César Milstein (1927-2002) and monoclonal antibodies: Father of modern Immunology

https://doi.org/10.51167/ice000018

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Abstract:

"It is not an exaggeration to describe César Milstein's contribution to science and medicine as the most important immunological advance of the century. His discovery of the method to produce monoclonal antibodies reinvented the field of immunology. The ability to make monoclonal antibodies at will in the test tube and in unlimited quantities, to any sort of antigen—whether an interesting chemical, infectious microorganism, cancer or normal cells—opened numerous new and unforeseen avenues for research, many with medical implications" [A. Karpas, American Association of Immunologists]. Milstein was born and raised in Argentina. In 1964, he fled the political upheavals and rising antisemitism to the UK, where he spent almost his entire career. In 1975, Milstein together with his post-doc Georges J. F. Köhler discovered the technique to produce monoclonal antibodies. For this discovery, Milstein shared part of the 1980 Wolf Prize in Medicine and in 1984, he and Köhler shared part of the Nobel Prize in Physiology or Medicine.

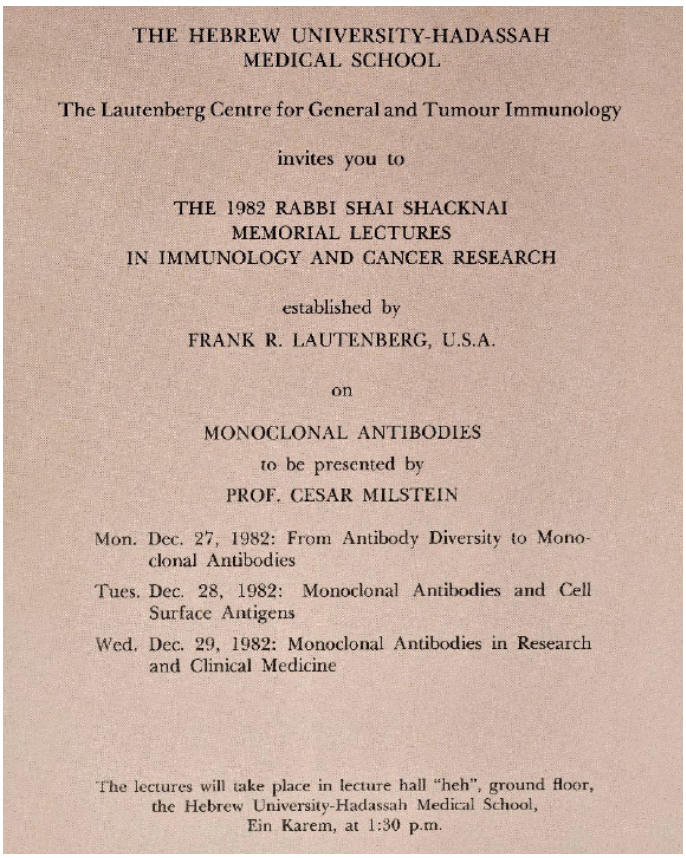
Introduction

The hybridoma technique is a method for producing large numbers of identical antibodies from a single clone of cells or cell line, also called monoclonal antibodies. This represents the most important immunological advance of the last century. Scientists for a long time hoped that it would become possible to produce monoclonal antibodies with predetermined specificities. This dream became a reality in 1975 when César Milstein and his post-doc Georges J. F. Köhler described the hybridoma technique for production of monoclonal antibodies [1]. They immortalized antibody-producing cells by fusing them with tumor cells. The method allows unlimited production of monoclonal antibodies with predetermined specificity. Monoclonal antibodies have opened up completely new fields for theoretical and applied biomedical research and allow precise diagnosis and treatment of disease. These cells are called *hybridomas* – from *hybrid* and *-oma* (meaning tumor) [2]; and since the antibodies obtained from hybridomas are produced by *clones* (meaning identical copy) derived from a *single* lymphocyte, they are called *monoclonal antibodies*. (The term *hybridoma* was coined by Leonard Herzenberg while on sabbatical in Milstein's lab [3].) For work on monoclonal antibodies, Milstein shared part of the 1980 Wolf Prize in Medicine, delivered the 1982 Rabbi Shai Shacknai Memorial Prize Lectures in Immunology and Cancer Research at the Hebrew University of Jerusalem, and Milstein and Köhler shared part of the 1984 Nobel Prize in Physiology or Medicine. Milstein is regarded as the "Father of Modern Immunology," see Figures 1–3 [4-7].

