what is biotechnology?

A HEALTHCARE REVOLUTION IN THE MAKING

The Story of César Milstein and Monoclonal Antibodies

Collated and written by Dr Lara Marks

Today six out of ten of the best selling drugs in the world are monoclonal antibody therapeutics. One of these, Humira®, which is a treatment for rheumatoid arthritis and other autoimmune conditions, was listed as the top selling drug across the globe in 2012 with a revenue of US$9.3 billion. Based on its current performance many predict the annual sales of the drug will surpass the peak sales of Lipitor, a treatment for lowering cholesterol, that is the best selling therapeutic of all time. Currently monoclonal antibody drugs make up a third of all new medicines introduced worldwide.

Portrait of César Milstein.
Photo credit: Godfrey Argent Studio

Monoclonal antibodies are not only successful drugs, but are powerful tools for a wide range of medical applications. On the research front they are essential probes for determining the pathological pathway and cause of diseases like cancer and autoimmune and neurological disorders. They are also used for typing blood and tissue, a process that is vital to blood transfusion and organ transplants. In addition, monoclonal antibodies are critical components in diagnostics, having increased the speed and accuracy of tests. Today the antibodies are used for the detection of multiple conditions, ranging from pregnancy and heart attacks, to pandemic flu, AIDS and diseases like anthrax and smallpox released by biological weapons. Beyond human healthcare, monoclonal antibodies help detect viruses in animal livestock or plants, prevent food poisoning and investigations into environmental pollution.

Monoclonal antibodies are indispensable in so many walks of daily life thanks to their ability to target a single type of cell. Produced in the laboratory, these antibodies are derived from naturally occurring proteins made by the body's immune system to recognise and fight foreign invaders, such as bacteria and viruses. The antibodies are generated through the fusion of a myeloma cancer cell with spleen cells taken from an immunised animal.

Yet the story of how these unsung microscopic heroes came into the world and helped change healthcare remains largely untold. Moreover, their significance was largely overlooked at the time of their creation. The journey of monoclonal antibodies all started when an Argentinian émigré called César Milstein arrived at the Laboratory of Molecular Biology in Cambridge, the same laboratory where Francis Crick and James Watson discovered the structure of DNA in 1953. It was to be here that Milstein, together with Georges Köhler, pioneered the seminal technique for the production of monoclonal antibodies in 1975 and showed their clinical application for the first time.

This exhibition of the life and work of César Milstein provides a window into the world where monoclonal antibodies were first developed. Showing Milstein's notebooks and writings for the first time, this exhibition provides first-hand the complexities that were involved in the creation of monoclonal antibodies and brings to life the many challenges scientists face in devising a viable biotechnological tool and its

application in healthcare. Transforming monoclonal antibodies, which started life as a laboratory tool into something that could be of use in the outside world was neither straightforward nor inevitable.

From Milstein's papers we learn first-hand how the newly-created monoclonal antibodies spread from the confines of Milstein's laboratory in Cambridge to scientists across the world and were then adapted for clinical applications. They highlight the logistical difficulties Milstein and his team faced in transporting monoclonal antibodies to other laboratories, and the fact that other scientists initially had little idea about how to grow and maintain the antibodies, let alone any idea what purpose they might serve.

Strikingly, initially Milstein had very few requests for monoclonal antibodies. By 1977, however, he was being inundated with requests for samples and had to search for outside support in the distribution process. This was to pave the way to the earliest commercialisation of the technology with the help of Sera-Lab, a small British company set up to supply animal serum reagents to the scientific community. The relationship between Milstein and Sera-Lab illustrates the process of technology transfer in biotechnology during its formative years. All of this was done with little public fanfare and no venture capital or government support. Yet, the collaboration between Milstein and Sera-Lab laid the foundation for the wide-scale commercialisation of monoclonal antibodies.

The exhibition also offers a way of understanding why the original technology developed by Milstein and Köhler was not patented in Britain and instead formed the basis of patents taken out by the Polish-American virologist, Hilary Koprowski, and his team based at the Wistar Institute in Philadelphia. The latter were thus the first scientists to be granted patents for monoclonal antibodies. Generating major controversy in the late 1970s, the patent story told in this exhibition reveals some of messy business of patenting research science and the implications this holds for those working in both the laboratory and the commercial world.

It also provides some insight into Milstein's very early efforts to demonstrate the practical application of monoclonal antibodies. He and his colleagues paved the way for the use of monoclonal antibodies as tools for the purification of natural compounds for drugs and as reagents for blood typing. Their work also demonstrated the use of monoclonal antibodies as probes to investigate the pathological pathway of neurological conditions and a wide range of other diseases. This paved the way to the adoption of monoclonal antibodies as diagnostic tools and an invaluable platform in the move towards personalised medicine. The final part of the exhibition shows how Milstein encouraged the use of genetic engineering to improve the safety and efficacy of monoclonal antibodies thereby enabling their use as therapeutics on a large scale.

Celebrating the first publication of monoclonal antibodies

It is now 41 years since César Milstein and Georges Kohler published their technique for producing monoclonal antibodies. To celebrate the occasion we invite you to watch the film *Un Fuegito* about the life and work of Milstein, produced by Ana Fraile, Pulpofilms. The film, **which you can find on vimeo.com**, has been released to help raise funds for a new educational film to promote greater understanding about monoclonal antibodies and how they have transformed the lives of millions of patients across the world.

Milstein's early life and work >>

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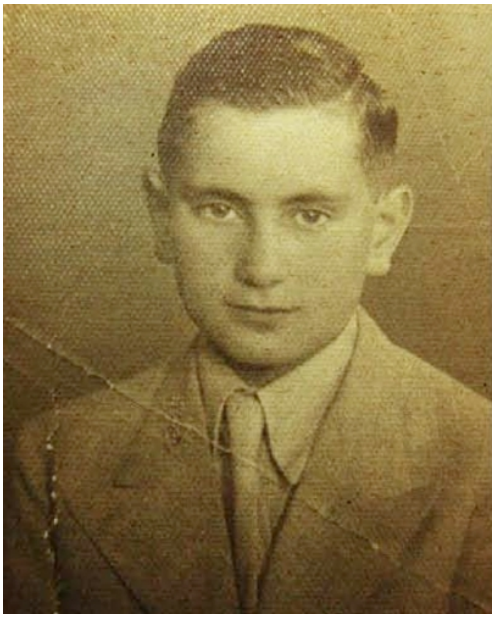
Milstein's early life

The journey from Argentina

The son of Jewish immigrants, César Milstein grew up in Bahía Blanca, a port town located by the Atlantic ocean some 500 miles south of Buenos Aires. Jews had begun to settle in Bahía Blanca from around 1900, many of them coming from central and eastern Europe.

Family background

Milstein's father, Lazaro, was born in a village in the Ukraine and migrated to Argentina in 1913 at the age of 14 with his aged aunt and uncle. For many years he lived in Jewish settlements near Bahía Blanca trying his hand at different trades, including farm labour, carpentry and railway work. During this time he taught himself Spanish and was an enthusiastic reader. He was also active in social and cultural activities, helping to preserve Yiddish literature and working for non-religious Jewish organisations, some with anarcho-syndicalist connections.



This photograph was taken when Milstein was a young man.

Photograph credit: Celia Milstein.

Lazaro met Maxima, his wife-to be, in Bahía Blanca. Maxima was born in Argentina. She was the daughter of poor Ukrainian immigrants who made great sacrifices to ensure she had a secondary school education and went to college. At the time Lazaro met Maxima she was a school teacher. Soon after their meeting, Maxima rose to become a head mistress. From 1926 to 1933, Maxima directed School No.3, the first co-educational school established in Bahía Blanca. Milstein, the middle of three brothers, was born at the family home on this school's premises. He also attended the school in his early childhood. Both of Milstein's parents spoke Yiddish at home, but Milstein was raised speaking only Spanish.

During his early childhood Milstein preferred playing with other children in the streets to reading books. With his mother's encouragement, however, he soon began to find pleasure in books, particularly adventure stories such as Rudyard Kipling's *Jungle Book*. Milstein developed a desire to pursue science at the early age of 8. This was prompted by a discussion he had with one of his cousins who had just completed her degree in Chemistry and was then working as a biochemist at the Instituto Malbran. Milstein was particularly fascinated by his cousin's description of her attempts to extract snake venom to treat snake bite victims. Milstein's interest in science deepened when on his ninth birthday he was given a Spanish translation of Paul de Kruif's *Microbe Hunters* by his mother. This book awakened his desire to have the same type of adventurous life like that of the scientists Antoni van Leeuwenhoek and Louis Pasteur described in the book.

Milstein grew up in a family which prized knowledge and education. Until his last year of school, Milstein attended schools close to home in Bahía Blanca, including the Colegio Nacional. In his final year, however, he moved to a secondary school in Buenos Aires to prepare for the entrance exam of the University of Buenos Aires.

Milstein's parents always supported his research, his mother helping to type up his first PhD thesis and his father offering him economic assistance so that he could dedicate himself to his doctoral research. Fiercely independent, Milstein declined his father's financial support.

Google Map showing the location of Bahia Blanca in Argentina. The town is a major trans-shipping and commercial centre, known for its large export trade of grains, wool, oil and fruit. Click to view a larger map.

Education

In 1945 Milstein started to study chemistry at the University of Buenos Aires. His undergraduate studies, however, were interrupted when, during a faculty picnic, he suffered severe injuries to his pancreas when he hit a log while diving into a shallow pool and had to take off some time off to recover. He finally received his BSc in chemistry in 1952.

During his undergraduate years Milstein was active in campaigns against the Peronist government's policies aimed at privatising education and their more general impositions on universities and student life and rose to be President of the Student Union. At the time the government was clamping down on any political activity, and the atmosphere was particularly tense. In 1951, for example, a chemistry student, Ernesto Mario Bravo, was arrested and tortured for 20 days as a result of protesting against the government. His arrest sparked a major student strike. More than 150 strikers were arrested and university administrators expelled the more prominent leaders of the student movement. In the end, however, Bravo was released. The student movement considered this a major achievement.

Three years later student unrest erupted once again when the Peronist regime imposed even greater control over the media, education system, trade unions and the legislative and the judiciary. In October 1954 students joined workers striking against the then deep economic and social crisis and increasing unemployment.

Shortly after Milstein returned to the University of Buenos Aires from his several months of convalescence, Milstein met Celia Prilleltensky, a fellow chemistry undergraduate. Their first encounter was at the laboratory bench, where they found themselves working alongside each other. Celia not only shared Milstein's scientific interests, but was similarly an ardent student campaigner for free education. A year after their graduation in 1952, Milstein and Celia married.

At the same time as getting married, Milstein began to look for a suitable doctoral supervisor. Initially he sought to work with Professor Luis Leloir, a distinguished Argentinian enzymologist. To this end he visited Leloir's workplace, an old house in Buenos Aires. On arrival he met what seemed to him an unassuming man carrying a basket. This turned out to be Leloir. Having no space to take Milstein on, Leloir instead referred him to the Argentinian biochemist Professor Andrés Stoppani.

Milstein recalled that Stoppani was 'one of the few and perhaps the only full-time Professor of the Faculty of Medicine in the University of Buenos Aires, perhaps the most important universities in Latin America, a full time professor who probably had a salary of about the same order of magnitude as a janitor, trying to do serious and honest research in a laboratory with no funds at all'.

This photograph shows Milstein early on in his courtship with Celia Prilleltensky. Photograph credit: Celia Milstein.

Stoppani advised Milstein to take some time off before he started his doctorate in view of the tense political climate which was hostile to students such as Milstein who had actively campaigned against the Peronist government's policies in education. His advice encouraged Milstein to take a year long honeymoon with Celia exploring Europe.

By 1954 the political environment had begun to improve and Milstein began researching enzymes for a doctorate in biochemistry although he had no funding as there was none for students in this period. He was forced to support his studies by working half-days in the Laboratorios Liebeschutz, a clinical biochemistry laboratory. In later years Milstein argued that this part-time job had taught him the value of organising his time.

During his doctoral research Milstein had access to only the most basic equipment. Some idea of how poor the facilities were at the time can be seen from his recollections that Stoppani had 'to pay, from his own meagre salary, for the pound of yeast … needed from time to time in order to prepare … [the enzyme] aldehyde dehydrogenase.' He recalled, 'We survived on what was inherited from the golden days … from the Medical School and with reagents justified by teaching requirements'. The most precious piece of equipment in the department was a Warburg apparatus, which Stoppani did not allow anyone but himself to use.

By 1955, the political situation in Argentina had improved still further and with this the conditions in Stoppani's department became slightly easier. This meant, for example, that the department was able to purchase a refrigerated centrifuge. The centrifuge proved useful to Milstein in his enzyme preparations.

Click here to see the additional photographs from Milstein's early life.

Nonetheless, problems remained. The nearest spectrophotometer, an instrument that Milstein needed for measuring enzyme activity, was located three blocks away from his department and Milstein had to trek every day between departments carrying reagents and enzyme preparations. Early on in his doctoral studies he came close to losing his position in the department when, in the process of making his enzyme preparation, he succeeded in consecutively breaking three of the department's five very expensive 5-litre flasks.

Despite these hurdles, Milstein completed his doctoral research and was awarded a prize in 1957 by the Associación Quimica Argentina for the best thesis in chemistry that year. His doctorate was an investigation of the enzyme dehydrogenase. He had focussed his research on one of the enzyme chemical bonds, known as a disulphide bridge. Between 1957 and 1959 Milstein would publish several papers with Stoppani arising from his doctoral research.

The relationship with Cambridge begins

In 1958, funded by a British Council scholarship, Milstein joined Malcolm Dixon and Edwin Webb at the Sir William Dunn School of Biochemistry in Cambridge. In part the decision was influenced by the fact that Stoppani had worked with Dixon before the Second World War.

Initially, Milstein had difficulty understanding what Dixon and Webb were saying because he lacked fluency in English, but with their advice he set out to study the kinetics and heavy metal activation of the enzyme phosphoglucomutase. This was inspired by Dixon's suggestion that Milstein follow up an odd observation made some years earlier in the department that phosphoglucomutase required two metals for full activity, magnesium and a trivalent metal like chromium.

Milstein was left to pursue his research on his own within Cambridge. The work was not without its pitfalls. Milstein lost his first large-scale enzyme preparation in an electric cold bath. According to Milstein this was caused by the distraction of attending a champagne party to celebrate the awarding of a Nobel Prize to Fred Sanger in 1958. Sanger had been awarded the Prize two weeks after Milstein's arrival in Cambridge. A central figure in the Cambridge Biochemistry Department, Sanger's achievement had been to show that proteins have a defined chemical composition.

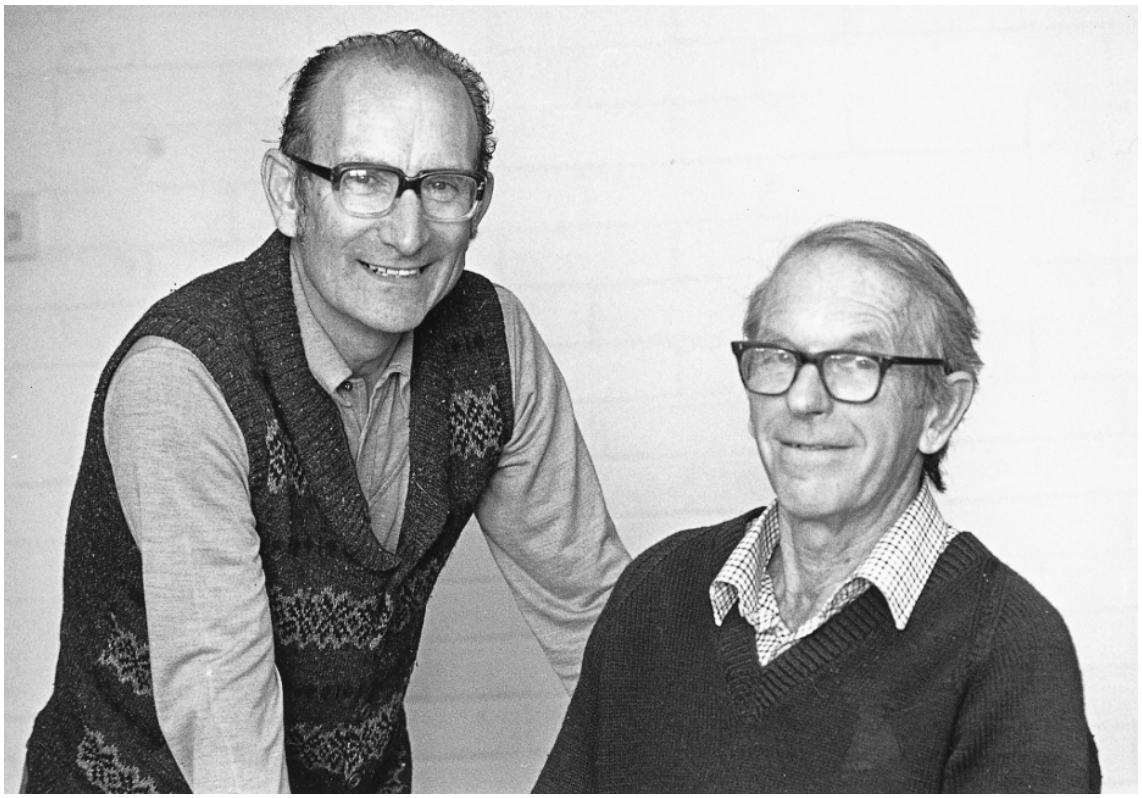
Despite his early disaster with his enzyme preparation, within a year Milstein's experiments on phosphoglucomutase had provided sufficient data for him to write up his research. This led to the award of a second doctorate, this time from Cambridge University. Based on this research he published three papers. Contrary to contemporary opinion, Milstein discovered that the activation of phosphoglucomutase was caused by the *displacement* of heavy metals by magnesium. Prior to Milstein's finding, scientists believed the enzyme was activated by the heavy metals themselves.

During his British Council fellowship in Cambridge, Milstein formed a strong bond with Sanger. At the time Sanger was a pivotal figure in the Department of Biochemistry, as he possessed the only functional pH meter. While an unassuming figure, Sanger was a dominant influence within the department. Milstein, for example, remembered a warning sign to the entrance of the department's high-voltage electrophoresis room reading 'Danger – High Power' which was altered by someone in the department to read 'Sanger – High Power'.

Malcolm Dixon, the biochemist who supervised Milstein at the Sir William Dunn School of Biochemistry, Cambridge University. Photo credit: Sir William Dunn School of Biochemistry, Cambridge.

Milstein was quickly drawn to Sanger not only because of the equipment he possessed but also by the fact that they shared the same research interests. They soon collaborated to define the active site of phosphoglucomutase and published a joint paper on this research. It appeared as C. Milstein, F. Sanger, 'An amino acid sequence in the active centre of phosphoglucomutase', *Biochemistry Journal,* 79 (1960), 456-69.

When it came time for Milstein to return to Argentina, Sanger offered to secure Medical Research Council money for him to extend his time in Cambridge. Milstein, however, decided to return to Argentina to take up a position he had been offered prior to coming to Cambridge.



This photograph was taken around 1980. It shows Milstein together with Fred Sanger. Photo credit MRC, Laboratory of Molecular Biology.

A new chapter in molecular biology in Argentina

In 1961 Milstein departed from Cambridge for the Instituto Malbran, where he headed up a newly-created Department of Molecular Biology in the National Institute of Microbiology. Celia also had a post in the department. The return of the Milsteins to Argentina coincided with a period of reform in the country following the fall of Peron, when many academic scientists who had been sidelined (or expelled) during Peron's rule returned; they included Bernardo Houssay, a physiologist who won the Nobel Prize in 1947, and Leloir who would go on to win the Nobel Prize in Chemistry in 1970.

As head of the Department of Molecular Biology, Milstein had a wide range of responsibilities, from employing his carpentry skills to install a laboratory to acting as mentor to 25 young scientists and bringing bacterial genetics into the research orbit of the department. In addition to his day-to-day to management of the department, he continued the research he had begun in Cambridge around phosphoglucomutase, developing techniques for studying the sequence of the enzyme and marking its active centres. He also started investigating another enzyme: alkaline phosphatase of bacteria. Much of this work was focused on understanding the enzyme's mechanism of action. His notable achievement at this time was elucidating the sequence around the active site of alkaline phosphate of bacteria with Noé Zwaig. This they accomplished ahead of scientists in the USA. They published their results in N. Zwaig, C. Milstein, 'On the nature of the phosphoenzyme intermediate in the phosphoglyceromutase reaction', *Biochimica et Biophysica Acta*, 73 (1963), 676-9.

In 1962 Milstein's life, like that of many other Argentinians, was thrown into turmoil as a result of a military coup. Most disturbingly for Milstein, Ignacio Pirosky, the director of his institute, was dismissed, as were many colleagues in Milstein's department who had defended Pirosky. With the coup, persecution began to mount against both political dissenters and Jews in Argentina. This had major implications for Milstein. Bearing a Jewish name, authorities immediately associated him with communist activists. When four of his own staff were expelled from his department, he found himself no longer able to concentrate on his own scientific research and he decided to resign from his position and return to Cambridge. He was just one amongst the many tens of thousands of intellectuals and scientists who left Argentina during the military regime.

