 1970s, Milstein had become convinced that antibody structure could not explain the genetic basis of diversity. So he switched to work on the biosynthesis of antibodies and, with George Brownlee, on antibody messenger RNA. This required culturing antibody-producing myeloma cells and analysing them with molecular biological methods. It was this marriage of cellular and molecular techniques that led to the ensuing breakthroughs.

As a prelude, a biosynthetic precursor of parts of antibodies — the light chains — was discovered, providing early experimental evidence that a 'signal sequence' is required for protein transport across membranes. Most importantly, however, although it was initially only a sideline, the cell-fusion method was established in the laboratory to show that in hybrids of different myeloma cells antibody chains from both parental cells are produced in their original form. This was an early indication that, in antibody-forming cells, genes encoding the *V* and *C* regions were brought together, presumably by some translocation event. When Köhler arrived at LMB from the Basel Institute for Immunology, he brought with him an assay for identifying single antibody-producing cells that had been invented many years before by Jerne, director of the Basel Institute. With that, everything was in place for the great experiment.

"This time I can tell you something really interesting," César said with a smile, when I met him in 1975 at an obscure conference in San Remo, the kind of meeting he liked to attend. That something was the production of continuously growing, cloned cell lines secreting monoclonal antibodies of predetermined specificity, achieved by fusing myeloma cells (contributing immortality and a high rate of antibody secretion) with antibody-producing cells from immunized mice (contributing antibody specificity). I felt the earth shaking.

This creation of hybridomas that produced antibodies of predetermined specificity was a dramatic advance. Antibody specificity and diversity could now be studied at the level of individual antibodies, and highly specific antibody reagents could be developed, allowing biological systems to be analysed with unprecedented resolution. Monoclonal antibodies have also become essential tools in medical diagnostics and are increasingly used in treating cancer and other diseases. A large part of the biotechnology industry is based on the monoclonal antibody technique.

Milstein contributed to these developments in manifold ways, seeing his invention as the beginning of a new era of 'antibody-based' molecules. But his real interest remained in antibody diversity, and he used the hybridoma technique to analyse how antibodies diversify during immune responses. This work showed the essential role of somatic mutation of antibody V genes in the affinity maturation of the antibody response. Mutations are introduced into these genes by a special hypermutation mechanism, as he and Brenner had speculated earlier. Now he tried to understand this mechanism through characterization of the pattern of mutations at the DNA level. He worked on this problem until the end of his life, and much of our knowledge of somatic hypermutation is based on the work of Milstein's Cambridge 'school'. Sadly, his wish to live long enough to see the solution of this problem was not fulfilled.

Milstein was awarded innumerable prizes and distinctions, but remained modest and easily approachable. He was not interested in academic power, always worked with a small group and liked to do experiments himself. He was insatiably curious, and his love of arguing about science — as well as about anything else in life — stemmed from that, and the belief and tradition that truth should be approached by dispute. He and his wife Celia liked to be with people, and to help them, and he had a special gift for friendship: his death on 24 March marks the loss not only of an outstanding scientist but of a man held in great affection by many.

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