Bob Weintraub was born in Brooklyn, New York and made aliyah in 1975 to Beer Sheva, where he remained. He earned the PhD in Physical Chemistry from MIT and the Diploma in Library Science from the Hebrew University of Jerusalem. He held positions in scientific and technical librarianship in industry, hospital and academic institutions. He is now retired. He has an interest in the history of chemistry.

Figure 1. César Milstein with a spinning culture flask containing monoclonal antibodies growing in a fluid, ca. 1990s. Image courtesy of MRC Laboratory of Molecular Biology.

Figure 2. César Milstein, left, at the award ceremony of the 1980 Wolf Prize in Medicine. Milstein is seen receiving the award from President Yitzhak Navon, Chagall State Hall, The Knesset, Jerusalem. Milstein's father came with him to the ceremony. Milstein: "On that occasion, however, he could not stop himself when I gave my three-minute speech of thanks. This was when I mentioned that I was a typical example of a Jew of the Diaspora, the beneficiary of the determination of the parents that were willing to make all sorts of sacrifices to see their children in higher education. He jumped from his seat, came to the rostrum (the ceremony took place at the Knesset and I received the award from the hands of the President of Israel) and to my great embarrassment gave me a kiss! The audience, however, loved it." [15] Photograph courtesy of the Wolf Foundation.



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MONOCLONAL ANTIBODIES

to be presented by

PROF. CESAR MILSTEIN

Mon. Dec. 27, 1982: From Antibody Diversity to Monoclonal Antibodies

Tues. Dec. 28, 1982: Monoclonal Antibodies and Cell Surface Antigens

Wed. Dec. 29, 1982: Monoclonal Antibodies in Research and Clinical Medicine

The lectures will take place in lecture hall "heh", ground floor, the Hebrew University-Hadassah Medical School, Ein Karem, at 1:30 p.m.

Figure 3. Invitation from commemorative booklet for the award to César Milstein of the *1982 Rabbi Shai Shacknai Memorial Prize Lectures in Immunology and Cancer Research* delivered at the Hebrew University–Hadassah Medical School, Ein Karem. Three lectures were delivered: From Antibody Diversity to Monoclonal Antibodies; Monoclonal Antibodies and Cell Surface Antigens; and Monoclonal Antibodies in Research and Clinical Medicine. Image courtesy of Cambridge University, Churchill Archives Centre.

César Milstein

César Milstein (1927–2002) was born in Bahía Blanca, Argentina, where he grew up. It was a Jewish family, his father from Ukraine and his maternal grandparents from Lithuania. Argentina at the turn of the last century was a frequent destination for Jewish families escaping antisemitic persecution in Russia. Baron Hirsch founded the Jewish Colonization Association, which sponsored Jewish immigration to Argentina. Milstein's parents spoke Yiddish at home and for several years he attended a Yiddish-speaking school. Milstein started his studies at the National College in Bahía Blanca and in 1952 earned his BS in chemistry from the University of Buenos Aires [8-10].

During his student years, Milstein was active politically and sided with left-wing student movements. He spoke out against the right-wing Juan Perón government. Milstein was popular on campus and in 1951 became president of the student union, which was risky as student leaders were being arrested. At Buenos Aries, Prof. Andrés Stoppani agreed to be Milstein's research advisor for PhD studies in biochemistry. Milstein began his research and was shocked to see, as a result of Peronist policies, how underfunded the research lab was. Stoppani feared that Milstein's political views and history of campaigning against the Peronist education policy would lead him into trouble; further, support was not available to university departments with doubtful loyalties. He advised Milstein to take time off from his studies until the political environment changed. As Milstein had recently married, he and his wife left on honeymoon. They were away for a year, hitchhiking through Europe and Israel, including several months as volunteers on kibbutzim. By 1954, the situation had calmed down, and Milstein started working with Stoppani [11].