More historical background can be found on Milstein and the rise and fall of molecular biology in Argentina in the late 1950s and late 1960s in P. Kreimer and M. Lugones, 'Pioneers and Victims: The Birth and Death of Argentina's First Molecular Biology Laboratory', *Minerva,* 41/1 (2003), 47-69.

Milstein's career begins at the Laboratory of Molecular Biology

Milstein departure from Argentina was facilitated by Fred Sanger. On hearing of Milstein's difficulties in Argentina, Sanger invited him to join him at the Medical Research Council (MRC) Laboratory of Molecular Biology, where he now headed the Protein Chemistry division. Milstein arrived at the Laboratory in 1963 on a three year contract paid for by the MRC. This was one year after the Laboratory had moved into its own premises.

For more on Milstein's recollections of his early days see C. Milstein, 'Messing about with isotopes and enzymes and antibodies', Lynen Lecture, Miami Winter Symposium Proceedings, in W. Whelan, ed., *From Gene to Protein: Translation into Biotechnology* (New York and London, 1982).

<< Introduction to the exhibition

Milstein's early antibody research >>

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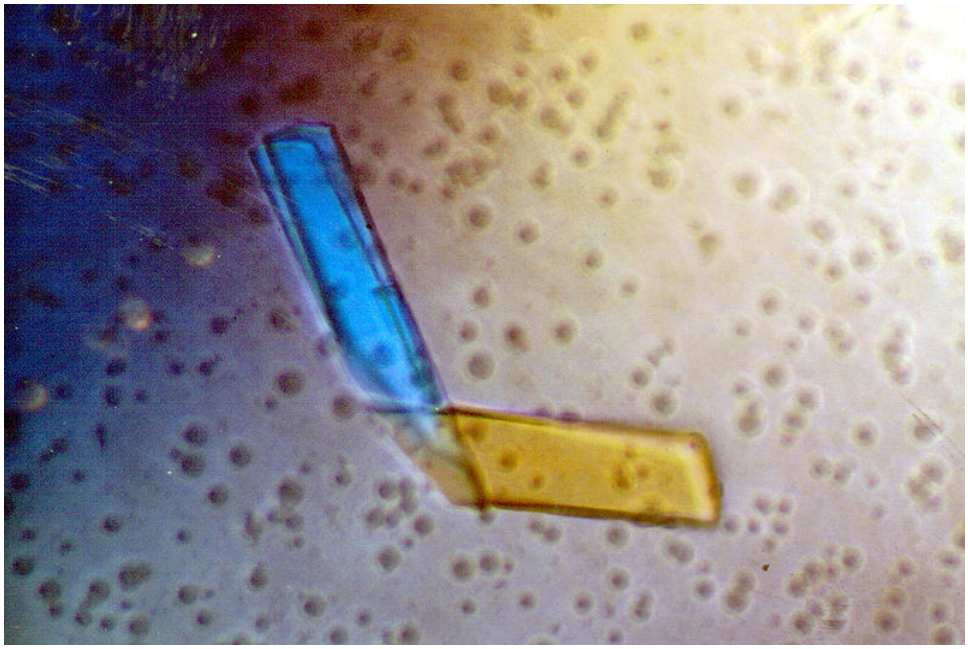
Early antibody research

The quest to understand antibodies diversity

Soon after arriving at the Laboratory of Molecular Biology (LMB) Milstein began an investigation into the Bence-Jones protein. His prime objective was to understand the molecular structure behind the formation and diversity of antibodies.



This photograph shows a crystal of a Bence-Jones protein. Photo credit: Alex McPherson, University of California, Irvine; National Institutes of General Medical Sciences, ID 2399.

The Bence-Jones protein is a substance found in the urine and blood of patients suffering from multiple myeloma, a cancer that results in the softening of bones. The protein was first described by the English physician Henry Bence-Jones in 1847 and its physical characteristics were determined during the 1950s. By the early 1960s, scientists had discovered that the Bence-Jones protein possessed the same structure as a light-chain, a sub-unit of an antibody molecule. Thereafter scientists began to use the protein as a tool for investigating the structure and function of normal antibodies. One of the advantages in exploring the Bence-Jones protein was that it was easily available. Scientists could gain access to the protein collected from the urine and blood of patients in hospitals.

Milstein's decision to investigate Bence-Jones proteins for the purposes of investigating antibodies was prompted in part by his supervision of a doctoral student working on antibodies when in Argentina and in part by discussions he had with Sanger on arriving at the LMB.

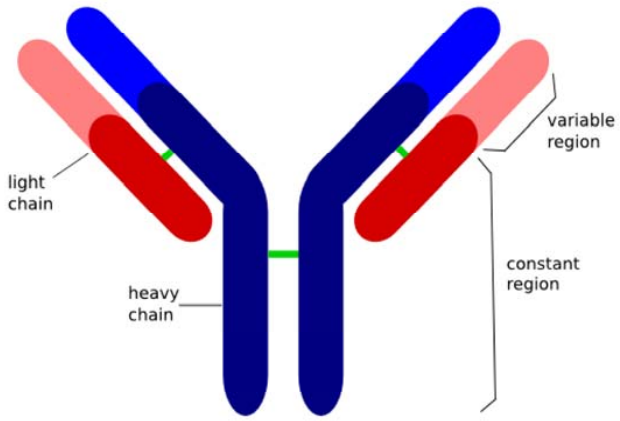
At the start of his research into Bence-Jones proteins, Milstein was one of many scientists then trying to understand the body's immune system by unravelling the structure and function of the billions of antibodies made by the body every day to fight off infections.

An antibody works by specifically targeting a foreign intruder, known as an antigen. Such antigens can range from bacteria, viruses and fungi, to pollen, dust, or food

proteins that cause allergic reactions. Not all antigens are foreign bodies, however; they can also be cancer cells made in the body itself.

Once an antibody recognises a particular antigen it will attach itself to a specific marker on the cell surface of the antigen so that the latter can be targeted for destruction. In many ways the binding of an antibody to an antigen can be likened to the insertion of a key in a lock.

By the time that Milstein began his research into antibodies, scientists were beginning to unravel the basic structure of an antibody. This they viewed as a Y-shaped formation composed of two protein sub-units, labelled as light and heavy chains (appearing as red and blue in the diagram), held together by disulphide bonds (indicated by green lines). They believed the antibody structure was divided into two regions, one that was constant, forming the stem of the Y, and one that was variable on the tip of the arms of the Y.



This shows the basic structure of an antibody.

Nonetheless, while the basic molecular structure of antibodies was beginning to be solved, what remained a puzzle was how such an apparently almost identical group of proteins could specifically target simultaneously any one of a multiple of antigens. This specificity as well as the diversity of antibodies was a question that had intrigued scientists ever since the late 19th century when antibodies were first observed.

What lay behind the heterogeneity of antibody molecules would be a recurrent subject for most of Milstein's laboratory investigations from the 1960s onwards. One of the attractions of this research for Milstein was that it could be achieved through very simple experiments, essentially comparing primary DNA sequences of two different antibodies. This gave Milstein the means to elucidate the diversity of antibodies at the level of their amino acid sequences.

Dissecting the antibody structure

Having decided upon his topic of research, Milstein began investigating different chemical techniques to dissect the structure of antibodies. He was assisted by John Jarvis, a biochemistry technician who had joined the LMB when he did. Jarvis would work as Milstein's research assistant until Milstein retired.

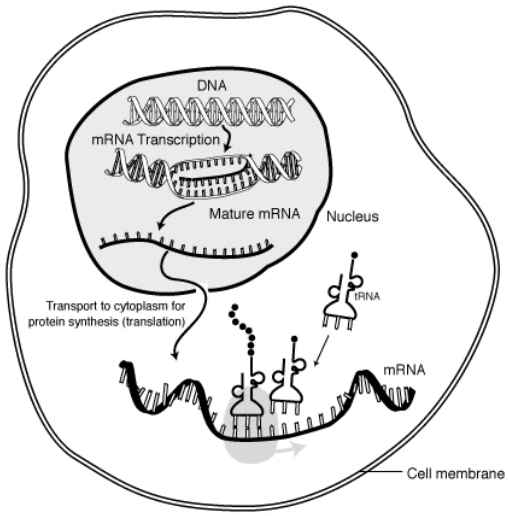
One of the first experiments Milstein conducted was to determine the amino acid sequence of the Bence-Jones protein in order to unravel the function of the disulphide linkages within the overall molecular structure of the antibody. He published his first results in C. Milstein, 'Disulphide bridges and dimers of Bence-Jones Protein', *Journal of Molecular Biology*, 9 (1964), 836-8. The paper provided the first sequence data for the Bence-Jones protein and was the first of the many papers that Milstein would write on antibodies over the next forty years.

Having analysed the dilsulphide bridges of antibodies, Milstein began to explore the differences in amino-acid sequences, the positions of carbohydrate attachments and mutations. All of this work was directed towards understanding the nature of the diversity of antibodies at the DNA level.

One of the tools that proved vital to Milstein's work in determining the structure of antibodies was the chromatography column. In column chromatography a sample is put through a glass tube filled with a liquid known to separate the different components within the sample. Because of molecule size and polarity, different components in the sample travel through the column at different speeds.

In Milstein's experiments, the chromatography column was used to separate out the subunits of light and heavy chains and other components of the antibody protein. Once put through the column, the separated components of the antibody were then collected and purified. After this, an enzyme was added to the collected sample in preparation for sequencing. This enabled digestion of the sample to separate its constituent elements. After being treated with an enzyme, the sample was run again through a chromatography column. Together these steps enabled Milstein to begin to determine the sequence of individual genes and larger genetic regions within the antibody.

Overall the sequencing of an antibody's amino acids was a laborious and time-consuming process. The procedure would become much easier and faster with the development of new sequencing methods in the early 1970s.



This diagram shows how mRNA is transcribed and translated into the nucleus of a cell, and then being processed and transported to the cell's cytoplasm, a gel like substance residing within the cell's membrane. Once in the nucleus the mRNA is translated and decoded for the formation of other proteins. Image credit: National Institutes of Health, Wikipedia.

In his elucidation of antibody diversity at the level of amino acid sequences, Milstein was aided by Richard Pink, his first English graduate student, and Blas Frangione, an American postdoctoral scientist. He was also helped by his wife Celia, with whom he published a joint paper in the *Journal of Molecular Biology* in 1970.

By the early 1970s Milstein had begun to shift his research towards investigating the role played by messenger ribonucleic acid (mRNA) and the encoding DNA in determining the diversity of antibodies. The mRNA is a type of chemical 'blueprint' for a protein product.

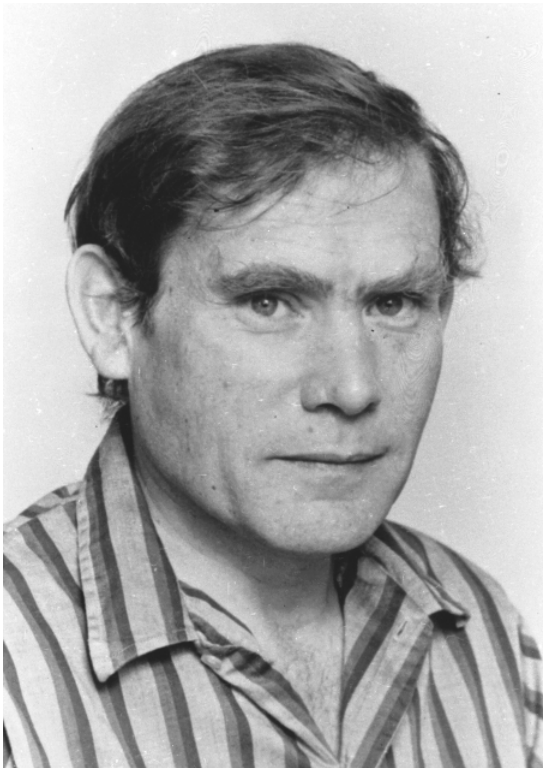
Somatic mutation and antibody diversity

One of the people Milstein worked with to understand what lay behind antibody diversity was Sydney Brenner. Milstein had been introduced to Brenner in the mid-1960s by Francis Crick, co-discoverer of the double helix of DNA and a close colleague of Brenner. Together Milstein and Brenner developed a theory to explain the phenomenon. This was published in S. Brenner and C. Milstein, 'Origin of antibody variation', *Nature*, 211 (16 July 1966), 242-3.

At the time Milstein and Brenner wrote their article, the work of both Milstein and other scientists suggested that sequence diversity in antibody polypeptide chains was primarily located in their amino-terminal portions. The question this raised was how such localised diversity was generated. Both Milstein and Brenner hypothesised that it originated from a mutation in the DNA of the antibodies, known as somatic mutation.

Somatic mutation occurs as a result of an accidental change in the genomic sequence of DNA. Such mutations cannot be passed on to offspring as the alterations of the DNA occur only in non-reproductive cells of the body. They can be caused by radiation, viruses, chemicals or errors that occur for other reasons during DNA replication. A somatic mutation can also be induced by the organism itself, through a cellular process known as hypermutation, a mechanism by which the immune system adapts to new foreign elements, such as microbes.

This photograph shows Sydney Brenner, a South-African-molecular biologist who in the 1960s was attached to the Molecular Genetics (now the Cell Biology) Division at the Laboratory of Molecular Biology. Photo credit: MRC, Laboratory of Molecular Biology.



[<< Milstein's early life and work]

[The making of monoclonal antibodies >>]

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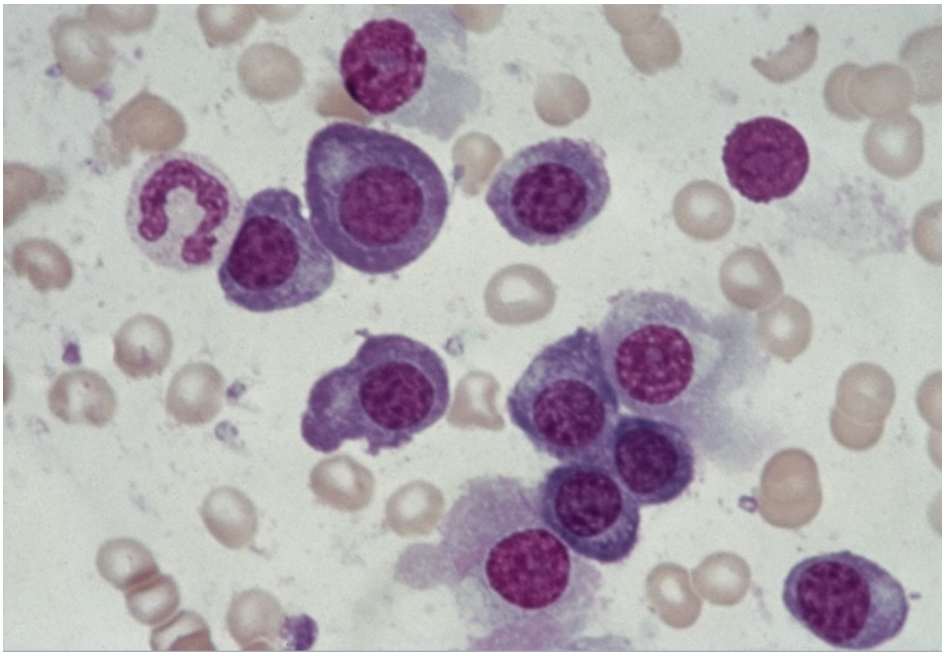
Making monoclonal antibodies

The hunt for a single antibody

From the late 1960s onwards Milstein would devote much of his research to understanding somatic mutation in antibodies. The idea that somatic mutation could help explain antibody diversity was not new. Indeed, Milstein and Brenner's suggestion that this could be what determined antibody diversity was in many ways an extension of a hypothesis first formulated by the American molecular biologist Joshua Lederberg in 1959.



This photograph shows myeloma cells stained purple as appears under a microscope. Photo credit: Wellcome Images, N0009977.

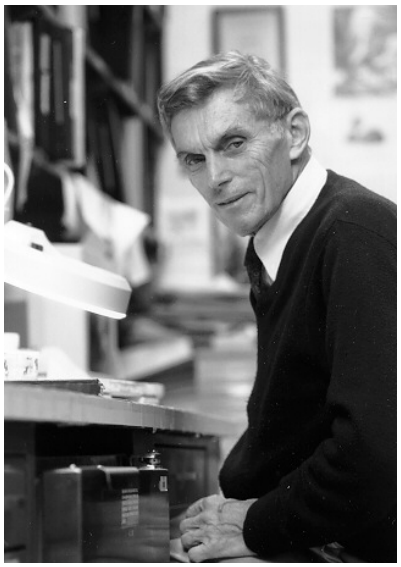
In his quest to understand somatic mutation and antibody diversity, Milstein was greatly helped by the availability of cells taken from the blood of patients with multiple myeloma. Such myeloma cells, which produce antibodies resembling normal antibodies, had been noted in 1951 by Henry Kunkel, an American immunologist based at the Rockefeller Institute in New York. Investigating the blood of myeloma patients, Kunkel was surprised to observe that malignant plasma cells of multiple myeloma appeared to produce just one antibody. This contrasted with normal plasma cells which produce a vast array of antibodies.

Kunkel's discovery was a significant breakthrough for antibody research. Ever since the early 20th century scientists had been struggling to isolate and purify single antibodies from the billions made by the body every day. One of the advantages of the antibodies produced by myeloma cells is that they are all identical. Moreover, they are fairly easy to obtain in large quantities from the blood or urine of patients with multiple myeloma. Based on this, Kunkel and his co-workers began using myeloma cells as a tool for investigating normal antibodies.

The availability of myeloma cells for antibody research greatly expanded after 1962 when the American molecular biologist Michael Potter made the serendipitous discovery that an injection of mineral oil into the peritoneal cavity of BALB/c mice, a particular strain of laboratory

mice, induced the growth of myeloma cells. This meant that the cells could be grown easily and indefinitely. Following this, Potter and his colleagues at the National Cancer Institute in Bethesda established a collection of myeloma cells lines for distribution to researchers around the world.

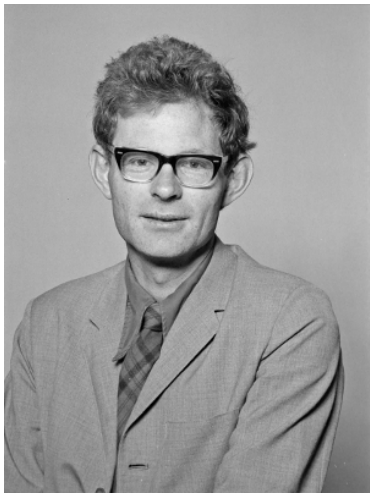
By the 1970s scientists' access to myeloma cells was made much easier by the work of Kengo Horibata and A.W. Harris under the supervision of Melvin Cohn at the Salk Institute in San Diego. They found a way of adapting Potter's mouse myeloma cells to grow in tissue culture, a technique that allows for the growth of tissues or cells outside an organism. Essentially, tissue culture provides for the growth of cells under controlled conditions outside their natural environment. The growth of such cells is helped by the use of a suitable medium, which provides the nutritional elements they require.



This photo shows Michael Potter, an American molecular biologist, whose myeloma cell line helped laid the foundation for the development of monoclonal antibodies. Photo credit: University of Maryland.

The first tissue culturing techniques date back to 1885, and had become a common method in laboratories by the 1940s and 1950s. Each cell type necessitated different conditions, such as temperature, and various types of culture medium for optimum growth. This was perfected by a process of trial and error. The tissue culture developed for the growth of Potter's myeloma cells is described in K. Horibata and A.W. Harris, 'Mouse myelomas and lymphomas in culture', *Experimental Cell Research,* 60 (1970), 61-77.

Overall the work undertaken by Horibata and Harris freed scientists from the laborious process of growing myeloma cells in mice. With the cultivation of myeloma cells *in vitro,* scientists now had access to a continuous supply of such cells.



This shows George Brownlee, a British pathologist, who worked with Milstein on mRNA. Photo credit: MRC, Laboratory of Molecular Biology.

Milstein had obtained Potter's mouse myeloma cell line (MOPC21) that Horibata and Harris had adapted to long-term tissue culture during the early 1970s from his LMB colleague Alan Munro, who had originally obtained them from the Salk Institute where he had spent a sabbatical year. Milstein grew these cells together with George Brownlee for the purposes of harvesting mRNA and as a source for studying the structure of antibodies.

Based on their growth of the myeloma cells, Brownlee and Milstein were able to establish the existence of a leading sequence in the RNA of the light chain. They reported their results in C. Milstein, G. Brownlee, G.G. Harrison and T.M. Mathews, 'A possible precursor of immunoglobulin light chains', *Nature New Biology,* 239 (1972), 117-120.

