RETROSPECTIVE IMMUNOLOGY

César Milstein (1927–2002)

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César Milstein, the coinventor with Georges Köhler of monoclonal antibodies, died on 24 March 2002 at the age of 74. Monoclonal antibodies form one of the pillars of modern biotechnology and are indispensable tools for biomedical research. They are also used extensively in diagnostics, including everyday applications such as home pregnancy test kits. Some have been approved for use as drugs, benefiting more than a million patients with cancer, rheumatoid arthritis, and heart disease.

Milstein received his first Ph.D. in biochemistry from the Universidad de Buenos Aires, Argentina, in 1957, and his second from the University of Cambridge in 1960. Working with Fred Sanger in Cambridge, he identified amino acid sequences in enzyme active sites. After a 2-year stint back in Argentina, he joined Sanger and others under the chairmanship of Max Perutz at the recently created Medical Research Council (MRC) Laboratory of Molecular Biology.

Milstein decided to work on a hot new topic—the molecular basis of antibody diversity and specificity. His early interest in somatic hypermutation as a mechanism for generating antibody diversity was the impetus that led him to invent monoclonal antibodies. He obtained early evidence for the variable and constant sequences that surround cysteines in antibodies, and pinpointed the different disulfide bridges between heavy chains that characterize each immunoglobulin (Ig) subclass.

In the 1970s, Milstein began to directly sequence Ig mRNAs from myelomas (tumor cells that produce antibodies). With George Brownlee, he discovered the junction between variable and constant Ig regions and thus showed that their union preceded protein synthesis. The development of recombinant DNA techniques and DNA sequencing opened up new ways of investigating the generation of antibody diversity, but, in characteristic fashion, César decided to embark down his own unique path.

Together with David Secher and Richard Cotton, Milstein analyzed mutations in cultured myeloma cells seeking evidence for somatic hypermutation. They did identify mutations, but these were not located in antibody variable regions. Thwarted, Cotton and Milstein decided to use the myeloma cells to investigate a different immunological problem. They selected myelomas producing distinctive IgG proteins for their susceptibility to various drugs, and fused them together in different combinations. Somatic cell hybrids, selected on the basis of their drug resistance, secreted Ig types from both myeloma parents. The fact that the variable and constant regions of the two different antibodies were not intermixed suggested that they had been joined at the DNA rather than the RNA level.

Image not available for online use.

Köhler arrived in Milstein's lab with the idea of screening for mutations in the antigen-combining sites of myeloma IgGs using antigens. However, there were only a few myeloma cell lines secreting antibodies that bound to antigen, and these cell lines failed to grow. Frustrated, Köhler and Milstein hit upon an ingenious idea. They made their own antibody-secreting cells by fusing myeloma cells with lymphocytes from animals immunized with a specific antigen. In the resulting hybrid cells, the immune lymphocyte parent contributed the specific antibody heavy and light chains and drug resistance, and the myeloma cell parent contributed immortality and secreted large amounts of the specific antibody. Hybridoma technology was born.

Despite this resounding success, Milstein worried that hybridoma technology was not being adopted by other labs, and resolved to establish it firmly in the research repertoire. Giovanni Galfré joined the lab, optimized hybridization, and made it reliable by substituting polyethylene glycol for Sendai virus as the fusing agent. A period of remarkable productivity followed, in which Milstein and Galfré fused, grew, and cloned hybridomas by the hundreds, with collaborators providing lymphocytes from immunized animals and screening for monoclonal antibodies of the required specificity.

At the time, cell surface proteins were thought to be of enormous importance, but the lipid "swamp" in which they floated hindered their study. The true strength of the monoclonal approach was that incredibly complex or "dirty" mixtures such as whole cells could be used for immunization. The only "purification step" required to obtain monospecific antibodies was to clone the hybridomas. A huge number of cell surface proteins were soon identified. Milstein, Galfré, and Alan Williams produced the first monoclonal antibody against a T lymphocyte subset antigen, CD4. Len Herzenberg, who had just introduced fluorescence-activated cell sorting to biology, was on sabbatical in Milstein's lab; the synergy between sorting and monoclonal antibodies immediately became apparent. Milstein and Jonathan Howard generated the first all specific monoclonal antibodies against the rat major histocompatibility complex. With Andrew McMichael, he obtained the first monoclonal antibody to a human leukocyte differentiation antigen, CD1. Milstein and I made the first monoclonal antibodies to cell adhesion molecules. In collaboration with Claudio Cuello, he worked on the immunocytochemistry of the nervous system, and with Herman Waldmann on making antibodies to human hematopoietic cell antigens. One of these was "humanized" by Greg Winter and was subsequently approved as a therapeutic for treating chronic lymphoblastic leukemia.

Despite Milstein's efforts, monoclonal antibodies were never patented. Prime Minister Thatcher, a chemist herself, was reported to have criticized this bureaucratic failure under her predecessor's watch. The indirect financial benefits for Great Britain were nevertheless large, and the benefits to humanity enormous.

Milstein was active in advancing science all over the world, particularly in Latin countries. He served on the committees of many international funding agencies, and was the force behind the Leukocyte Workshops that fostered adoption of the CD nomenclature. His contributions to science and humankind were recognized with almost every prize imaginable, including the Nobel Prize in 1984, the Copley Medal of the Royal Society, the first MRC Millennium Prize, and a Companion of Honour for services to molecular biology.

César was open, approachable, and loved to discuss scientific issues. His early activity in politics while at University in Argentina fostered his ability to see a problem from all angles. He steered clear of approaches taken by others, seeking unique ways of attacking scientific questions. He achieved greatness with a laboratory of modest size. His students were the privileged few who, along with his equally fortunate MRC colleagues, cherished his scientific wisdom, generosity, and charm.

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