Milstein earned his PhD in 1957 from the medical school at Buenos Aires with a thesis on disulphide bonds and thiol groups in dehydrogenases. He then earned a second PhD in 1960 at the Department of Biochemistry at Cambridge University on the mechanism of metal activation of enzyme kinetics and heavy metal activation of phosphoglucomutase. At Cambridge he met the prominent biochemist Fred Sanger, and they formed a lifetime association. A short time after completing his PhD, Milstein returned to Argentina as Head of the Division of Molecular Biology, National Institute of Microbiology, Malbrán Institute, Buenos Aires, where he continued research on projects that he had worked on at Cambridge, and further developed techniques for the study of sequencing and marking the active centers in phosphoglucomutase, phosphoglyceromutase and alkaline E. Coli. It was a time of reform after the Peronist government fell. Soon afterwards, there was the military coup of 1962, which put in another right-wing government. With the coup, persecution began to mount against political dissenters and Jews. Milstein remained at the institute for two years until government political interference in his laboratory became unbearable and he resigned [12].

Milstein recalled the visit of the Minister of Public Health to the institute, "It was during [José María] Guido's administration. …He came to interview us, the rebels, who were writing letters against him, because he had fired the director, and he said to us, 'But you are good kids, brilliant scientists. You don't have a future in this country, why don't you leave? Intellectuals should leave. It's best if they leave as they are all communists and Jews.' " People under Milstein's direction were fired for "ridiculous" reasons. Milstein said, "Either you reinstate them, or I quit." Milstein then returned to Cambridge where he rejoined Sanger at the newly established Medical Research Council Laboratory of Molecular Biology. He remained there for the rest of his career [13].

Myeloma fusions and monoclonal antibodies

The experiment

Neuberger and Askonas: "Research into fusion between different myeloma cell lines was originally initiated out of pure intellectual curiosity. But it was this curiosity-driven research that led to the technology for the derivation of monoclonal antibodies of predefined specificity, a technology with a huge impact on research, therapy, diagnostics and industry." [14]

Milstein, in discussing his early work on monoclonal antibodies, "In 1970, we started experiments using myeloma cells in culture. These are B-cell tumors which secrete tumors with myeloma proteins. These proteins are structurally the same as antibodies, but, since they are made by tumors, they are directed against unknown antigens. The idea was to see if such cells mutated their myeloma proteins as we predicted for antibodies." This experiment required analysis of 7000 individual clones. The results did not explain the expected diversity of antibodies.

"In parallel we also started using myeloma cells in culture to understand why only one of the two chromosomes produced antibody. This is known as allelic exclusion. Only one of the two alleles makes the antibody. The question we set out to investigate was whether fusion of two myeloma cells will produce hybrid cells co-expressing both antibodies or not. … The results showed that hybrid cells were capable of expressing both myeloma proteins and indeed light and heavy chains of both parental cells." [15] The antibody molecule is made up

of four polypeptide chains, two identical light chains and two identical heavy chains, and can be thought of as forming a flexible Y-shaped structure. Antibodies produced by myeloma cells are all identical.

At this time Georges Köhler joined the group as a post-doc. His project was to grow a myeloma cell in culture capable of recognizing an antigen, in order to derive mutants with altered affinity for the antigen. The project was not successful.

Milstein: "[Köhler] started a side project that involved variations on the theme of hybridization of two myeloma cells. The combination of the need of antibody-producing cells in tissue culture, which could not grow, and the experiments with hybrid myelomas, did the trick. Why not try to make the cell we needed? Perhaps we could substitute one myeloma cell for an antibody producing normal B-cell? Normal antibody-producing B cells die very quickly, but perhaps we could immortalize the antibody production by fusion with the myeloma cell line? To our surprise, the experiments were a resounding success from start to finish. Within a short time, we derived cell lines in culture making antibodies against preselected antigen." [15-16]

Preparing cell lines against a pre-selected antigen

The steps in the production of cell lines against a preselected antigen are summarized on the Nobel Prize website for 1984: "Spleen cells are prepared from animals, usually mice, which have been immunized with a selected antigen. These cells are then fused with myeloma cells maintained in culture in the laboratory. The product of this fusion is referred to as a hybridoma. Surprisingly, a hybrid of two cells can survive and also continue to divide. In this particular hybrid the myeloma cells contribute the capacity for survival, whereas the spleen cells direct the synthesis of antibodies with the preselected specificity. By special arrangements it is possible to achieve a multiplication of hybridoma cells but not of isolated myeloma cells. The hybrids obtained are propagated in a highly diluted state so that colonies deriving from single hybrid cells can be isolated. By use of a sensitive method the clones which produce the specific antibodies are identified. A particular hybridoma can then be used for future, unlimited production of a highly specific antibody." [6]