Fusing myeloma cells to pinpoint somatic mutation

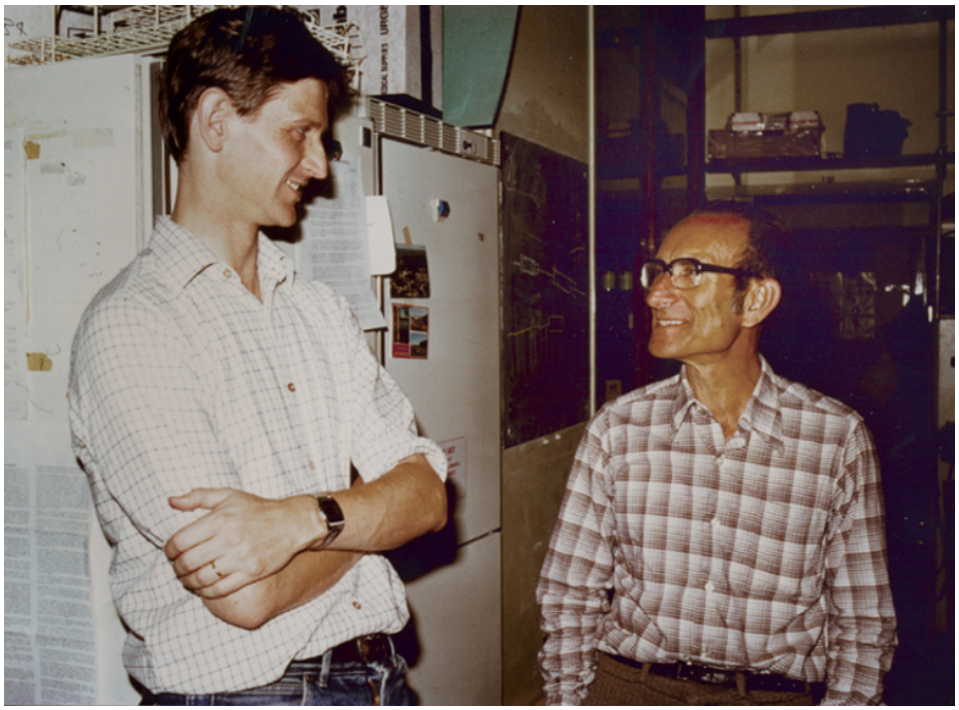
After his letter with Brenner in *Nature,* which had hypothesised that antibody diversity was the result of somatic mutation, Milstein began looking for a way to carry out experiments to validate the theory. This he decided to do using the MOPC21 myeloma cell line. He was joined in the venture by David Secher who joined the LMB to do a doctorate in the summer of 1970.

What Milstein and Secher wanted to determine was the rate of somatic mutation that occurred in the antibodies produced by the myeloma cell line and to identify and characterise any variants that emerged. This they hoped would help them unravel the process of antibody diversity as it occurred in nature.

Shortly after Milstein and Secher began their project to investigate somatic mutation in MOPC21 myeloma cells, they were joined by Dick Cotton, a postdoctoral scientist from Australia who was interested in immunogenetics. Rapidly picking up the skills for cell culture, with the help of Abraham Karpas who worked in a nearby laboratory, Cotton soon succeeded in cloning MOPC21 cells in soft agar. This laid the basis for the isolation of MOPC21 mutants.

This shows Milstein speaking with his postdoctoral researcher David Secher in 1980. Credit: MRC

Laboratory of Molecular Biology.

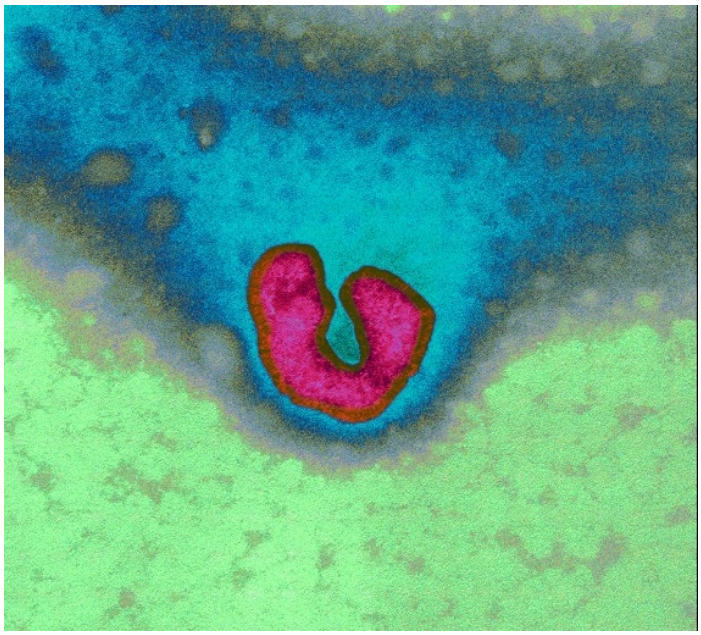


With Cotton's technique in place, the team then spent many months looking for somatic mutants. However, this proved frustrating. The difficulty was that such mutants rarely occur. Just five structural variants were isolated, for example, after three months of continuous culture and analysis of antibodies produced by 7000 clones.

While providing a disappointing yield of mutants, the myeloma cells nevertheless provided scientists with a window through which to see somatic mutation in cells. Importantly, mutation had occurred without any addition to the myeloma cell line. It had taken place on its own accord. The difficulty, the team found out later, was that the mutants observed in the myeloma cell line bore no resemblance to the major types of mutants found in nature.

Alongside this experiment a second project was launched. Undertaken by Cotton, this involved the fusion of two different myeloma cell lines. It was carried out using inactivated Sendai virus obtained from Abraham Karpas. This reagent was used for the promotion of cellular fusion.

This image shows a colour-enhanced image of the Sendai virus. It is a virus that usually infects mice, but which can also infect human cells without causing disease. Inactivated Sendai virus was a common reagent for cellular fusion by the 1970s. Photo credit: University of Edinburgh, Wellcome Images, B0004621.



One of Cotton's objectives was to understand why antibody-producing cells appeared to use only one set of parental genes to produce a functional antibody. Called allelic exclusion, this phenomenon was particularly puzzling as in most cases cells inherit a copy of both sets of genes from their parental cells. What scientists assumed was that in the process of antibody reproduction one gene was silenced while the other was transferred across. By fusing two myeloma cells lines, Cotton and Milstein wanted to see which genes would be transferred and which would be silenced. They were also interested in what effect such fusion would have on the structure of antibodies in terms of their variable and constant regions.

In the fusion experiment, the first myeloma cell line was a variant of the mouse MOPC21 line that was sensitive to the chemical bromodeoxyuridine. The second was a rat myeloma cell line obtained from Hervé Bazin in Belgium.

To Cotton and Milstein's surprise, the fusion of the mouse and rat myeloma cells resulted in a hybrid clone, known as a hybridoma, that produced antibodies carrying the genes of both parental cells. Indeed, they could find no evidence for allelic exclusion. Moreover, there appeared to be no scrambling of the variable and constant regions in the structure of the antibodies produced. Based on this, they theorised that the linkage between variable and constant regions occurred early in the process of cell differentiation, before antibody production began.

Following the fusion of the rat and mouse myeloma cells, Cotton, with the help of Shirley Howe, one of Milstein's laboratory technicians, continued to perfect the fusion method. One of the fusions resulted in hybridomas derived from mouse-mouse myeloma cell fusions. These yielded the same results as the earlier mouse-rat fusions.

Cotton and Howe's fusion efforts were greatly enhanced by the selection of a different myeloma clone, known P3-X63Ag8. The clone was a variant of a sub-clone of the P3 myeloma cell line originally developed by David Secher. Secher had made this from the cell line originating from Horibata and Harris's adaptation of Potter's MOPC21 myeloma cell line. A key advantage of P3-X63Ag8 was that it was resistant to azaguanine (Ag), a reagent that helps promote fusion. Other myeloma cell lines were liable to be destroyed by azaguanine.

This shows Milstein with Georges Köhler, a German biologist with whom he worked, during a trip to Kenya in 1979.Photo credit: Celia Milstein, MRC Laboratory of Molecular Biology.

In 1973 Milstein presented the results from the myeloma cellular fusion experiments conducted with Cotton to the Basel Institute of Immunology. It was here that Milstein met Georges Köhler. At the time Köhler was on a temporary secondment to the Institute while completing a doctorate at the University of Freiburg. Excited by Milstein's presentation and taking an instant liking to him, Köhler asked to join Milstein's research team in Cambridge as a post-graduate. This he did in April 1974, and he soon joined in the research efforts to understand somatic mutation and the mechanism underlying antibody diversity.

Finding an antibody with defined specificity

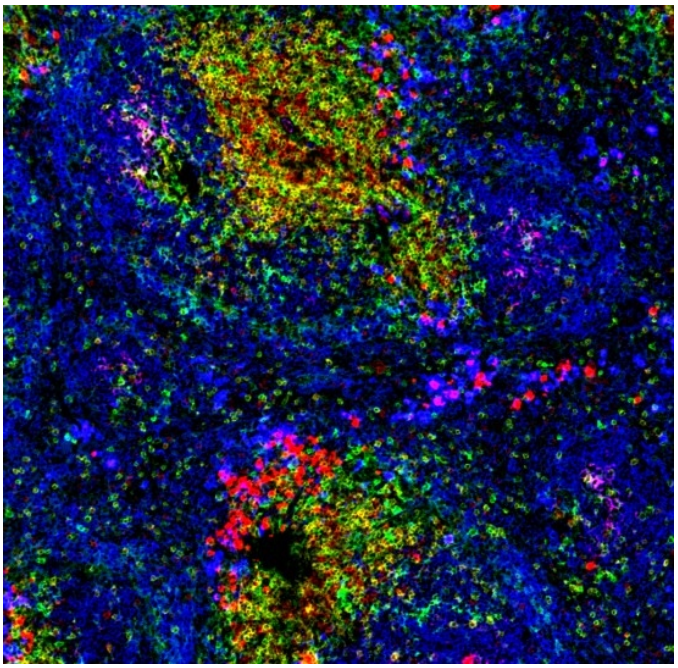
Prior to Köhler's arrival, much of Milstein's research into somatic mutation and the diversity of antibodies had focused on a search for mutant genes within the variable region of antibodies, as this was the portion of the antibody understood to be responsible for binding to antigens. Yet this had proven a laborious process, akin to looking for a needle in a haystack.

What was needed was an antibody with a clearly defined specificity. This would provide the most effective means for detecting slight differences caused by such mutations. Such an antibody, however, was not readily available.

Until the mid-1970s most of the studies Milstein and his team had undertaken on somatic mutation in antibodies were still being conducted with myeloma cells, dictated by their abundance and the fact that this was the closest scientists had come to a source of natural antibodies. However, such cells had certain limitations. The difficulty was that no one knew which specific antigens the myeloma cells bound to. Part of the problem was the fact that such cells are triggered by malignancy, a process that affects cells at random.

Not knowing which antigen was targeted by myeloma cells was a major problem for Milstein and others interested in understanding the molecular basis of antibody specificity. Some scientists had attempted to get around the problem by trying to induce tumours to produce antibodies to an injected antigen. These efforts, however, had come to nothing.

This shows a confocal image of the spleen of a mouse showing B cells stained in blue. Photo credit: Peter Lane and Fiona McConnell, Wellcome Images B0003949.



By the time that Milstein and Köhler began their collaboration, a number of scientists had begun to devise ways to make antibodies with known specificity to a particular antigen.

Discovery of a natural hybrid cell producing antibodies

One of the earliest was Joseph Sinkovics, a Hungarian immunologist based at the M.D. Anderson Hospital and Tumor Institute in Texas funded by the National Cancer Institute (NCI). Between 1960 and 1962, while conducting research into simple viral mouse leukaemia, Sinkovics happened to come across a mouse lymphoma cell with virus like particles on its surface, a highly unusual characteristic, which prompted an immune response in mice able to destroy the cell. Keen to learn more Sinkovics and his team began looking for ways to grow the cells in tissue culture. Initially this proved an uphill struggle, but by 1966-67 they had managed to grow a new cell line in suspension spinner cell cultures. This they achieved by cultivating the original cells in culture with tissue taken from the spleen of mice that had rejected such cells. Much to the surprise of the team the new cells appeared to contain sets of chromosomes unlike the previous lymphoma cells which had just 2 sets of chromosomes. The new cells also grew more vigorously when injected into the adult mouse than the previous cells, forming huge tumours which presented as abnormal fluid or ascites in their abdomens. This work was published in J.G. Sinkovics, B. Drewinko, E Thornell, 'Immunoresistant tetraploid lymphoma cells', *Lancet* (17 Jan 1970), 139-40.

Joseph Sinkovics, 1960s, credit: Sinkovics. Born in Budapest in 1924, Sinkovics obtained his medical degree from the University of Petrus Pazmany. Following this he set up a laboratory to study viruses in the Institute of Microbiology in Budapest. In 1956 Sinkovics left Hungary following the uprising against the Soviet imposed communist government and took up a Rockefeller Fellowship in the US. In 1979 he was appointed a consultant oncologist in the University of Texas M.D. Anderson Hospital and Tumor Insitute, where he was to remain for the rest of his career. For more about Sinkovics click here.

On further investigation the team discovered that the new cells not only produced virus particles but also antibodies against the mouse leukaemia virus. Further experiments with mice indicated the antibodies were highly specific against the virus. Indeed, they were better at neutralising the mouse leukaemia virus than antiserum taken from rabbits or mice immunised with the virus. Sinkovics hypothesised that the antibodies were the result of a natural fusion of a splenic plasma cell with the mouse lymphoma cell. Such a fusion he argued was highly exceptional and would only occur under special conditions. He believed it was due to a 'specific immune reaction between the budding virus still embedded in the cell membrane and the antibodies retained before their release on the surface of the immune spleen cells'. This process generated a hybrid cell (later known as a hybridoma) capable of producing antibodies which incorporated the immortal qualities of the malignant cell. (For more details see J. Sinkovics, 'Discovery of the hybridoma principle in 1968-69. Immortalization of the specific antibody producing cell by fusion of a lymphoma cell', *Journal of Medicine*, 16 (1985), 16: 509-524).

Credit: Sinkovics. This diagram outlines the hypothesis Sinkovics developed to account for the formation of the natural hybridoma in murine lymphomas. He presented the diagram to various international conferences between 1968 and 1969, including the International Tumor Conference in Perugia, Italy and at two annual conferences at the University of Texas M.D. Anderson Hospital, one on basic science and another for clinical medicine. The image was also published on the front of the *Leukemia-Lymphoma Year Book* (1970).

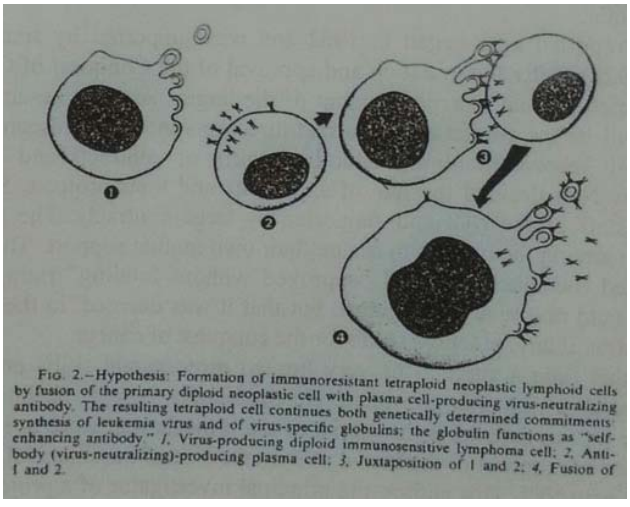
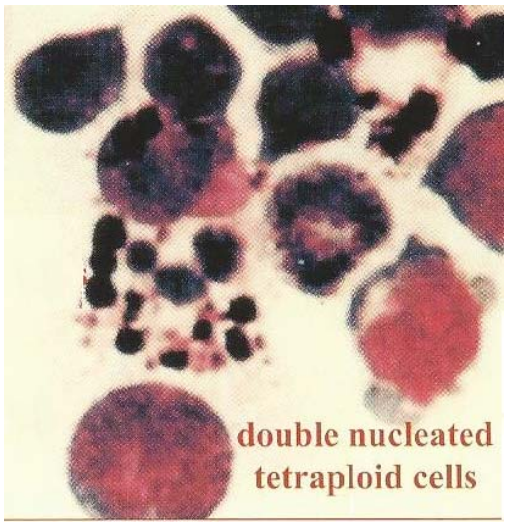


FIG. 2.—Hypothesis: Formation of immunoresistant tetraploid neoplastic lymphoid cells by fusion of the primary diploid neoplastic cell with plasma cell-producing virus-neutralizing antibody. The resulting tetraploid cell continues both genetically determined commitments: synthesis of leukemia virus and of virus-specific globulins; the globulin functions as "self-enhancing antibody." *1*, Virus-producing diploid immunosensitive lymphoma cell; *2*, Antibody (virus-neutralizing)-producing plasma cell; *3*, Juxtaposition of 1 and 2; *4*, Fusion of 1 and 2.

Between 1968 and 1969 Sinkovics presented his findings to several international and American conferences during which he showed a sketch of his hypothesis. Yet, to his disappointment, the significance of what he had found seemed to by-pass everyone who heard him. As he recalled, no one rose to ask a question or comment on what he had found. In spite of this, Sinkovics and his colleagues continued their work on the cells. (J.G. Sinkovics, 'On the threshold of the door of "no admittance", in A. Szentivanyi, H. Friedman, eds., *Immunologic Revolution: Facts and Witnesses* (Florida, 1994).



Photograph showing the spontaneous fusion of lymphoma cells with leukaemia virus immune plasma cells which continued to secrete specific mouse leukaemia virus-neutralising antibodies. The double nucleated tetraploid cells are the new hybrid cells. Credit: J.G. Sinkovics, *Cytolytic Immune Lymphocytes* (Budapest, 2008), figure 7D, p.100.

By 1970 Sinkovics and his colleagues had shown that the fused cells could grow in suspension culture for many years and continued to produce specific mouse leukaemia virus-neutralising antibodies. Furthermore they had devised a method to purify the antibodies. That year, however, they were forced to abandon the project because the NCI refused funding to extend the project despite finding it to be scientifically sound. Moreover, the team could not envisage any therapeutic applications for the antibodies. (J.G. Sinkovics, 'On the threshold of the door of "no admittance", in A. Szentivanyi, H. Friedman, eds., *Immunologic Revolution: Facts and Witnesses* (Florida, 1994).

Antibodies from cloned B cells

Soon after Sinkovics and his team had developed their antibodies, another group at the National Institute of Medical Research led by Brigitte Ita Askonas (1923-2013), an Austrian-Canadian biochemist, hit upon a way of generating antibodies by cloning B lymphocytes, a type of white blood cell, in genetically identical irradiated mice. This they did as part of a wider project to understand the process underlying the generation of B cells and antibody diversity. Called E9, these clones produced antibodies that bound to a hapten antigen, a small molecule that can only stimulate an immune response when attached to a larger molecule like a protein. Labelled E9, the clones had a major drawback – they could only be maintained for a maximum of six months. (B. A. Askonas, A. R Williamson, B. E. G. Wright, 'Selection of a single antibody-forming cell clone and its propagation in syngeneic mice', *Proceedings US National Academy of Sciences,* 67/3 (1970), 1398-1403).

Brigette Askonas, credit: Cambridge University. Born in Vienna in 1923 to Czech parents, Askonas undertook a biochemistry degree at McGill University and then landed up as a post-graduate researcher at Cambridge University and the Basel Institute of Immunology. In 1952 she joined the National Institute of Medical Research where she remained until 1989. Her research was focused on understanding the process underlying the generation of B cells and antibody diversity. For more about Brigitte Askonas click here.

Splenic fragment technique for antibody production

As had happened with Sinkovics, the generation of antibodies to a specific antigen by the Askonas team would remain largely overlooked by the scientific community. More attention was paid to a technique published in 1969 by Norman Klinman, an American immunologist based at the University of Pennsylvania with an attachment to the Wistar Institute. His method required a number of steps. First a mouse would be treated with radiation to destroy its antibody-producing capability. It would then be injected with new antibody-producing cells, some of which lodged in its spleen. Once this was done the mouse's spleen was cut into cubes and individually grown as hybrid cells in tissue culture with an added antigen. If an antibody-producing cell was present within a given fragment, it would produce antibodies specific to that antigen. Called 'monofocal antibodies', these antibodies could then be isolated from the culture medium and used for experiments.

Klinman published his technique in R. Klinman, 'Antibody with Homogeneous Antigen Binding Produced by Splenic Foci in Organ Culture', *Immunochemistry*, 6/5 (1969), 757-9. The splenic fragment system marked a major milestone. Importantly, it showed that one cell produced only one antibody. The technique was soon adopted by a number of scientists. One of the first to do so was Walter Gerhard, a Swiss-trained physician, who had a post-doctoral research position in Klinman's laboratory in the early 1970s. By early 1975 Gerhard, by now based at the Wistar Institute, had successfully generated single antibodies with known specificity against influenza viruses.

The difficulty with Klinman's method, however, was that it produced only a minute quantity of antibodies. Moreover, the cells that produced the antibodies survived for a maximum of only three months.

This shows Norman Klinman, an American immunologist. Photo credit: Scripps Research Institute.

The creation of monoclonal antibodies

On pondering how to take their investigations into the somatic mutation of antibodies further, Milstein and Köhler started searching for a means to create an antibody with the desired specificity. Their starting point was Cotton's myeloma cell fusions and the experiments by Jerrold Schwaber and Edward Cohen at the University of Chicago. In 1973, these men had succeeded in producing a hybrid cell line able to secrete both myeloma and lymphocyte-derived antibodies by fusing human lymphocytes with mouse myeloma cells. These earlier techniques, however, had a major limitation. Crucially the antibody producing cells did not survive long and the antigens that the antibodies targeted remained unknown.