Köhler and Milstein: "We used sheep red blood cells (SRBC) as immunogen (An *immunogen* is a specific type of antigen that is able to elicit an immune response.), which enabled us, after culturing the fused lines, to determine the presence of specific antibody producing cells by a plaque technique. The hybrid cells were cloned in soft agar and clones producing antibody were easily detected by an overlay of SRBC and complement. Individual clones were isolated and shown to retain their phenotype as almost all the clones of the derived purified line are capable of lysing SRBC. The clones were visible to the naked eye." [1]

Successful results

Köhler and Milstein: "Three different experiments were successful in producing a large number of antibody-producing cells. Three weeks after the initial fusion, 33/1,086 clones (3%) were positive by direct plaque assay. The cloning efficiency in the experiment was 50%. In another experiment, however, the proportion of positive clones was considerably lower (about 0.2%). In a third experiment the hybrid population was studied by limiting dilution analysis. From 157 independent hybrids, as many as 15 had anti-SRBC activity. The proportion of positive over negative clones is remarkably high. It is possible that spleen cells which have been triggered during immunization are particularly successful in giving rise to viable hybrids. It remains to be seen whether similar results can be obtained using other antigens. (The technology was soon extended to every conceivable immunogen [7] .)

"The cells used in this study are all of BALB/c (laboratory-bred strain of the house mouse) origin and the hybrid clones can be injected into BALB/c mice to produce solid tumors and serum having anti-SRBC activity. It is possible to hybridise antibody-producing cells from different origins. Such cells can be grown in vitro in massive cultures to provide specific antibody. Such cultures could be valuable for medical and industrial use." [1]

The hybridoma revolution: an offshoot of basic research

Milstein, in the introduction to the 1999 paper called "The hybridoma revolution: an offshoot of basic research," wrote, "The production of monoclonal antibodies against predefined and, even more importantly, novel antigens has had an enormous impact in biology, medicine, and industry. Indeed, the hybridoma technique has been one of the pillars of the biotechnology revolution. Yet, none of the current applications were the goal of the research that made it possible. With hindsight, it may seem obvious that the invention of a method to immortalize cells that produce specific antibodies should have such potential. At the time, however, these most important applications were neither in our minds nor in the minds of biologists or even immunologists. When we stated in the original study that 'Such (monoclonal antibody) cultures could be valuable for medical and industrial use' we were thinking about immunoassays and passive therapy. It was only later that we started to consider seriously other possibilities. The technology was based on methods of somatic cell genetics, which we were using to analyse the origin of

antibody diversity, and arose from a practical need, namely to have an antibody secreting cell line that was suitable for studies of somatic mutation of antibody genes." [3]

Argentina declared 2021 as year of tribute to César Milstein

The President of Argentina declared 2021 as the year of tribute to Milstein. This is in context of the World Health Organization declaration of the role of scientific research as a tool against the Covid-19 pandemic. This marked the 60th anniversary of Milstein's return to Argentina when he was appointed Head of the Department of Molecular Biology of the Carlos Malbrán National Institute of Microbiology.

Declaration: "That the legacy of Dr. César Milstein transcended the borders of the country, and his discovery of monoclonal antibodies set a milestone in the history of medicine and influenced various specialties such as immunology, oncology, biotechnology, as well as the industry.

"As a result of these findings, in recent years it was possible to develop various innovative drugs, such as drugs to prevent transplant rejections, passive immunization for respiratory syncytial virus, therapies for asthma and for immune-mediated diseases such as rheumatoid arthritis, psoriasis and Crohn's disease or hidradenitis suppurativa, and improved survival rates and quality of life for cancer patients.

"Dr. Milstein maintained a deep commitment to science and promoted universal access and availability of knowledge for the benefit of society as a whole, renouncing personal economic benefits and rewards." [17]

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