Milstein and Köhler wondered whether one way to resolve some of the difficulties encountered by previous researchers was to fuse a normal B cell from the spleen of a mouse immunised with a certain antigen, which itself produced an antibody with known specificity, with a mouse myeloma cell. By doing this they hoped to transfer to the antibody the trait of immortality from the myeloma cell. If it worked, they would have access to a hybrid cell, or hybridoma, not only capable of secreting antibodies with known specificity but also of surviving indefinitely.

Initially three different myeloma cell lines (labelled P3, 289 and P1) were chosen as the fusion partners for the spleen cells in the experiment. After a number of trials and errors, however, Köhler determined P3-X63-Ag8, the cell line used by Cotton, to be the most promising myeloma fusion partner.

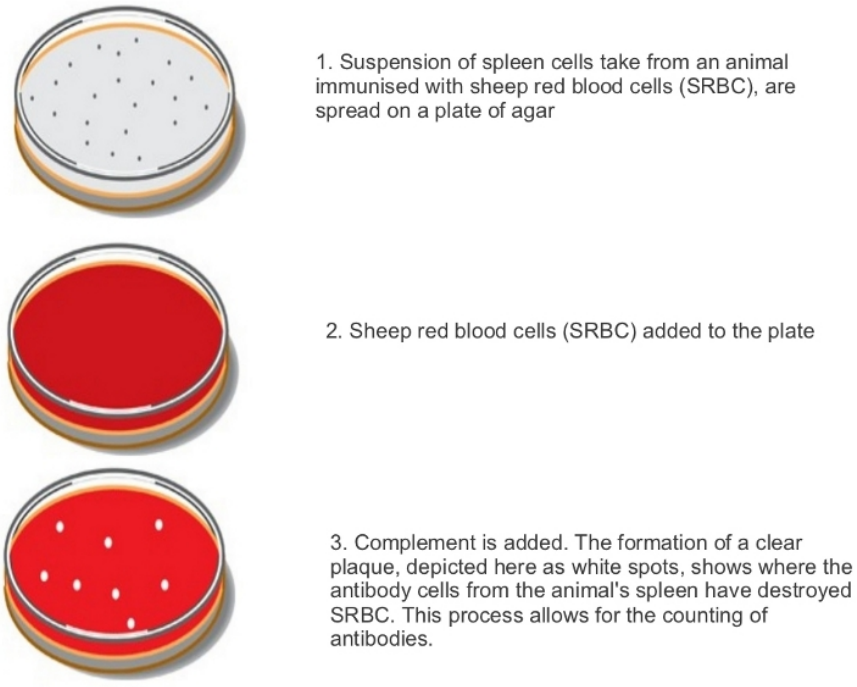
Köhler chose to grow the fused cells in a hypoxanthine-aminopterin-thymidine (HAT) medium. The medium had first been used for the fusion of tumour cells back in the 1960s. Like Cotton before him, Köhler also added inactivated Sendai Virus to the culture as this was known to disrupt the membranes of cells and thereby promote the fusion process.

The antigen Milstein and Köhler decided to target was sheep red blood cells (SBRC) because the mouse's immune system was known to react vigorously against them. Moreover, antibodies against such cells could be easily detected by a plaque essay test, a procedure developed in 1963 that had become common in laboratories by the mid-1970s.

Milstein and Köhler's fusion project was well under way by December 1974. It was undertaken with the skilled technical assistance of Shirley Howe. Towards the end of the month Köhler could see a number of cells growing in the medium, but he did not know if any had the desired specificity for the SRBC antigen. Before he could test the mixture with the plaque assay, however, Köhler spotted contamination of the mixture.

With the cells no longer viable, Köhler was forced to start the fusion process again. By the end of January 1975 he finally had some cells for testing and he decided to perform the first plaque assay test for the experiment on January 24 1975. This involved testing cells he had cultivated four days earlier through the fusion of X63 with the cells taken from a mouse immunised with SRBCs.

This diagram shows the steps involved in the plaque assay test.



Köhler started the plaque assay test at 5pm and returned home in the expectation that the process would take some hours before the results would materialise. A few hours later he returned to the laboratory bringing his wife as company to see what he anticipated to be boring results. Much to his amazement, Köhler spotted green halos on the two plates on which the two tests had been performed. He was so happy with what he saw that he shouted and kissed his wife! The test not only showed the hybrid cells were capable of secreting antibodies to SRBCs, but they produced large amounts. The frequency of antibodies was far greater than anything he or Milstein had ever predicted.

Following this success, Köhler and Milstein repeated their experiment twice more to check the technique was reproducible. When these experiments proved positive, the two scientists realised that they possessed a tool scientists had been striving to make for many years. Critically, they had created an immortal cell line capable of producing an endless supply of identical antibodies with known specificity. The

method would later be dubbed 'hybridoma technology' and the antibodies it produced 'monoclonal antibodies' to signify the fact that they were derived from a single hybrid cell.

In May 1975 the two scientists submitted a paper announcing their experiment to Nature, one of the most prestigious scientific journals in the world. In this paper they pointed to the fact that their technique could have major benefits for both medicine and industry. Yet the importance of their achievement was missed by the journal's editors, who asked for the article to be shortened and failed to include it in the section reserved for findings considered to be of leading significance. In the end the article was published in Nature in August 1975.

This shows the first photographs of hybridoma cells secreting antibodies grown in petri dishes as appeared as Figure 2 in G. Köhler and C. Milstein, 'Continuous Cultures of Fused Cells Secreting Antibody of Predefined Specificity', *Nature*, 256 (5517): 495-97. Photo credit: Nature.

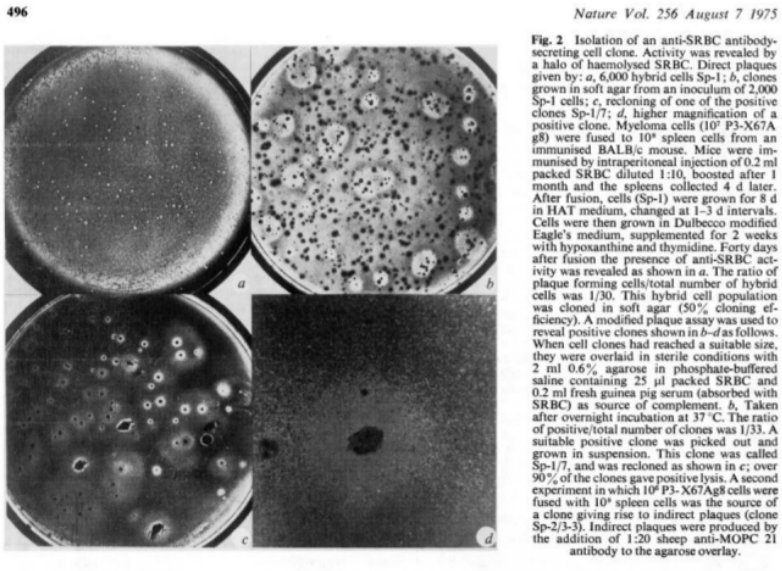


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Fig. 2 Isolation of an anti-SRBC antibody-secreting cell clone. Activity was revealed by a halo of haemolysed SRBC. Direct plaques given by: *a*, 6,000 hybrid cells Sp-1; *b*, clones grown in soft agar from an inoculum of 2,000 Sp-1 cells; *c*, recloning of one of the positive clones Sp-1/7; *d*, higher magnification of a positive clone. Myeloma cells (10^6 P3-X67A g8) were fused to 10^8 spleen cells from an immunised BALB/c mouse. Mice were immunised by intraperitoneal injection of 0.2 ml packed SRBC diluted 1:10, boosted after 1 month and the spleens collected 4 d later. After fusion, cells (Sp-1) were grown for 8 d in HAT medium, changed at 1–3 d intervals. Cells were then grown in Dulbecco modified Eagle's medium, supplemented for 2 weeks with hypoxanthine and thymidine. Forty days after fusion the presence of anti-SRBC activity was revealed as shown in *a*. The ratio of plaque forming cells/total number of hybrid cells was 1/30. This hybrid cell population was cloned in soft agar (50% cloning efficiency). A modified plaque assay was used to reveal positive clones shown in *b–d* as follows. When cell clones had reached a suitable size, they were overlaid in sterile conditions with 2 ml 0.6% agarose in phosphate-buffered saline containing 25 µl packed SRBC and 0.2 ml fresh guinea pig serum (absorbed with SRBC) as source of complement. *b*, Taken after overnight incubation at 37 °C. The ratio of positive/total number of clones was 1/33. A suitable positive clone was picked out and grown in suspension. This clone was called Sp-1/7, and was recloned as shown in *c*; over 90% of the clones gave positive lysis. A second experiment in which 10^6 P3-X67Ag8 cells were fused with 10^8 spleen cells was the source of a clone giving rise to indirect plaques (clone Sp-2/3-3). Indirect plaques were produced by the addition of 1:20 sheep anti-MOPC 21 antibody to the agarose overlay.

In later years there would be much speculation as to who originally conceived of the experiment to generate the first successful monoclonal antibodies. Köhler and Milstein responded in 1984 when they were jointly presented with the Albert Lasker Basic Medical Research Award, stating publicly: 'both the conception and execution of the work was the result of close collaboration between us with the skilled technical assistance of Shirley Howe'. Milstein would later emphasise that monoclonal antibodies had been made as a tool for answering a basic scientific question and not for any particular practical application.

A crisis in reproducibility

Click here to see documents relating to Köhler and Milstein's statements.

Just at the point that Köhler and Milstein's paper was accepted for publication by *Nature*, the two scientists faced a major crisis. Suddenly their technique stopped working and they were unable to achieve any fusions. This came as a total surprise as it had worked on seven previous occasions. Increasingly concerned as subsequent attempts failed, Milstein began to wonder whether in fact they should withdraw their paper from *Nature*. With the validity of their results threatened, the two scientists faced six stressful months trying to fathom what had gone wrong.

Click here to see Milstein's notebook recording some of the work undertaken at the time of the crisis.

Their anxiety was not helped by the very basic conditions in which they were performing their experiments. In his manuscript, entitled 'In the early days', Milstein's recalls:

'In the early days, the “tissue culture laboratory” was a room in the basement where the power switches of the laboratory were located. Effectively we only had one hood, and several feet of bench. The laboratory where we were working was so overcrowded, and I was so concerned about the bacteria which were being used all over the place that I preferred the situation, located four floors away from my own laboratory. It contained the electricity switch board so we were not allowed to have water services inside, or any other device which could mean flooding or splashing. It had no windows, the covering of the floor and painting of the walls was totally unsuitable. My only argument in its favour was a report I read somewhere saying that in a microbiological laboratory which had been suitably tested, the least contaminated area was found to be the lift shaft. How did my assistant Shirley Howe survive those conditions? I don't know. But I should point out that at one stage we became somewhat concerned about her health.' (Milstein's early manuscript for 'Messing about with Isotopes', presentation to Miami Winter Symposium, 1981, Churchill Archives Centre, Milstein Papers, file MSTN/D23).

Not only did Milstein and his team have to work in very basic and hazardous conditions, the technique for producing monoclonal antibodies required multiple steps and ingredients. While some of these procedures and ingredients could be carefully pretested and monitored, others could not.

The process for teasing out what had gone wrong was further hampered because by then Köhler had returned to the Basel Institute of Immunology. In the end, it would be Giovanni Galfré, a postdoctoral student who had recently joined Milstein, who identified the source of the problem – an incorrectly-prepared stock solution of HAT medium which was proving toxic to the cells.

Soon after identifying the problem with the culture medium, Galfré had to resolve another issue. This was the fact that the original preparations of Sendai virus were running out. He chose to resolve the shortage by using instead polyethylene glycol (PEG), a reagent that had been used successfully in other cell-cell fusions.

Click here to view a letter outlining the advantages Milstein believed monoclonals brought over conventional antibodies.

Following Galfré's changes, Milstein and Köhler's hybridoma technique began working again. In fact Galfré's introduction of PEG helped enhance the success of the method. Scientists now had a viable technique for generating immortal monoclonal antibodies on an unprecedented scale. Their arrival heralded a major advance over conventional polyclonal antibodies.

<< Milstein's early antibody research

The patent saga >>

what is
biotechnology?

A missed opportunity?

The patent saga

In July 1975 Milstein gave a presentation on monoclonal antibodies at an internal Medical Research Council (MRC) meeting convened to discuss the safety of genetic engineering. After his presentation, he was approached by Tony Vickers, a scientist by training and an administrative official of the MRC . Vickers was interested in learning more and requested the proofs of the article Milstein and Köhler were about to have published in *Nature*.

This shows the configuration of equipment that was set up for the continuous growth of myeloma cells which was important in the generation of monoclonal antibodies. To the right is the spinner culture which was drip fed continuously from the container on the left. This enabled the cells to be kept at an optimal density. The cells could be removed as required. Overall the set-up was optimized so as to avoid contamination and to allow long-term cultures to accumulate mutants. The myeloma X63/Ag8 cell clone was isolated from a 3-month-old culture. In some cases some cultures were grown for up to 1 year. Photo credit: John Jarvis.

Struck by the commercial possibilities for hybridoma technology, Vickers quickly looked to patent the technique and immediately alerted the National Research Development Corporation (NRDC), the body responsible for patenting MRC inventions, to see if they could file a patent. Action needed to be swift because British patent law does not allow for the disclosure of any work such as publication of an article prior to filing an application for a patent.

While Vickers was quick off the mark in his attempt to get a patent for Köhler and Milstein's technique, intervening before the *Nature* article went into print, it was to take the NRDC several months and some prompting by the MRC before any action was taken. The response came in the form of a letter shown here, written in October 1976. By this time the opportunity for patenting the technique in Britain had been lost as by now the method had been published in *Nature*. The NRDC staff made it clear that while they recognised the possible medical and commercial value of hybridoma technology, they were unable to 'identify any immediate applications' so had taken no action to file for patent.

Political storm

Click here to see the correspondence between Milstein, Vickers and the NRDC.

The failure of the NRDC to patent Köhler and Milstein's technique was to become the subject of much controversy in the late 1970s, a time of economic and political anxiety in the wake of the decline in Britain's manufacturing sector, and consequent rising unemployment. One of the most vehement critics of the failure to patent the technique for monoclonal antibodies was Margaret Thatcher, a chemist by training, who was elected Prime Minister in 1979.

This photograph shows Margaret Thatcher, the British prime minister during a conversation with Max Perutz, a Nobel Prize winner and founder and Chairman of the LMB. The photograph was taken during a private visit to the LMB on August 27 1980. Thatcher's husband, Denis is standing in the background. Photo credit: MRC Laboratory of Molecular Biology.

Thatcher's criticism became much more damaging as a result of the publication of a report in 1980 by a joint working party then investigating the commercialisation of biotechnology in Britain. The report pinned much of the blame for the failure to patent on the shoulders of the scientists themselves. The paragraph in the report that incensed Milstein read as follows:

'There appears to be a lack of awareness in practice of the obligations on recipients of government money and of the rights of the NRDC. This must be remedied. We are concerned that a lack of appreciation of the NRDC, particularly by young scientists, may continue to result in situations such as that which occurred over monoclonal antibodies where patent protection was not sought early enough and British advantage was reduced.'

Click here to see Milstein's views about the patent affair that he expressed to Thatcher.

The Wistar Patents

The anger over the non-patenting of Köhler and Milstein's technique was reinforced by the granting of two patents to Hilary Koprowski, Carlo Croce and Walter Gerhard for the making of monoclonal antibodies against tumours and influenza virus antigens in October 1979 and April 1980. These were the first ever patents awarded for the making of a monoclonal antibody.

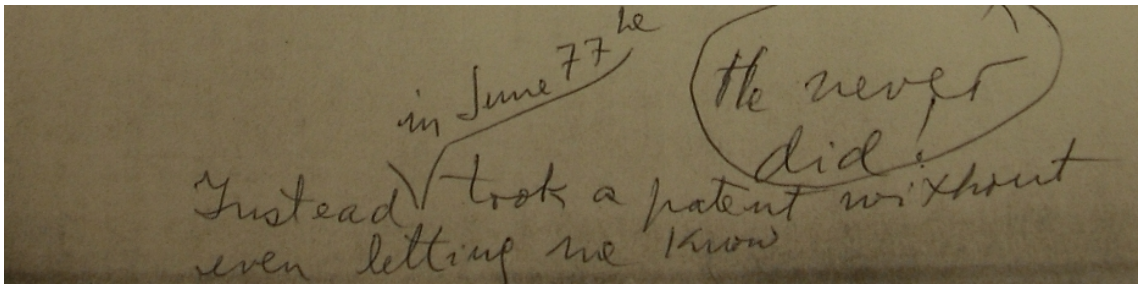
What was particularly galling about the Wistar patents for monoclonal antibodies was the fact that they had been created using the X63 myeloma cell line originally supplied by Milstein to Koprowski back in September 1976. Making broad claims, the Wistar patents provoked major controversy in Britain and in the international scientific community. Many regarded the Wistar patents as patenting what many saw as effectively Milstein and Köhler's technique. Questions were accordingly asked about why Milstein and Kohler's technique had not been patented, leading to a major shake-up of British government policy on patenting and the commercialisation of biotechnology.

Milstein's supply of the cell line to Koprowski was in keeping with the tradition among many scientists at the time. The only conditions Milstein imposed on recipients was that they acknowledge in publications where they had sourced the cells and that they seek permission from him if passing them on to other scientists. He also made it clear that recipients should not patent any products from the cells. This stipulation, however, was difficult to enforce as demonstrated in the case of Koprowski.

Click here to see the first Wistar patent and Milstein record of sending of samples to Koprowski.

At the heart of the debate over the British failure to patent monoclonal antibodies was the extent to which Koprowski had ignored Milstein's request that no products be patented from samples sent by Milstein to other scientists.

Some of Milstein's frustration over the affair can be seen from a note he scribbled on a letter from Koprowski in November 1976, requesting his permission to pass on Milstein's cell line to another colleague, Norman Klinman. This was written next to a comment by Koprowski stating, 'Dr Carlo M Croce's and my studies with our line progress satisfactorily and if we get any positive results in producing monoclonal antiviral antibodies we will let you know immediately.' To which Milstein retorted, 'He never did! Instead in June 77 he took a patent without even letting me know.'



This shows the words Milstein pencilled on the letter written to him by Koprowski in November 1976. He wrote them some time later when trying to work out what had happened in the patent affair. Source: Churchill Archives Centre, Churchill College, Milstein's Papers, file MSTN/C324.

In 1980 Nicholas Wade, a science journalist, published an article in the widely disseminated journal *Science*, capturing some of the sentiment around the patenting of monoclonal antibodies. He wrote, 'Milstein did not apply for a patent on his technique. He gave away his plasmacytoma cells in the usual scientific tradition of free exchange, asking only that recipients should not patent any hybridomas made from the cells and that they should not pass them on to third parties.'

Wade quoted Milstein as stating, 'We were too green and inexperienced on the matter of patents', indicating that in the past the British Medical Research Council encouraged its scientists in the past to make methods freely available. As Milstein put it 'We were influenced by that psychology. We were mainly concerned with the scientific aspects and not giving particular thought to the commercial applications.'

Wade went on to argue: 'The Wistar Institute seems in a sense to have jumped into the gap which Milstein and Köhler left. A broad patent for monoclonal antibodies raised against tumour cells was granted on 23 October 1979 to Hilary Koprowski and Carlo Croce. A similar broad patent covering the antibodies to viral antigens was issued on 1 April 1980 to Koprowski, Croce, and Walter Gerhard.' Wade also cited Milstein's unhappiness over the breadth of the Wistar patent application, for, as he put it, 'they are essentially patenting our procedure'.

Overall, Wade's article raised concerns about the extent to which Koprowski had broken an agreement with Milstein not to patent any product based on the cell line he had supplied. Angered by the implication that they had stolen Milstein's

procedure, Koprowski and Croce responded in a letter to *Science* denying they had contravened an agreement.

The difficulty Milstein had in countering Koprowski and Croce's argument was because he could not find any letters in his files to verify whether Koprowski had explicitly signed a letter consenting to the general conditions he placed on all recipients of his cells not to patent any products.

Nonetheless, other letters between the two scientists indicate that Milstein was upfront that this was a condition in sending his cells. For example, a letter written by Milstein to Koprowski in May 1977 concerning the transfer of his cell line to another colleague states very clearly that products arising from such cells should 'not be made the subject of any patent rights'. Given that Koprowski received this letter a month before the Wistar Institute applied for a patent for their monoclonal antibodies this is particularly striking. As Milstein poignantly wrote later in pencil on this letter, 'Yet Koprowski took up a patent in June 1977!'

Milstein only became aware of the Wistar patent after receiving a letter written by Eric Tridgell from the NRDC in March 1979. The news came as a total surprise. What he found particularly shocking, as he wrote in response to Tridgell's communuication, was the fact that the Wistar application represented,'a blanket for deriving any clone directed against any virus and using myeloma cells from any origin.'

This shows Milstein with a flask containing monoclonal antibodies growing in a fluid. Photo credit: MRC, Laboratory of Molecular Biology.

Click here to see various correspondence Milstein had with Koprowski and others concerning the patenting of the technique.

Patents and the sharing of cells

In many ways the problems that occurred with Koprowski reflects the casual arrangements that were in place for sharing cell samples in these years. A more formal procedure was only put into place in 1978 at the request of the NRDC when they began seeking a patent for Milstein's development of a new line of monoclonal antibodies derived from rats. From this time on recipients were expected to sign a form waiving the right to patent before the cells were actually sent. As one MRC official admitted to Milstein, however, the difficulty was how far this could be enforced.

Like many scientists at the time, Milstein viewed patents as slightly distasteful, a matter best kept separate from scientific discovery and invention and sorted out by lawyers. Moreover, Milstein believed he had done his duty by having sent a preprint of his *Nature* article when asked to do so by Vickers in July 1975. Some years later when asked whether he was unhappy not to have patented his and Köhler's technique Milstein was heard to reply, 'I was not unhappy, Margaret Thatcher was'.

Looking back in his later life, Milstein would come to see the NRDC's failure to patent as in some ways a blessing. Crucially, it allowed him greater freedom to publish and get on with his research. Had the NRDC moved forward on a patent, he might have been forced to become more secretive about his work. Moreover, in not patenting the cells he and Köhler had created, many scientists were able to move much faster in working out their application.

In many ways Milstein's attitude was reflective of the more general dislike of commercialisation within the Laboratory of Molecular Biology in the 1970s. The situation was reinforced by the fact that scientists working within British government-funded biomedical laboratories were not entitled to any royalties accruing from patenting their innovations at the time. This sentiment only really began to change in the 1980s when the MRC established a new scheme allowing for the sharing of royalties with inventors.

While stimulating intense political debate in the late 1970s and early 1980s, some doubt the degree to which the missed patent for Köhler and Milstein's technique contributed to any real loss in the long-term. They argue that there would have been only minimal financial gain from a patent for the type of monoclonal antibody that the two scientists created because it had limited applications and required considerable refinement before it could be clinically applied.

Click here to see some of Milstein's sentiments on patents.

For both Milstein's recollections and those of his contemporaries of the patent saga see E.M. Tansey et al., eds,Technology Transfer in Britain: The Case of Monoclonal Antibodies *Wellcome Witnesses to Twentieth Century Medicine,* vol 1 (London, 1997

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<< The making of monoclonal antibodies From Milstein's laboratory to the world >>

what is biotechnology?

Into the wider world

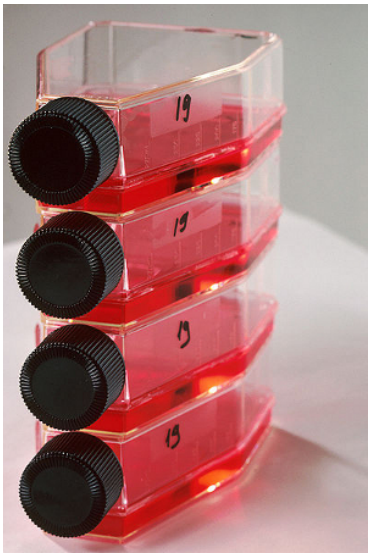
The journey of the monoclonal antibodies from Milstein's laboratory

Few could imagine how fast monoclonal antibodies, which were developed originally as a tool to answer a basic research question, would travel from Milstein's laboratory around the globe. Within a matter of months of their first development, Milstein was receiving requests from scientists worldwide for access to the cells. Yet the transfer of the monoclonal antibodies was not a smooth process and recipients initially found the technique difficult to apply.

Milstein sent cells in different forms. Some scientists were given myeloma cells or serum containing antibodies taken from immunised mice. Others received the whole hybrid cell (hybridomas) secreting monoclonal antibodies. Alternatively a sample might be given from the medium in which the antibody producing cells were growing, known as 'supernatant', from which the cells could be isolated.

Of all the samples provided, hybridomas were the easiest to send as they were being grown all the time in cultures in flasks. Their survival however, was dependent on quick transportation in cold conditions which did not go below freezing; this was difficult to ensure particularly if flasks were placed in the baggage hold of an aeroplane where temperatures were regularly freezing. Other samples needed slightly more preparation, requiring first to be taken out of liquid nitrogen and placed on dry ice. Nonetheless, these samples were slightly easier to transport as they were packed in polystyrene containers that could be placed in the baggage holds.

In the early days Milstein recorded the sending of his monoclonal antibody samples in a notebook he kept informally. Each sample was assigned a letter and number according to the type of cell line being sent. Those with the letters 'SP' referred to cloned hybrid cells which had been made from the fusion of a myeloma cancer cell with antibodies taken from the spleen of an immunised mouse. The original hybrid cell was labelled 'SP1' and all subsequent cells were given a new number as they were further generated. Samples labelled with 'X63' referred to the myeloma cell line used to generate monoclonal antibodies in Milstein's laboratory. Samples referred to as 'NSI' were hybrid cells developed later by Milstein with his colleagues, and 'PEG' was the added reagent used to encourage the growth of the monoclonal antibodies.



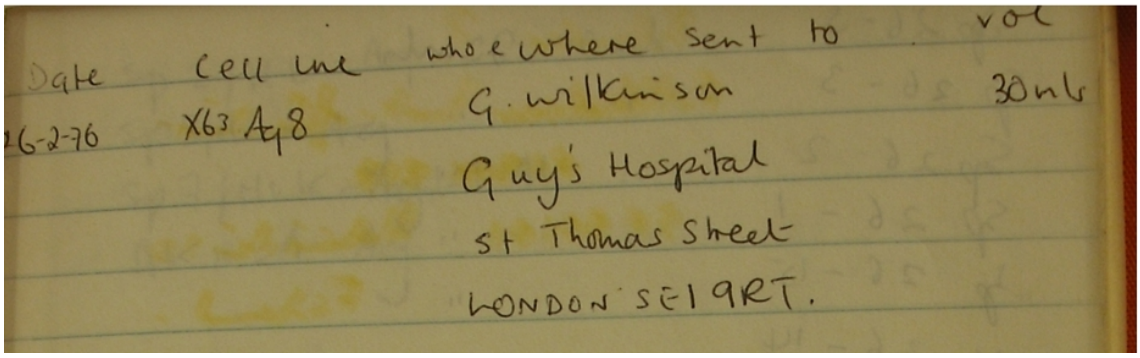
This shows vials containing tissue culture, a reagent used in the production of monoclonal antibodies. Photo credit: National Cancer Institute, Wikipedia.

Click here to see some pages from Milstein's notebook recording his early sending of various cell lines.

The replication struggle

One of the first researchers to whom Milstein sent a sample of some myeloma cells was G. Wilkinson, a junior pathologist at Guy's Hospital, London. These were to be used as a fusion partner for the generation of the hybrid cells to produce monoclonal antibodies. Wilkinson had learnt about the technology from Matthew Scharff, a scientist with whom Milstein had close relations but whom he regarded as a competitor.

Yet, while Wilkinson reported success in establishing the myeloma cell line in his laboratory, he was unable to replicate Köhler and Milstein's technique to produce monoclonal antibodies. This was despite numerous attempts over nine months.



This shows the first X63Ag8 myeloma cell line Milstein recorded sending. It was the myeloma fusion partner used for creating the first hybridoma capable of secreting antibodies. The recipient was Wilkinson, a junior pathology researcher based at Guy's Hospital, London. Source: Milstein's notebook, Churchill Archives Centre, Milstein Papers, file MSTN/C.282.

The correspondence between Wilkinson and Milstein reveals how hard it can be to replicate a technique before it becomes a standardised process. The letters reveal that even Milstein and his team experienced difficulties in replicating the method. Milstein's papers also show that others had similar difficulties.

When Wilkinson continued to experience problems, he was advised to visit Milstein's laboratory personally in March 1977 to learn the technique. The difficulties Wilkinson and others faced are evidence of the complexity of transferring knowledge and practice from one laboratory to another. Their experiences show how hands-on experience in the laboratory is not easily replaced by the supply of a set of ingredients and written instructions.

Even after Milstein and his team had made improvements to the process and cell lines, other scientists struggled to produce monoclonal antibodies. This led some to question the viability of the technique and its value for their work. Many of the difficulties encountered, however, reflected the more general complexities associated with the tissue culture, which is plagued by problems such as contamination and the difficulties of stabilising cells. This suggests the fact that tissue culturing may be as much an art as a science.

Click here to see correspondence between Milstein and Wilkinson and others concerning the difficulties they had reproducing Milstein and Köhler's technique.

The requests begin

The number of samples that Milstein sent out remained small in the first year. In 1976 different variations of the cell samples were only sent to 8 different individuals, most of whom had some connection with Milstein or his close colleagues. One person Milstein noted as recipient of the cells in December 1977 was Brigitte Askonas who in the 1970s had been among some of the first scientists to find a way of making monoclonal antibodies. These antibodies however only survived a short time.

In the first half of 1977, Milstein sent out relatively few cells. From September that year, however, the number began to increase. In that month alone he sent samples to seven people. By November 1977, Milstein estimated that he had sent out his myeloma cell line for making monoclonal antibodies to a total of 50 people.

The rise in requests for samples in part reflected the growing interest in monoclonal antibodies awakened by the article Milstein, Galfré and other colleagues had published in Nature the previous April. In it, they reported on the successful development of monoclonal antibodies against rat histocompatibility antigens and this was of considerable clinical interest. Histocompatibility antigens are important as they can be used to establish the unique identity of an organism's tissues and thus to determine compatibility between a donor and a recipient in the process of organ transplants. The importance of this paper can be gauged from the fact that it had been cited in more than 1,490 publications by 1993.

A valuable asset

Click here to see extracts from the articles by Milstein and Galfré and by Welsh in *Nature*

By generating monoclonal antibodies against histocompatibility antigens, Milstein and his team laid the foundation for the use of monoclonal antibodies in many other clinical applications. Their paper generated great excitement. An article in *Nature* publicised the significance of its research. It was written by Ken Welsh, an immunologist then based at East Grinstead Hospital in Sussex, UK, whose inspiration for penning the piece was in part stimulated by his fury at learning of the NRDC refusal to seek a patent for the technology. Welsh believed the technique provided a 'major step forward', particularly for tissue typing which was critical in the care of transplant patients.

Welsh was not alone in publicising the potential advantages of the new technique. An editorial in *The Lancet* in June 1977 also claimed it could 'have profound implications for medical practice.' In addition to its possible uses in transplants, the editorial pointed out its potential for improving diagnostics and therapeutics.

The growing enthusiasm for monoclonal antibodies in the scientific world was further reinforced by a paper published by Milstein with his colleagues in November 1977. This was A. F. Williams, G. Galfré, C. Milstein, 'Analysis of Cell Surfaces by Xenogeneic Myeloma-Hybrid Antibodies Differentiation Antigens of Rat Lymphocytes', Cell 12 (Nov 1977), 663-73. It highlighted how monoclonal antibodies could be used to identify and distinguish between different markers found on the surface of cells. By 1993 it had been cited 660 times. Milstein himself later admitted that his research in this area marked a turning point in his thinking about the potential application of monoclonal antibodies.

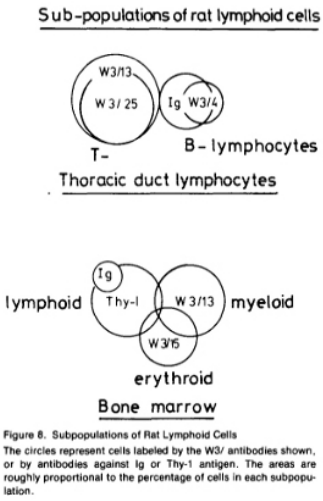


Figure 8. Subpopulations of Rat Lymphoid Cells
The circles represent cells labeled by the W3/ antibodies shown, or by antibodies against Ig or Thy-1 antigen. The areas are roughly proportional to the percentage of cells in each subpopulation.

This shows the different cell surface markers monoclonal antibodies helped to differentiate. It was published as figure 8 in A.F. Williams, G. Galfré and C. Milstein, 'Analysis of cell surfaces by xenogeneic myeloma-hybrid antibodies: Differentiation antigens of rat lymphocytes', *Cell*, 12/3 (1 Nov 1977), 663-73.

Once scientists realised the significance of monoclonal antibodies to their work, Milstein received a flood of requests for cell samples. Those recorded in his 1977 notebook were just a few of the many vials that were now being dispatched from the Laboratory of Molecular Biology.

The demands of distribution

Click here for an illustration of the kinds problems that scientists reported when receiving cells from Milstein's laboratory.

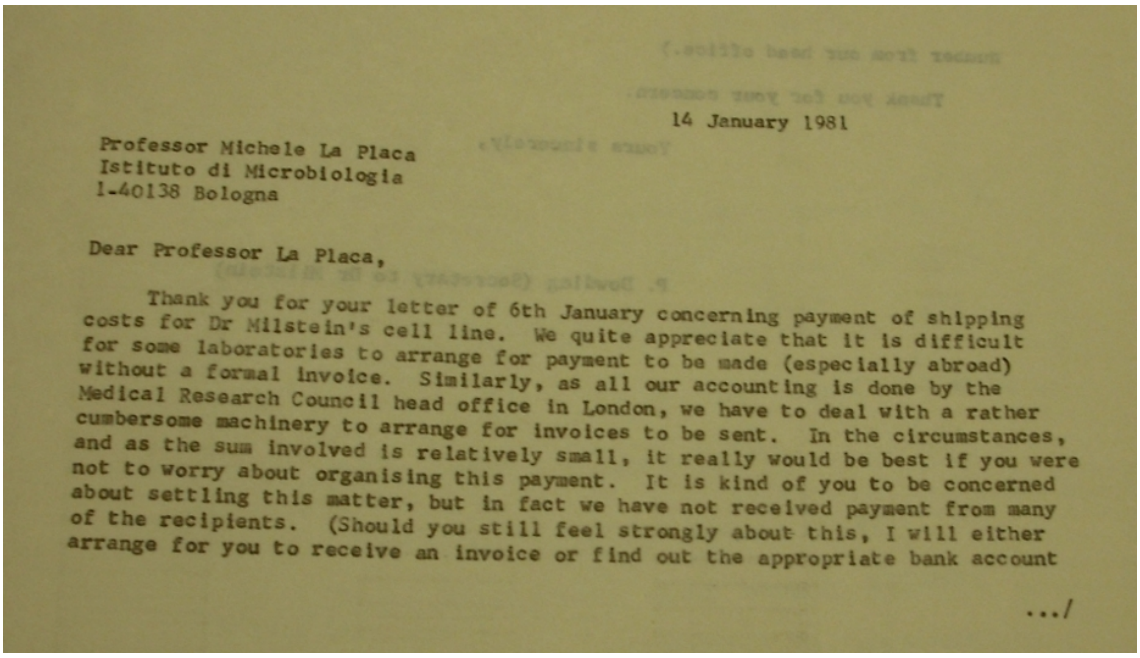
The way in which cells were distributed varied greatly. Some were collected in person by individual researchers in a cell suspension carried in a test tube in the pockets of individual researchers. Others were sent in parcels through postal services, on trains or aeroplanes. Inevitably the logistics of transportation were not straightforward. Not only were there strikes to contend with, on occasion military coups disrupted transport networks. Less dramatically, transport could break down or customs officials could hold up the process. Any delay or change in environmental conditions could jeopardise the survival of the cells that were dispatched.

The growing requests for cells therefore imposed significant demands on Milstein and his staff, requiring a high degree of organisation. Not only did they have to grow the cells and then package and label them, but there was also the headache of transport which could involve numerous telexes and phone calls to transport carriers for pick up and delivery. Each batch of cells dispatched also had to be followed up with letters to ensure their safe arrival.

On top of all of this, international transactions required permits to take packages through customs. All of this could take time and sometimes caused the death of the cells. Even sending samples through the post could be difficult.

Click here to see the conditions Milstein's laboratory laid down to recipients of cells.

In providing the cells, Milstein's laboratory asked recipients to cover the costs of their transport. This was frequently difficult to arrange. One of the problems was the bureaucracy involved in issuing invoices. In the early years the laboratory was willing to overlook non-payments for shipping. By 1983, however, the financial burden of the work involved in sending cells had become so great that recipients were asked on receiving their samples to send a cheque to cover costs. Two years later the rules were changed so that recipients were expected to send cheques prior to dispatch of the cells. And once the patenting of Milstein's cells became more systematised the process for sending out cells became much more bureaucratic.



14 January 1981

Professor Michele La Placa
Istituto di Microbiologia
I-40138 Bologna

Dear Professor La Placa,

Thank you for your letter of 6th January concerning payment of shipping costs for Dr Milstein's cell line. We quite appreciate that it is difficult for some laboratories to arrange for payment to be made (especially abroad) without a formal invoice. Similarly, as all our accounting is done by the Medical Research Council head office in London, we have to deal with a rather cumbersome machinery to arrange for invoices to be sent. In the circumstances, and as the sum involved is relatively small, it really would be best if you were not to worry about organising this payment. It is kind of you to be concerned about settling this matter, but in fact we have not received payment from many of the recipients. (Should you still feel strongly about this, I will either arrange for you to receive an invoice or find out the appropriate bank account

.../

This letter from Milstein's secretary, Dowding, to a scientist in Italy, makes clear it was frequently difficult to arrange payment to cover the costs of transferring cells from Milstein's laboratory. Source: Churchill Archives Centre, Milstein Papers, file MSTN/C303.

<< The patent saga

The early commercialisation of monoclonal antibodies >>

what is biotechnology?

Monoclonal antibodies become a marketable product

The first commercialisation of monoclonal antibodies

With the number of requests for cell lines flooding into Milstein's laboratory, Milstein and his team soon realised they could not handle the distribution of the cell alone. Help would come from an unexpected source: David Murray, founder of Sera-Lab, a company producing and marketing antisera as a research reagent for scientists.

Click here to see Murray's diary and his initial correspondence with Milstein.

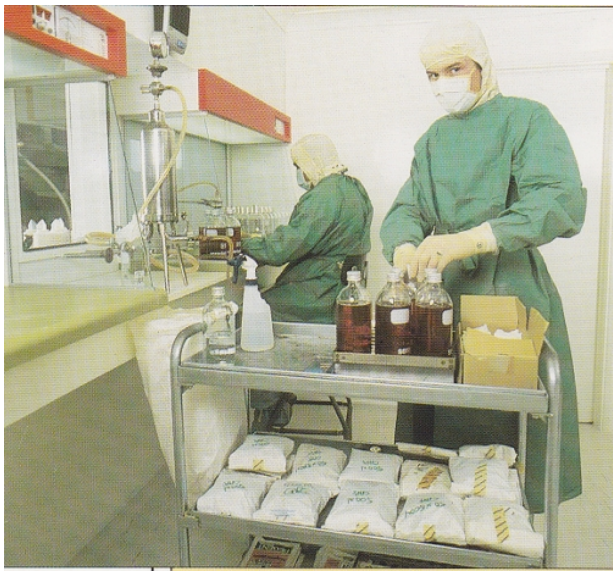
Murray had first been alerted to Milstein's work by a meeting he had with the immunologist Ken Welsh, who was based at Queen Victoria Hospital in East Grinstead, not far away from Crawley Downs in Sussex where Sera-Lab was located. Murray already had an association with Welsh's department, being a regular supplier of rabbit antiserum for research purposes to Richard Batchelor, Welsh's superior. He also had strong links with the hospital's chief technical officer, Ron Chambers, who helped him found his company Sera-Lab in 1971.

One of Murray's first meetings with Welsh was in January 1977 shortly after the latter learnt of the NRDC's decision not to pursue a patent for Köhler and Milstein's technique for producing monoclonal antibodies. At that time Welsh was in the midst of a series of negotiations with a number of company executives to see if they could help and he urged Murray to look at the article published by Köhlerand Milstein in *Nature* in August 1975. Excited by the prospect monoclonal antibodies promised for his own business, Murray immediately wrote to Milstein to find out whether they could collaborate.

This shows David Murray in 1978 when he was awarded best business award on Thames Television award. Photo credit: Jenny Murray. Click here to see David Murray's profile.

In February 1977, shortly after posting his letter, Murray encountered Milstein in person by chance on one his sales rounds for his company's foetal calf serum, a critical reagent for tissue culture. Knocking on the door of what, unbeknown to him, was Milstein's office, Murray, not recognising Milstein, launched into a sales pitch for the serum. He was quickly interrupted, however, by Milstein, who withdrew Murray's letter from under his pile of papers. Murray recalled that it was only at that moment that he realised whom he was speaking to. The discussion soon shifted to the headache Milstein was experiencing in meeting the flood of requests for his cell lines. On hearing of Milstein's frustrations, he quickly realised his company could probably help with their distribution.

According to Milstein, although he had been aware of the commercial potential of monoclonal antibodies, prior to this meeting with Murray he given little thought to the practicality of such an endeavour. What Murray offered him was an arrangement whereby Sera-Lab would distribute the monoclonal antibodies for Milstein, at an agreed cost and would return a proportion of the Sera-Laboratory's profit on the antibodies to Milstein's laboratory. Attracted by Murray's



This shows staff at Sera-Lab bottling up foetal calf serum. The photo appeared in Sera-Lab's catalogue, 1986. Credit: Jenny Murray.

proposal, Milstein immediately got in touch with the Medical Research Council, which agreed to the proposal.

For Milstein his meeting with Murray was, as he put it, 'the starting point at which I started to think more seriously about the [commercialisation of monoclonal antibodies]. In my innocence, I thought if we could do that at the level I was talking about, a minor antibody being distributed by a commercial firm, we might do it on a larger scale, with a future, more important monoclonal antibody.' (Milstein's draft manuscript entitled, 'Messing about with isotopes', p.30, Churchill College Archives, Milstein Papers, file MSTN/D23).

Monoclonal antibodies: A business opportunity

Murray believed that monoclonal antibodies could slot into the already well-established market for antisera, that is blood serum containing antibodies. The antibodies contained in such sera were an important tool in the diagnosis of many diseases. They had been used for diagnostic tests since the late 19th century, based on observations that they could disintegrate (lyse), separate (precipitate) or clump together (agglutinate) bacteria in a solution. The reaction between an antigen and an antibody also had predictable biochemical reactions. Within this context the antibodies in antisera could be used as diagnostic probes to define, isolate and measure a wide variety of immunological molecules.

The first antiserum-based test was the Widal Test, introduced for diagnosing typhoid in 1896. This was to be followed by the Landsteiner Test for blood grouping (1900-1) and the Wassermann Test for detecting syphilis (1906). Over the years many other tests followed. One of the most important was the Coombs anti-globulin test developed in 1945, widely used for detecting antibodies that can cause premature red blood cell destruction. The Coombs test is particularly important in blood transfusions and detecting Rhesus (Rh) incompatibility between the blood of mother and foetus. By the 1960s the use of antisera for diagnostics had become widespread in clinics and hospitals, aided in part by the emergence of electrophoresis and labelling techniques using firstly enzymes and then radioisotopes.

Some idea of how large the market for antisera was can be seen from the fact that Murray believed there to be around 20 major international companies competing in the space and that many hospitals had specific budgets set aside for the purchase of antisera. This, he pointed out, provided a ready market for monoclonal antibodies.

This photograph shows Sera-Lab's headquarters. The photo appeared in Sera-Lab's catalogue, 1986. Credit: Jenny Murray.

According to Murray, monoclonal antibodies offered exciting prospects for routine hospital diagnostics. He argued they offered the chance for more accurate and more replicable results. This is particularly striking because at this stage Milstein and his colleagues were only just

beginning to investigate the practical utility of monoclonal antibodies for diagnostic applications. Nor was everyone at this time convinced that monoclonal antibodies provided any particular advantage over conventional antibodies as diagnostic reagents.

Click here to see the business proposition Murray sent to the MRC headquarters for monoclonal antibodies.

For Murray's own serum production business, however, Milstein and Köhler's technique not only opened up the possibility of producing standardised identical antibodies with known specificity, it also promised to cut down on the labour intensity of the production process. This, in turn, would reduce costs and increase potential profits.

A further important advantage Murray ascribed to the new method was the possible reduction in the number of animals needed in the production of antisera. Before Milstein and Köhler developed their technology, animal-based antiserum could only be made in small quantities, so that large numbers of animals had to be immunised for production, and each batch of antiserum was dependent on the life-time of a particular animal. Every time an animal died, other animals had to be tested for another source of antiserum. All of this was time-consuming as well as laborious.

A reciprocal relationship

Click here to see the correspondence relating to Sera-Lab's launch of monoclonal antibodies, the contract it established with the MRC and a list of the types of cells it marketed.

The contract between Sera-Lab and the MRC took time to be finalised. First drafted in November 1977, it was only finalised in October 1978. By this time Sera-Lab was already well under way, with the commercialisation of Milstein's first cell lines. As early as February 1978, the company included Milstein's myeloma cell line in its marketing catalogue, alongside the company's other antiserum products. This represented the first commercialisation of cell lines for the production of monoclonal antibodies. The number and type of samples from Milstein distributed by Sera-Lab increased over time. Murray also moved quickly to source monoclonal antibodies from other scientists for distribution.

Taken as a whole, the relationship between Sera-Lab and Milstein was to their mutual advantage. Milstein's laboratory helped Sera-Lab set up its facilities for the supply of monoclonal antibodies. In return Sera-Lab sponsored a research scientist to work at the Laboratory of Molecular Biology in Cambridge.

Click here to see some correspondence sent to Sera-Lab outlining how to maintain monoclonal antibodies.

In addition, Sera-Lab's distribution of the cell lines helped shift some of the burden of distribution Milstein and his team had experienced in the early years. Nonetheless, he was cautious about which cells he was willing to release. He was, for example, reluctant to give Sera-Lab cell lines with significant commercial potential, like those that would be useful for the purification of interferon.

Despite this, however, within a short space of time the company had become the focal point of call for researchers. Robert Tindle, the head of research at Sera-Lab from 1984, recalls that the company essentially acted as a clearing house, like a newsagent where a customer can buy any newspaper. As early as April 1979, Sera-Lab was receiving requests daily for the monoclonal antibodies made by Milstein and his colleagues. Sera-Lab's catalogue for 1979 listed 22 antibodies, most of them originating from MRC researchers.

Click here for Milstein's thoughts on the most appropriate means for distributing his cells.

By 1983 the Sera-Lab catalogue listed 68 monoclonal antibodies and many other reagents for making monoclonal antibodies. Three years later, Sera-Lab's catalogue list had grown to 221 monoclonal antibodies. At that time the company had one of the most extensive collections of monoclonal antibodies then in existence. Researchers could buy such monoclonal antibodies either in purified form, or as ascites or supernatant. Production conformed to the US Federal Drug Administration good manufacturing practice standards where monoclonal antibodies were supplied in large volume.

Competitors enter the space

Despite its being at the forefront of commercialising monoclonal antibodies, Sera-Lab was largely overlooked by the British government and policy-makers eager to make up for the lost opportunity of patenting the technology. Its existence was ignored, for instance, in the publicity surrounding the establishment of Celltech, a British biotechnology company founded to exploit British innovations such as monoclonal antibodies. Unlike Sera-Lab, which had been built up on a boot-strap basis, Celltech was founded with government backing and £12 million venture capital.

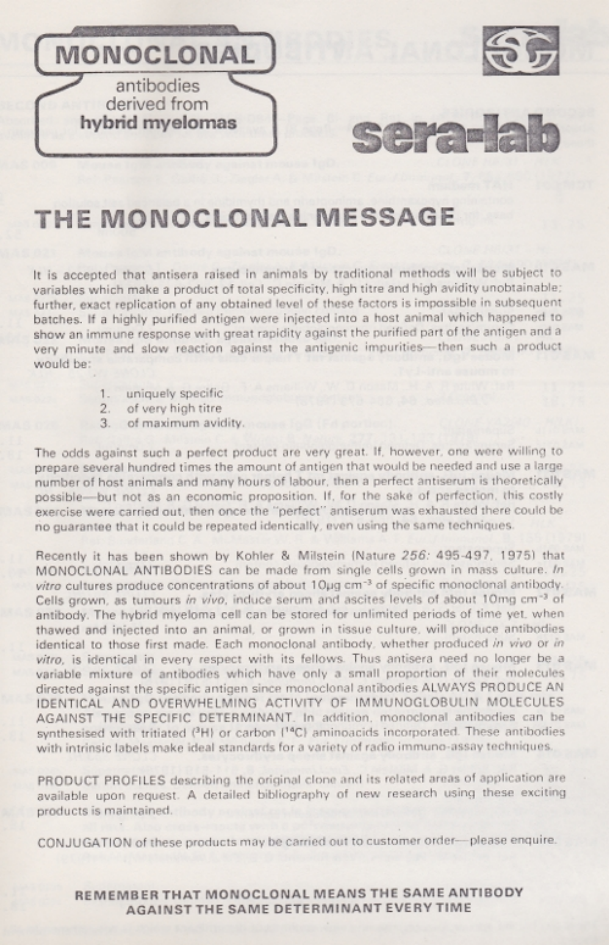
Click here to see Murray's overview of the role Sera-Lab played in the early commercialisation of monoclonal antibodies.

Murray was highly critical of the way his company's pioneering role in the commercialisation of monoclonal antibodies went unrecognised. From his perspective, the company had laid an important foundation for the industrial development of the technology, which the company promoted not only as reagents for laboratory use, but also for the development of diagnostics. He expressed his feelings in a letter that he sent to Milstein in July 1980 and copied to Sir Keith Joseph, the Conservative Minister, then involved in trying to protect British interests in biotechnology. In it, he pointed out that Sera-Lab was a British company, exploiting a British invention, and therefore deserved government support. Its failure to do so was particularly striking given the government's concern over the failure of the National Research Development Corporation (NRDC) to patent to the technique, which it blamed for the slowness of British companies to commercialise biotechnology.

Sera-Lab was one of several companies that Milstein and the MRC enaged to distribute the cell lines from the Laboratory of Molecular Biology. One of the companies that they also worked with was Flow Laboratories in Scotland, a supplier of cells, media and sera. It was a subsidiary of Flow General Inc an American company founded by Michael Wall, who would later help establish Centocor in 1979, one of the first American companies to commercialise monoclonal antibodies for diagnostics and therapeutics.

Over time, commercial distributors replaced Milstein's laboratory as the main source of cell lines for the making of monoclonal antibodies. This inevitably entailed new financial arrangements. In the early days Milstein's laboratory had been largely happy to provide cell lines provided transport costs were paid, but this was to change. With patents at stake and monoclonal antibodies now commercially valuable, new procedures had to be put in place.

Click here to see correspondence concerning other distributors and the implications this had for scientists in terms of access and payment.



MONOCLONAL
antibodies
derived from
hybrid myelomas

sera-lab

THE MONOCLONAL MESSAGE

It is accepted that antisera raised in animals by traditional methods will be subject to variables which make a product of total specificity, high titre and high avidity unobtainable; further, exact replication of any obtained level of these factors is impossible in subsequent batches. If a highly purified antigen were injected into a host animal which happened to show an immune response with great rapidity against the purified part of the antigen and a very minute and slow reaction against the antigenic impurities—then such a product would be:

1. uniquely specific
2. of very high titre
3. of maximum avidity.

The odds against such a perfect product are very great. If, however, one were willing to prepare several hundred times the amount of antigen that would be needed and use a large number of host animals and many hours of labour, then a perfect antiserum is theoretically possible—but not as an economic proposition. If, for the sake of perfection, this costly exercise were carried out, then once the "perfect" antiserum was exhausted there could be no guarantee that it could be repeated identically, even using the same techniques.

Recently it has been shown by Kohler & Milstein (Nature *256:* 495-497, 1975) that MONOCLONAL ANTIBODIES can be made from single cells grown in mass culture. *In vitro* cultures produce concentrations of about 10μg cm^{-3} of specific monoclonal antibody. Cells grown, as tumours *in vivo*, induce serum and ascites levels of about 10mg cm^{-3} of antibody. The hybrid myeloma cell can be stored for unlimited periods of time yet, when thawed and injected into an animal, or grown in tissue culture, will produce antibodies identical to those first made. Each monoclonal antibody, whether produced *in vivo* or *in vitro*, is identical in every respect with its fellows. Thus antisera need no longer be a variable mixture of antibodies which have only a small proportion of their molecules directed against the specific antigen since monoclonal antibodies ALWAYS PRODUCE AN IDENTICAL AND OVERWHELMING ACTIVITY OF IMMUNOGLOBULIN MOLECULES AGAINST THE SPECIFIC DETERMINANT. In addition, monoclonal antibodies can be synthesised with tritiated (^3H) or carbon (^{14}C) aminoacids incorporated. These antibodies with intrinsic labels make ideal standards for a variety of radio immuno-assay techniques.

PRODUCT PROFILES describing the original clone and its related areas of application are available upon request. A detailed bibliography of new research using these exciting products is maintained.

CONJUGATION of these products may be carried out to customer order—please enquire.

REMEMBER THAT MONOCLONAL MEANS THE SAME ANTIBODY AGAINST THE SAME DETERMINANT EVERY TIME

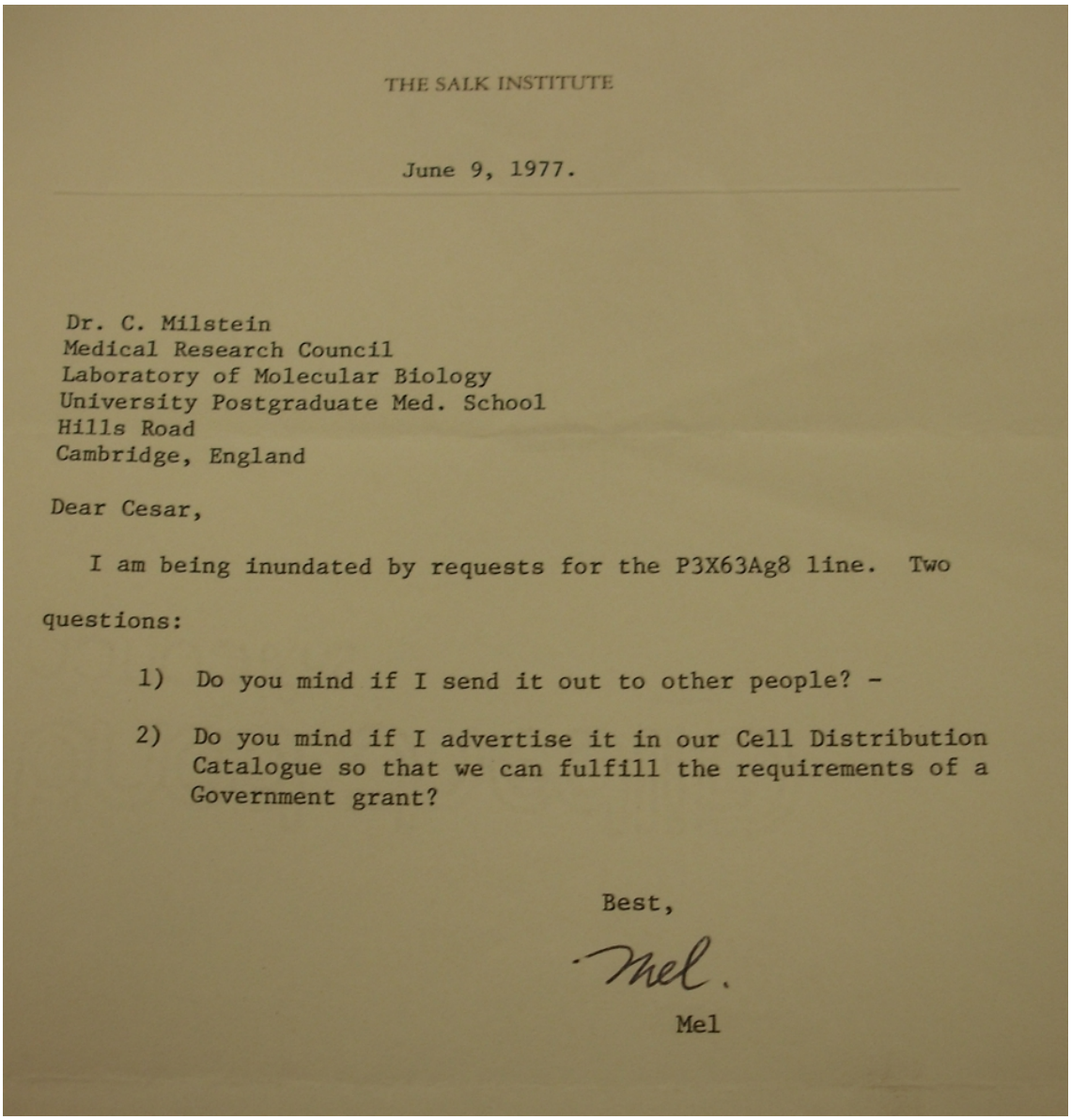
This shows a page from Sera-Lab's catalogue, 1981, advertising the advantages of monoclonal antibodies.
Credit: Jenny Murray.

Companies expected to be rewarded from the process of distribution, and royalties had to be paid on the cell lines that were now being patented. This inevitably made the transactions more commercial. Researchers were increasingly discouraged from obtaining their cells directly from Milstein's laboratory or from passing cells between themselves. One of the reasons for the shift was that any exchange between scientists reduced revenue for the companies supplying the cell line, thereby decreasing any royalties that could be passed on to the MRC.

Milstein not only distributed his cell lines through commercial channels. He also partnered with Melvin Cohn, an immunologist based at the Salk Institute in San Diego, to supply his cell lines through a cell bank Cohn had set up in the early 1970s to serve as a repository for murine immune-related culture and tumour cell lines, many of which originated from Michael Potter at the National Institutes of Health. It had funding from the National Cancer Institute.

Cohn first received the P3-X63Ag8 myeloma cell line from Milstein in January 1977. P3-X63Ag8 was in fact a derivative of one of the myeloma cell lines supplied from Cohn's Cell Bank and was the fusion partner Köhler and Milstein had used to produce the hybrid cell that secreted the first successful monoclonal antibodies.

By June 1977 Cohn reported that he was so inundated with requests for the P3-X63Ag8 myeloma cell that he wished to advertise it in the catalogue of the Salk Institute Cell Bank. For Milstein and his staff, Cohn's offer to distribute the cell line was very helpful. It meant that they could now refer scientists based in America to Cohn's laboratory, thereby circumventing the need to negotiate the international shipping of the cells and bypassing the need for customs clearance. By 1979 the catalogue had seven hybridomas listed. Over the following years, the Salk Institute would become a major repository for many different monoclonal antibody cell lines.



THE SALK INSTITUTE

June 9, 1977.

Dr. C. Milstein
Medical Research Council
Laboratory of Molecular Biology
University Postgraduate Med. School
Hills Road
Cambridge, England

Dear Cesar,

I am being inundated by requests for the P3X63Ag8 line. Two questions:

1) Do you mind if I send it out to other people? -

2) Do you mind if I advertise it in our Cell Distribution Catalogue so that we can fulfill the requirements of a Government grant?

Best,

Mel.

Mel

This letter from Melvin Cohn based at the Salk Institute in San Diego requesting permission to advertise Milstein's P3-X63Ag8 myeloma cell line in his cell bank's catalogue indicates how popular the cell line was becoming by June 1977 among researchers. Churchill College Archives, Milstein Papers, file MSTN/C285.

<< From Milstein's laboratory to the world

The first clinical applications of monoclonal antibodies >>

Sponsored by the Medical Research Council as part of its Centenary Programme.

Supported by the Department of Social Science, Health & Medicine, King's College, London

what is biotechnology?

Harnessing the value of monoclonal antibodies

Using monoclonal antibodies for clinical applications

Early on, Milstein recognised that the hybridoma technology he and Köhler had devised might have useful applications, but that he would have to demonstrate what it could do before it would be adopted. Temporarily, he had to drop his basic scientific ivestigation into antibody diversity. The key challenge he faced was proving the value of monoclonal antibodies over conventional polyclonal antibodies for different applications. This would not be straightforward and would require the help of others.

This photograph shows Milstein with Claudio Cuello in earnest discussion during one of the many walks they took together. Milstein and Cuello established a long lasting collaboration and friendship while they were both scientific MRC staff in Cambridge.

One of Milstein's first collaborators in demonstrating the practical applications of monoclonal antibodies was Claudio Cuello, a fellow British-Argentinian neuroscientist who was then attached to the MRC's Neurochemical Pharmacology Unit in Cambridge. Their collaboration continued even after Cuello moved to the Departments of Pharmacology and Human Anatomy at Oxford University in 1978.

A probe for neuroscientific investigation

Milstein and Cuello started thinking through how they might work together soon after Köhler and Milstein published 'Continuous cultures of fused cells secreting antibody', *Nature*, 256 (7 August 1975) 485-7. At the time, Cuello was wrestling with the difficulty of characterising Substance P (SP), known from the 1930s to be involved in pain and neurotransmission. His objective in this research was to determine whether SP was a peptide transmitter and to map its pathway within the body so that he could unravel the relationship between SP and disease.

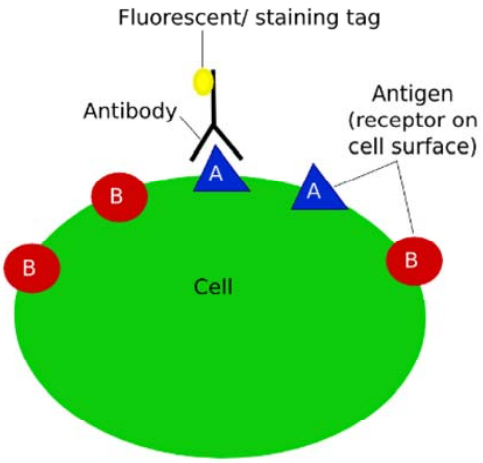
When Cuello began his collaboration with Milstein, he and his team had already gone some way to characterise and visualise SP. Yet this work had been laborious and time-consuming and their method was unreliable. The technique they were using for the research was immunohistochemistry, an immuno-based test, which by then was a commonplace procedure. The method exploits the binding mechanism between antibodies and specific receptors on the surface of cells to enable the analysis and identification of different cell types. In this context, antibodies are tagged with a label, such as a fluorescent dye, to serve as markers for locating receptors on the surface of a cell. This allows scientists to measure and track particular cells in biological samples such as tissue or organs.

The major advantage of the immuno-based test was that it could be done in conjunction with an electron microscope. However, the technique had a major drawback in the early 1970s: it was reliant on the use of

Click here to see Milstein's discussion of the difficulties

antibodies sourced from animal serum. Known as polyclonal antibodies, such antibodies are difficult to work with. They are heterogeneous and vary from preparation to preparation, which means they require extensive purification before use. Not only are polyclonal antibodies hard to standardise, they have a limited shelf-life. Such characteristics made it difficult for scientists in the early 1970s to reproduce their own results, and compare them with those of other scientists running similar immuno-based tests.

with early immuno-based tests.



This image illustrates the binding of a tagged monoclonal antibody with an antigen receptor on a cell surface, which is the mechanism exploited in immuno-based tests.

What Cuello and his team were using for their immuno-based tests to characterise SP were antibodies taken from guinea pig serum. This, however, posed certain challenges. Firstly, the antibodies taken from such serum were limited in supply. Secondly, they often cross-reacted with other substances, as they were not specific to SP.

On chatting to Cuello in 1975, Milstein quickly realised that the technique for raising monoclonal antibodies presented a potential breakthrough for the difficulties Cuello was then facing. Crucially, it offered the possibility of generating unlimited standardised antibodies which specifically targeted SP. By helping Cuello create a monoclonal antibody specifically for SP, Milstein not only hoped to aid Cuello in his work, but also to satisfy his wider agenda, which was to demonstrate the practical utility of monoclonal antibodies.

When Milstein and Cuello started their experiment to develop a monoclonal antibody specific to SP, many were sceptical about the utility of monoclonal antibodies over polyclonal antibodies. By 1979, however, the two scientists had succeeded in generating a monoclonal antibody specific to SP that proved a highly effective tool for characterising the neurotransmitter peptide.

Milstein and Cuello's collaboration on SP represented the first of many applications of monoclonal antibodies in neuroscience. Within a short time, Cuello and Milstein, together with other colleagues, were using the hybridoma technology to develop monoclonal antibodies for the detection and mapping of other neural proteins, including serotonin and enkephalin.

With their monoclonal antibodies, Milstein and Cuello had opened up a new world in neuroscientific dissection. This would help lay the foundation for better understandings of diseases like Alzheimer's and Parkinson's providing the pathway to improving neuropharmacological drugs.

A new tool for immuno-based diagnostic tests

If monoclonal antibodies could be generated to target specific neuropeptide transmitters, Milstein realised monoclonal antibodies raised against other targets could be used in a whole range of immuno-based tests. This, he believed, would help encourage the adoption of monoclonal antibodies into what had already become a routine procedure for diagnosis in the 1970s in fields such as parasitology, virology and cancer. Monoclonal antibodies offered such immuno-based diagnostics an unlimited standardised reagent which could be used in thousands of tests with the same reliability and reproducibility.

Click here for letters between Cuello and Milstein and others about monoclonal antibodies for immuno-based tests and examples of immuno-based tests.

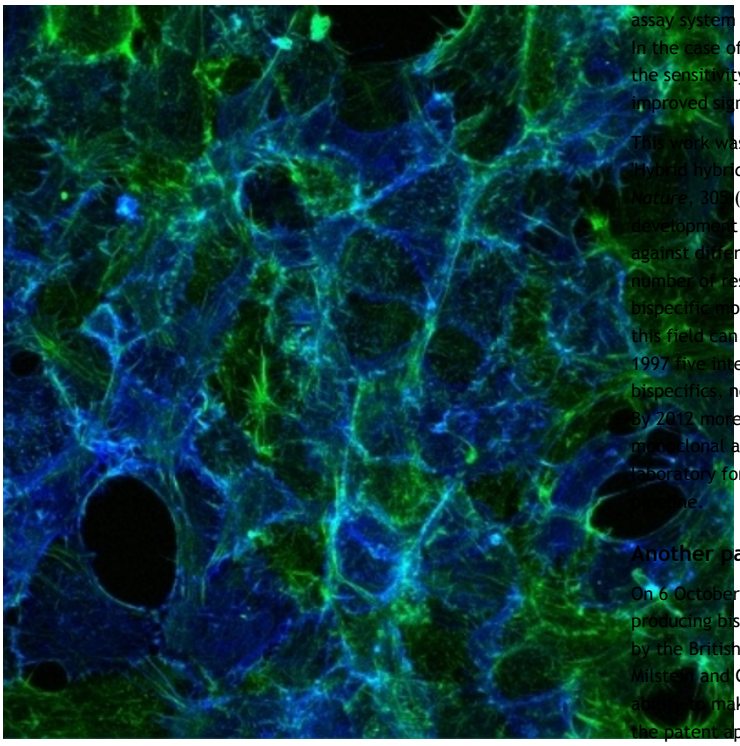
In addition to extending the boundaries of neuroscientific investigation, Milstein and Cuello's efforts helped improve immuno-based tests overall. Significantly, their work inspired the revival of many techniques which had been neglected because sources of polyclonal antibodies were inadequate.

One of Milstein and Cuello's key contributions was to help cut down the number of procedural steps involved in the making of immuno-based tests. This they achieved by demonstrating the possibility of conjugating monoclonal antibodies with enzymes and radioisotopes to act as tags in the immuno-based test.

This photograph shows cells with serotonin transporter proteins stained in green. The image was achieved with a monoclonal antibody based immunoassay. Photo credit: Wellcome Images, B0004135.

Bispecific antibodies

The power of immuno-based tests was further enhanced by Milstein and Cuello's development of bispecific antibodies. This combined two antibodies that recognised two different antigen epitopes, that is the portion of the antigen that binds to an antibody. The bispecific antibody was advantageous because it provided a tool that bound two different antigens simultaneously. This enabled the development of a single-step



assay system as it eliminated the need for a second antibody. In the case of immunohistochemistry it also helped improve the sensitivity of the test, simplified staining procedures and improved signal detection.

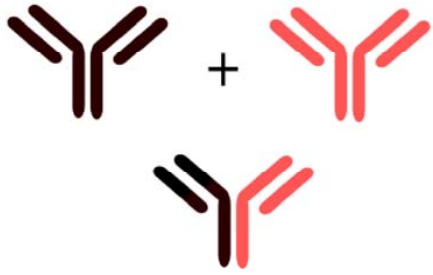
This work was published in C.M. Milstein and A.C. Cuello, 'Hybrid hybridomas and their use in immunohistochemistry', *Nature*, 305 (1983), 537-40. The paper inspired the rapid development of various bispecific monoclonal antibodies against different targets. Within a short space of time, a wide number of researchers had become involved in raising bispecific monoclonal antibodies. Some idea of the upsurge in this field can be seen from the fact that between 1989 and 1997 five international conferences were convened to debate bispecifics, not only for diagnostics but also for therapeutics. By 2012 more than 40 different formats of bispecific monoclonal antibodies had been designed and produced in the laboratory for clinical investigation and more were in the pipeline.

Another patent opportunity missed

On 6 October 1983 Milstein and Cuello's technique for producing bispecific antibodies was filed for patent protection by the British biotechnology company Celltech. Although Milstein and Cuello had been the first to demonstrate the ability to make bi-specific antibodies, to their great surprise the patent application was later abandoned by Celltech and the MRC. The application was dropped on the grounds that a patent had already been filed by the University of Texas. Although this application had been filed on the basis of a theoretical proposition rather its practical demonstration, in legal terms, it implied that Milstein and Cuello had only put into practice an experiment predicted sometime before. This undermined any possibility of success with the patent application.

Click here to see Milstein and Cuello's patent application for bispecific antibodies and Celltech's letter announcing its abandonment of the application.

The abandonment of the patent for Milstein and Cuello's technique is particularly striking given that today bispecific antibodies are growing in popularity for therapeutics. It could be argued that, yet again, an important patent opportunity was missed. Some researchers doubt, however, that any substantial revenue could have been earned from any bispecific monoclonal antibodies produced from hybrid hybridomas during the life-time of such a patent.



This diagram illustrates the process for making a bispecific antibody.

A means for sorting and identifying cell subtypes

Soon after starting his work with Cuello, Milstein began another collaboration that would help demonstrate the utility of monoclonal antibodies in another field. His partner in this endeavour was Leonard Herzenberg, an American from Stanford University Medical School, who arrived in Milstein's laboratory in 1976 for a brief sabbatical.

During his visit Milstein and Herzenberg began working together on the development of monoclonal antibodies to improve the utility of a fluorescence-activated cell sorter (FACS). This was a machine that Herzenberg and some engineering colleagues had devised in the early 1970s to automate the separation of cells. It was designed to help characterise, isolate and count different subsets of cells, notably white blood cells, that co-exist in blood and various organs.

This shows the American immunologist and geneticist Leonard Herzenberg together with his wife Leonore Herzenberg, a fellow geneticist and immunologist. Photo credit: Stanford Medical School.

By the time Herzenberg arrived in Cambridge, his FACS could sort 5,000 live, functional cells per second, and it was being marketed by the medical device company Becton-Dickinson. The FACS, however, still had some teething problems.

One of the key challenges was that the FACS, like other immuno-based methods at that time, relied on polyclonal antibodies. These antibodies, conjugated with fluorescent tags, were integral to the sorting mechanism in the instrument. Such antibodies targeted receptors on cells to facilitate the picking up and sorting of such cells with the use of a laser detecting fluorescence. The difficulty was that these antibodies were not standardised and were in limited supply. Moreover, they had a tendency to cross-react. Conjugating these antibodies with staining agents was also laborious and slow.

On arriving in Milstein's laboratory, Herzenberg immediately grasped the utility of monoclonal antibodies for improving the performance of the FACS. To this end he partnered with Milstein to develop monoclonal antibodies for use in the FACS machine. Much of the research was undertaken with the help of Herzenberg's wife, Lenore, and Vernon Oi, a graduate student from Stanford University.

Click here to see FACS in operation.

By 1977 Milstein and the Stanford scientists had generated the first monoclonal antibodies in mice capable of use in the FACS. Designed to screen determinants on the surface of many cells on a large scale, these monoclonal antibodies helped improve the reliability of FACS and boosted their popularity for many different purposes.

Today the FACS is a ubiquitous tool within the laboratory. In biological research, for example, the instrument is used to investigate cellular functions, measure metabolic processes in cells and work out how viruses infect cells. On the medical front, it is used for routine clinical tests involving the counting of white blood cells, as is needed in diseases like AIDS, and for the assessment of the efficacy of chemotherapeutic treatment for diseases like leukaemia.

Exploring the unknown terrain of the surface of the cell

In addition to helping to develop monoclonal antibodies to enhance the sorting and counting of different cell types, Milstein became immersed in another project that would open up a new understanding of the surface of the cell. His work in this area was triggered by a chance conversation he had at a conference at Coldspring Harbour (USA) in June 1976 with Alan Williams, an Australian immunochemist based at Oxford University.

Prior to meeting Milstein, Williams had spent many years attempting to purify antigens and antibodies in order to develop a probe that would distinguish between different white blood cells (lymphocytes) and to work out their functions. Such efforts were also directed towards identifying the cell surface molecules involved in the mediation of lymphocyte function. This he saw as a means to unravelling the biochemistry behind immunological reactions.

By the mid-1970s, Williams and his team had made a number of advances. Specifically they had purified an antigen known as Thy-1 that proved an effective tool for locating T-lymphocytes in mice. They had also identified a leucocyte-common antigen using antibodies taken from the serum of rabbits immunised against rat lymphocytes. Overall, however, they found it difficult to secure antibodies with the right strength and specificity for their target. Williams believed the technique developed by Milstein and Köhler might resolve such problems.

Soon after meeting in Coldspring Harbour, Milstein and Williams set up a collaborative project. The first monoclonal antibodies they chose to produce targeted a rat's thymus. Their choice of the thymus was deliberate as it is known to play an instrumental role in the immune system. Notably the thymus produces T-lymphocytes (T cells) which identify and attack foreign substances.

This shows Alan F Williams, Honorary Director from 1977 of the MRC Cellular Immunology Research Unit based at the Dunn School of Pathology, Oxford University. Photo credit: Royal Society.

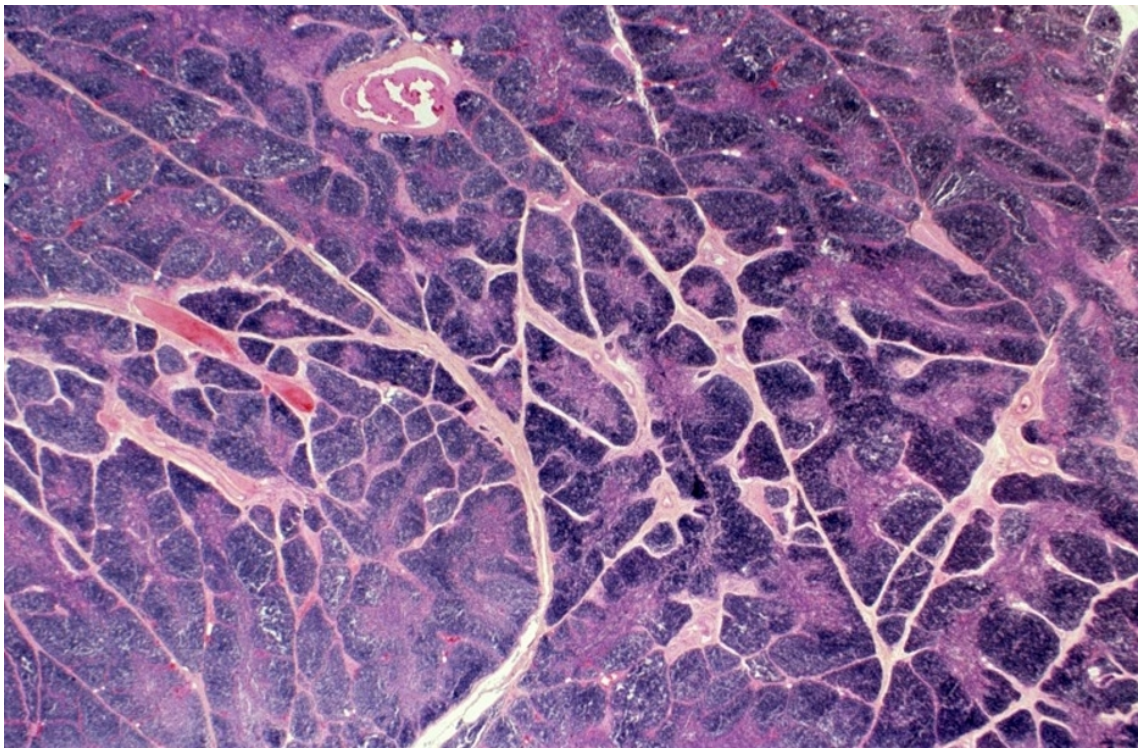
Milstein and his team took responsibility for the tissue culturing involved in the project, while Williams's team in Oxford oversaw the serological work. The work was logistically difficult because 85 miles separated Milstein's laboratory from that of Williams. Writing about the work later, Milstein recalled:

'The basic protocol of the collaboration was as follows. We produced the cultures and clones and sent the supernatants by urgent mail. As soon as he got them, Alan proceeded to do the binding assays, and usually within 24 hours he would phone (no faxes in those days) the results (perhaps 100 assays per round) for us to proceed with clonal selection. Speed turned out to be essential. We discovered that contaminating clones or chain-loss mutant all too often conspired against us. Indeed, literally hundreds and hundreds of failed attempts taught us a lot about the technical problems involved and how to tackle them.'(C. Milstein, 'The Hybridoma Revolution: An offshoot of basic research', *BioEssays,* 21/11 (1999), 966-73.

Within months of having immunised mice with cell membranes taken from a rat thymus, the two groups of scientists in Cambridge and Oxford had succeeded in generating mouse-based antibodies to rat T cells. The experiment yielded three monoclonal antibodies for distinct subsets of T-cells in rats. One of the most promising monoclonal antibodies was code named 'W3/25'. This later turned out to be the rat equivalent of a human antigen labelled CD4, which in the mid 1980s was identified as the receptor for the AIDS virus.

Click here to see pages from laboratory notebooks from Williams and Milstein relating to the development of a monoclonal antibody against a rat's thymus.

When Milstein and Williams began their collaboration, it could be argued that scientists had as much knowledge about the surface of immune cells as they did of the surface of moon. What was most exciting about their experiments was that they had shown it was possible to produce monoclonal antibodies to unknown cell surface antigens, and that such antibodies could be deployed to investigate and understand the function of such antigens on cell surfaces.



This shows a segment of tissue taken from a normal thymus in a child. The thymus is a pink-grey glandular organ at the base of the neck that helps to generate lymphocytes. Photo credit: Wellcome Images, W0047313.

Results from the Cambridge-Oxford collaboration were published in A.F. Williams, G. Galfré, C. Milstein, 'Analysis of cell surfaces by Xenogenic myeloma-hybrid antibodies: Differentiation antigens of rat lymphocytes', Cell 12 (1977), 663-73. In this article the scientists suggested that monoclonal antibodies might be developed in the future not only for investigating immune cells, but 'for the analysis of cell surface molecules of any sort'.

Having generated monoclonal antibodies to rat T-cells, Milstein began to investigate whether the process could be replicated for making such antibodies to antigens found on human cell surfaces. His partner in this work was Andrew McMichael, an immunologist based in Oxford who had an interest in human T-cell immune responses to viruses. Started in the spring of 1977, Milstein and McMichael's project was an extension of the research undertaken by Milstein with Willliams. What Milstein and McMichael were interested in developing was a monoclonal antibody against human T-cells. This they believed could help further knowledge about the immune response in human health and disease.

This shows Andrew McMichael, senior fellow in clinical science, Nuffield Department of Medicine 1977-79. Photo credit: Andrew McMichael.

McMichael and his team in Oxford were given the task of immunising mice with human thymocytes (precursors to T cells that develop in the thymus) to generate antibodies against human leucocytes. The antibodies were then sent through the post for fusion with myeloma cells in Milstein's laboratory. Once fused, the cells were then returned by post to McMichael's laboratory in Oxford, where they were analysed.

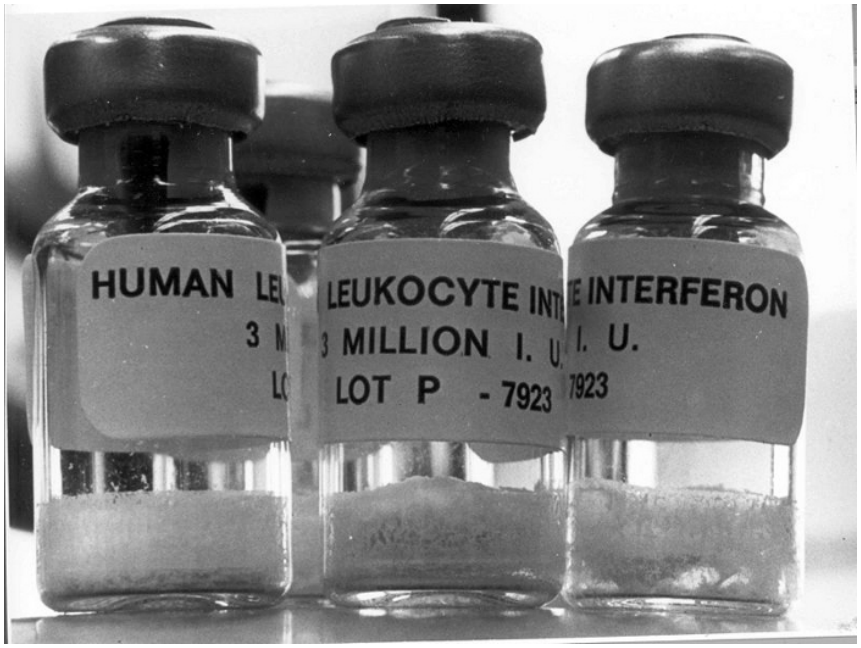
The work was not easy. Initially, the scientists battled to perfect a good fusion with the cells. Having finally achieved a good fusion, the project nearly collapsed when the cellular culture became contaminated with fungus. By chance the team had earlier stored some cells in the freezer. These cells helped rescue the project.

By late 1978, Milstein and McMichael's teams had produced a monoclonal antibody that was highly specific for human thymocytes. This represented the first discovery of a human leucocyte differentiation antigen using hybridoma technology.

Despite their achievement, Milstein and McMichael initially struggled to find a journal that would publish their findings. Notably editors of the *Journal of Experimental Medicine* rejected their paper without seeking referee's comments. This they did on the grounds such work was of 'little scientific interest'. Overall the editors believed the paper did not describe anything particularly different from what was then being done with polyclonal antibodies methods. In part, the editors' attitude reflected a more general lack of appreciation of the significance of differentiation antigens. It also stemmed the failure in the early years to grasp what hybridoma technology could achieve and from the fact that many scientists were still struggling to establish stable and viable monoclonal antibodies.

Milstein and McMichael's research was finally published as A.J. McMichael, J.R. Pitch, J.W. Fabre, David Y. Mason, G. Galfré, 'A human thymocyte antigen defined by a hybrid myeloma monoclonal antibody', European Journal of Immunology, 9/3 (March 1979), 205-10. The article would go on to become a citation classic.

A means to purify interferon



Vials containing human interferon. Photo credit: National Institutes of Health, Wikipedia.

In addition to exploring the use of monoclonal antibodies for probing the surface of cells, from 1976 Milstein began to wonder about their potential to purify natural products. He began working on this together with David Secher, who had been one of Milstein's doctoral students and was now his postdoctoral researcher.

Curious to establish their utility for purifying natural products, Milstein and Secher established a collaboration with Derek Burke, a biologist at the University of Warwick who had a long-standing interest in interferon, a group of natural proteins released by an organism's cells in response to pathogens such as bacteria, viruses or parasites. The objective of the partnership was to develop a monoclonal antibody that might be used to purify human leukocyte interferon.

Since the late 1950s, researchers had deployed several methods for purifying interferon, but most could not yield the several thousand fold purification for turning interferon into a pure and clinically-useful product. One of the most promising techniques for purification was immuno-adsorbent chromatography. The method allowed for the isolation and selection of the protein based on the binding properties between antibodies and antigens. However, the technique had a downside: it depended on the use of conventional polyclonal antibodies. What made matters worse was the fact that scientists only had access to impure interferon. Most antibodies available were therefore made

by immunising animals with impure interferon. This meant that most antibodies available for the immuno-adsorbent chromatography targeted contaminants in interferon preparations rather than the interferon itself.

Encouraged by Milstein from the sidelines, Secher and Burke next launched a project to produce a monoclonal antibody that could improve the immuno-adsorbent chromatographic method for the purification of interferon. The work was painstaking and there were many false starts. Just before Christmas in 1979, Secher and Burke were so disillusioned with their progress they considered abandoning their research altogether. By March 1980, however, they had created a system that yielded interferon that was about 100 times purer that anything managed before.

Patented, Secher and Burke's method for purifying interferon was just one of the many stepping stones on the way to the commercialisation of different genetically-engineered versions of interferon as a drug in the mid-1990s. It would be approved for marketing as a drug to control multiple sclerosis, and as a treatment for cancer as well as various infectious diseases, including hepatitis B and C.

A reagent for blood-typing

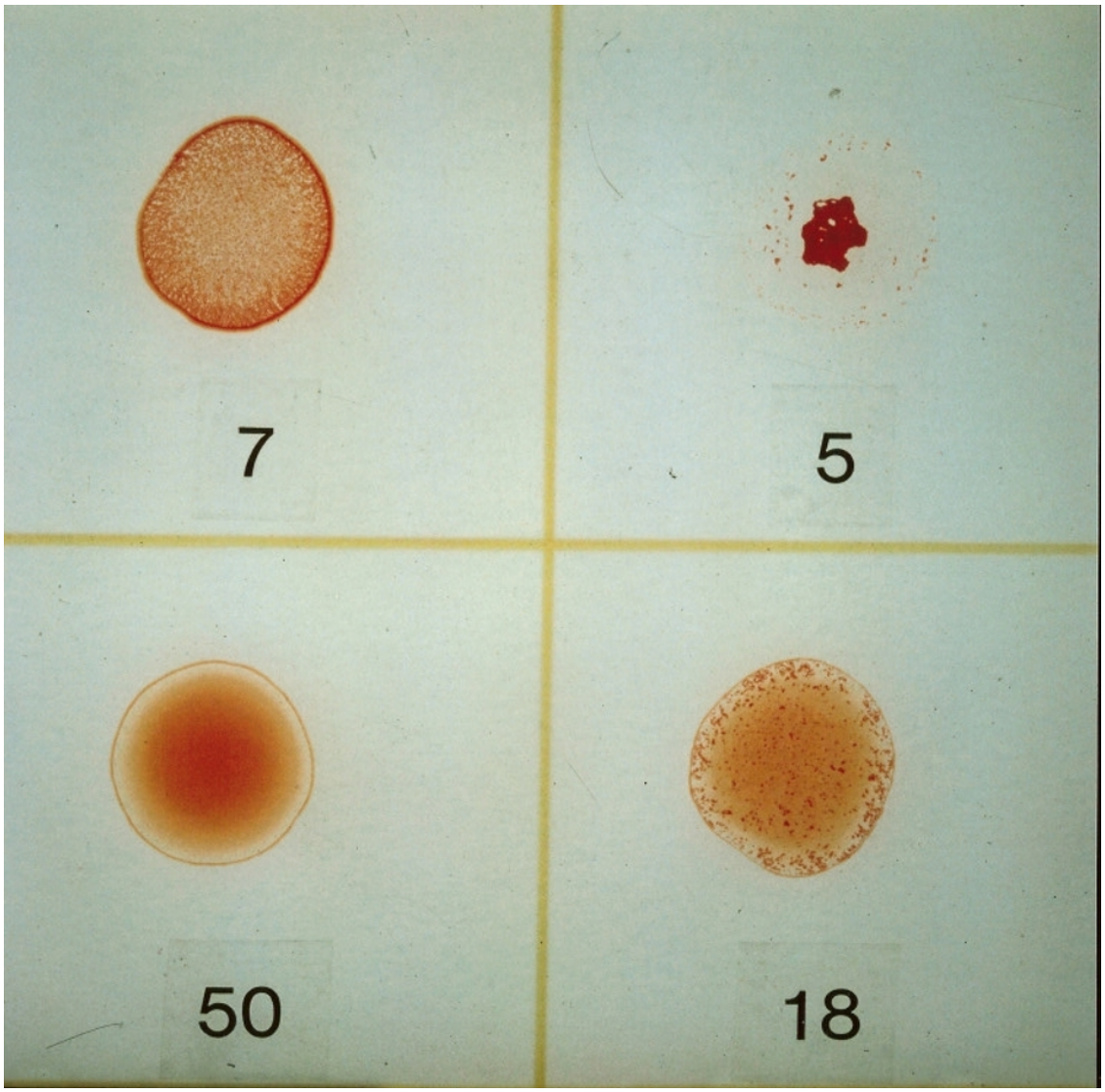
As early as 1975, Milstein attempted, with Köhler, to make monoclonal antibodies for typing the Rhesus (RH) blood group. They realised this could be helpful for blood typing, a technique that was important for ensuring safe blood transfusions. Little progress, however, was made. More success was to be had in generating a monoclonal antibody against another group of blood cells known as type A. This emerged by chance out of the work Milstein did with Williams to generate monoclonal antibodies for differentiating antigens on the surface of immune cells found in rats.

Milstein quickly grasped the potential monoclonal antibodies might hold for blood typing. At the time, all blood grouping tests were performed using conventional polyclonal antibodies drawn from antiserum. These antibodies acted as markers that would bind and clump specific determinants of the red blood cells. This helped determine the blood group classification.

Up to the late 1970s, the bulk of antiserum used in blood typing was sourced from human volunteers. This supply, however, was limited. Moreover, the little that was available was under severe strain. In part this reflected an upsurge in blood typing to meet the increasing frequency of major surgery and the fact that human blood serum was also critical for other medical purposes. The shortage was not helped by the fact that no two human sera are alike and sufficient volumes of antiserum are difficult to secure with adequate antibody potency for giving reliable results.

For Milstein, monoclonal antibodies provided an important tool for standardising blood group typing. To this end he collaborated with Douglas Voak, a pathologist based in the Regional Transfusion and Immuno-Haematology Centre which was located at Addenbrooke's Hospital, just over the road from his laboratory. Initially, the monoclonal reagent they created had some problems, but within a short time they had produced one that showed itself an experimentally effective tool for typing blood group A.

Soon after Milstein and Voak started their collaboration, they were approached by Steven Sacks, a medical graduate who had joined the Laboratory of Molecular Biology in 1978 to work as a doctoral student under Edwin Lennox, an American immunologist and founding member of the Salk Institute who had arrived in Cambridge in 1974. Sacks was curious to understand a strange phenomenon he had observed among some monoclonal antibodies that had been generated against different cancer tumours. These had been made as a tool to investigate the factors involved in the stimulation of the immune response against a tumour. When placed together with a mixture of cancer cells on a small plastic plate, the cells showed an unusual clumping in a disc-like formation. Surprisingly, he found that in addition to targeting tumour cells, the monoclonal antibodies appeared to target other cells. With the help of Voak and Milstein, Sacks discovered that he had inadvertently identified a monoclonal antibody that bound powerfully to antigens found on the surface of the human blood group type A.



The type of clumping that Sacks saw can be seen from this photo, which he took to record his observation. In the top row is a spot of blood from a person with a common type of blood group A (known as A1). To the left is the speckled clumping pattern following the addition of a conventional anti-A typing serum. To the right is a much stronger reaction causing the cells to clump into a single ball after the addition of the monoclonal anti-A. On the bottom row is a reaction with samples of blood expressing a weaker form of blood group A (known as A2). Red cell clumping is easily detected when the monoclonal anti-A is applied (bottom right), but more difficult to detect after the addition of the conventional serum (bottom-left). Photo credit: Steven Sacks.

With Milstein's encouragement, Sacks set about developing monoclonal antibody reagents, together with Lennox and Voak, for routine typing work. By 1982 the team had developed a number of different monoclonal antibodies suitable for routine use in ABO blood typing and had evaluated these agents against thousands of blood samples, both manually and through machines.

These monoclonal antibodies were patented and commercialised by the newly created British biotechnology company Celltech, which saw the reagents as fundamental to its growth. In 1982 the company's executives estimated that the world market for such products was worth £7.5 million in terms of hospital sales and £3 million for other sales. Within seven years, over half of the world's blood-typing reagents would be based on monoclonal antibodies derived from those first developed in Cambridge and produced by Celltech.

By 1989 it had become possible to produce 1,000 litres of monoclonal antibody supernatant in just 18 days, the equivalent of 2,000 individual human donations gathered through an automatic collection process. Importantly, the monoclonal reagents helped eliminate the

workload involved in evaluating large numbers of small individual donations. The successful creation of monoclonal antibody reagents also eliminated the need to continue the risky practice of immunising volunteers to secure conventional blood typing reagents.

This shows blood bags stored by different blood types. Photo credit: Wellcome Images, C0009182.

[<< The early commercialisation of monoclonal antibodies]

[The legacy of Milstein's work >>]

what is biotechnology?

Milstein's legacy

A technology for healthcare

In 1980 Milstein wrote:

'all that we seem to have acquired is the potential ability to select from an animal any of the antibodies of his repertoire. It is somewhat like selecting individual dishes out of a very elaborate menu: antibodies 'à la carte'... A gastronome worth his salt... wants to experiment with new ingredients, new combinations. His dream is to invent new dishes and not only to taste what others are doing. I am sure our next step will be to move from the dining table, where we order and consume our antibodies 'à la carte' to the kitchen, where we will attempt to mess them up.' (C. Milstein, 'Monoclonal antibodies from hybrid myelomas: Wellcome Foundation Lecture 1980', *Proceedings Royal Society of London,* 211 (1981), 393-412, 409).

As these words suggest, from the time that Milstein first developed the hybridoma technique with Köhler, he began looking for ways to improve the technology.

By the late 1970s, most monoclonal antibodies were being generated by using the protocol in the diagram shown here. Such monoclonal antibodies are produced by fusing animal myeloma tumour cells with spleen cells derived from mice or rats previously immunised with an antigen. Monoclonal antibodies created in this way are known as murine monoclonal antibodies, denoting their rodent origin.

The advantage of murine monoclonal antibodies was that they could be made to target almost any antigen and in vast quantities. Indeed, the rodent system possessed great flexibility to generate an antibody with any number of determined specificities. The system, however, had a downside: it produced non-human antibody molecules. Seen as foreign by the human body, such antibodies ran a high risk of prompting adverse immune reactions if administered to patients. Moreover, these adverse reactions could result in the rapid destruction and clearing of the monoclonal antibody from the body, which reduced their effectiveness for therapy.

Early on, Milstein and his colleagues had attempted to generate human monoclonal antibodies. This, however, had proven both very difficult and impractical. Manifestly, humans could not be immunised and manipulated like laboratory animals. Immunising humans with cancer antigens for the purpose of raising human antibodies against cancer to be converted into monoclonal antibodies, for example, was full of risks and raised serious ethical concerns.

Making monoclonal antibodies à la carte

By the late 1970s, when scientists were grappling to produce more human-like monoclonal antibodies, Milstein began to encourage his colleagues to think about using recombinant DNA (rDNA), or genetic engineering, to solve the problem.

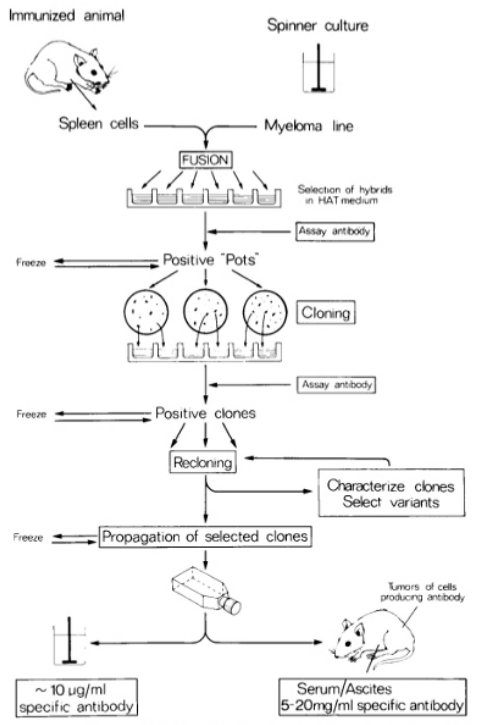
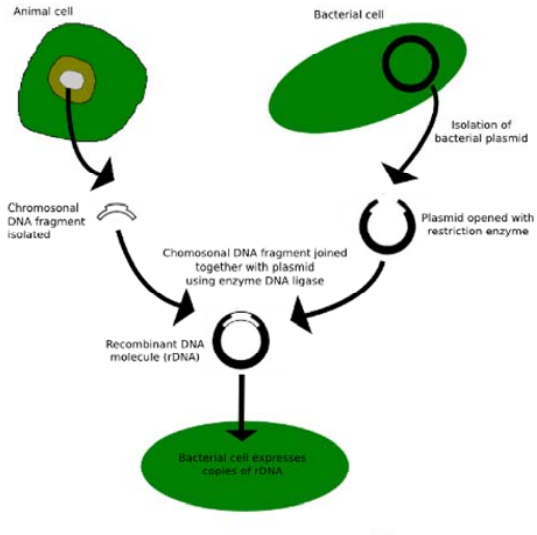


FIG. 3. Basic protocol for the derivation of monoclonal antibodies from hybrid myelomas.

This diagram illustrates the basic protocol developed by Köhler and Milstein for making monoclonal antibodies. It was first

First devised by Stanley Cohen at Stanford University and Herbert Boyer at the University of California in 1973-74, the technique involved using restriction enzymes to isolate a DNA fragment containing a gene of interest from an animal cell and then fusing this with a bacterial plasmid (an independent, self-replicating DNA molecule) that had been cleaved open with restriction enzymes. This method created what was called a recombinant DNA molecule, or rDNA. Once made the molecule could be put into a bacterial cell for amplification alongside the host cell DNA.

published in G. Galfré and C. Milstein, 'Preparation of monoclonal antibodies: Strategies and procedures', *Methods in Enzymology*, 75 (1981), p.15.



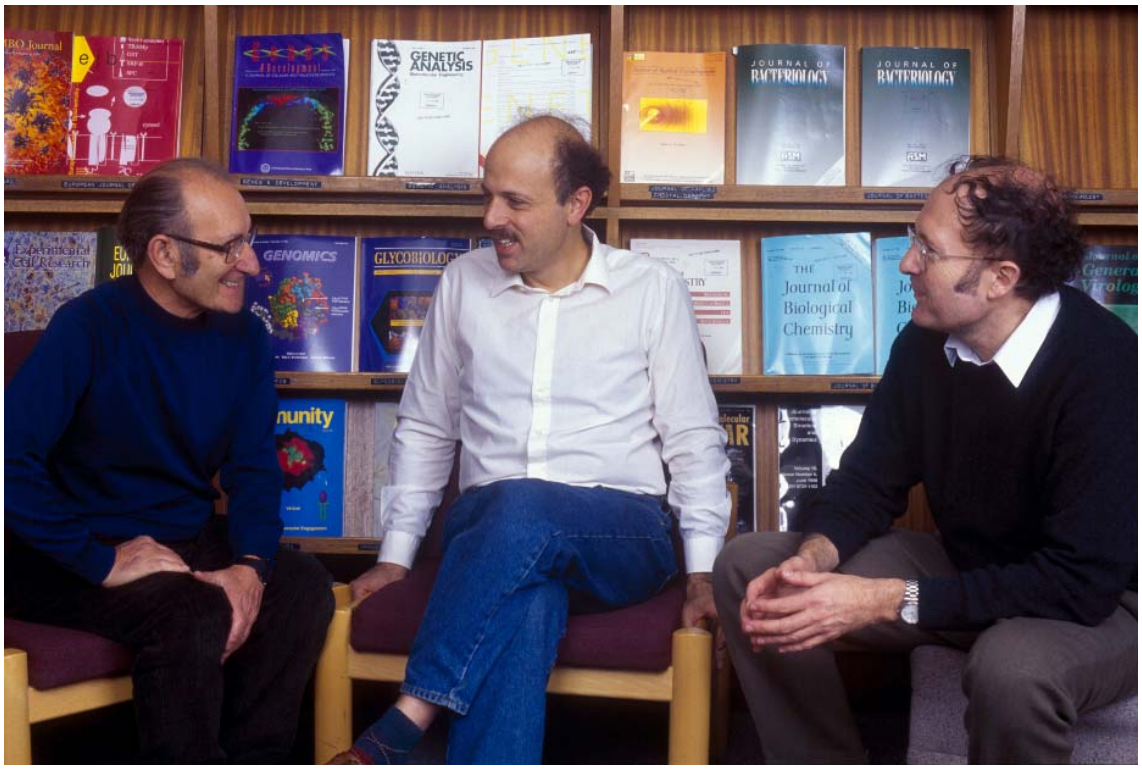
This diagram shows the steps involved in making rDNA.

Milstein argued that by combining genetic engineering with hybridoma technology scientists could shift from being restricted to immortalising naturally occurring antibodies as he and Köhler had done, and begin to design tailor-made antibodies. He believed this method could provide a way of engineering the human antibodies scientists had so far failed to produce.

One of the advantages scientists had in using genetic engineering to improve monoclonal antibodies was that antibodies possess a basic uniform modular structure. This meant that scientists could cut genes present on one part of the antibody and paste them on to the constant region of another antibody. For many scientists steeped in antibody research and immunology, such a shuffling of genes merely mimicked what already happened naturally in the immune system.

Milstein himself would make relatively modest efforts in the application of genetic engineering to monoclonal antibodies. Nonetheless, his vision inspired many others to begin the process. This included Michael Neuberger and Gregory Winter based at the Laboratory of Molecular Biology. They were instrumental in developing techniques for re-engineering monoclonal antibodies. This helped reduce their non-human protein components, thereby enhancing the efficacy and safety of monoclonal antibodies for use in therapeutics.

With Milstein's encouragement, the process for making monoclonal antibodies underwent a radical transformation over the next few years yielding antibodies that were much more compatible with the human body. By the 1990s scientists had gone a long way towards remoulding the basic monoclonal antibody first developed by Köhler and Milstein.



This photograph captures Milstein (far left) talking to Michael Neuberger (centre) and Gregory Winter (far right). Photo credit: MRC, Laboratory of Molecular Medicine.

With antibody engineering, scientists and clinicians found a way of producing a much safer and effective form of monoclonal antibody, one that could be used as therapeutically for many different diseases. Today monoclonal antibody drugs account for a third of all new treatments being introduced worldwide, and are beginning to bring in far greater revenue per product than older and more traditional small molecule pharmaceutical products. They have also been more successful in completing clinical trials than small molecule drugs.

Learning from past mistakes, both the Laboratory of Molecular of Biology and the Medical Research Council ensured patents were filed for the re-engineered forms of antibodies generated. This has generated an important source of revenue. To date the estimated MRC net income from monoclonals is £360 million (after deduction of revenue share and awards to inventors).

Recognition

In 1975 Milstein was elected a Fellow of the Royal Society. Two years later he was awarded an honorary doctorate by the Universidad Nacional del Sur based in the city of his birth Bahía Blanca. This was given in recognition of his contribution to science. Milstein received several other awards after this, including the Wolf Prize in Medicine in 1980, the Royal Medal in 1982, the Lasker Award in 1984 and the Copley Medal in 1989. He was jointly awarded the Nobel Prize in Physiology or Medicine in 1984 with Georges Köhler and Niels K. Jerne 'for theories concerning the specificity in development and control of the immune system and the discovery of the principle for production of monoclonal antibodies'.

By the time the Nobel Prize was awarded to Milstein and his colleagues, monoclonal

This shows Milstein with Köhler at the time of their receiving the Nobel Prize in 1984 together with Nils Jerne. Photo credit: MRC, Laboratory of Molecular Biology.

antibodies had become a ubiquitous method for researchers in a multitude of disciplines. The technology not only provided the tool for those involved in basic science, but also for those looking to develop new diagnostics and therapies.

Some idea of how valuable the technique had become can be seen by the fact that as early as 1980 the British biotechnology company, Celltech, calculated the world market for monoclonal antibodies would soon reach £6 million. Four years later, in 1984, the US Office of Technology Assessment indicated that the US market for monoclonals was worth between $5 and $6 million, predicting that this would rise to between $300 and $500 million by 1990.

Yet, the rise of monoclonal antibodies had not been a straightforward or inevitable process. It had taken years of work on the part of scientists to demonstrate its utility. Milstein, himself had been at the forefront of this process. Realising he could not leave such a task to others, Milstein quickly shifted, temporarily, from his long-time basic scientific interest in understanding antibody diversity to exploring, with many collaborators, the practical application of monoclonal antibodies.

Science for the world

This shows an Argentinian stamp, dated 2005, celebrating Milstein's Nobel Prize.

Conscious of his own difficulties as a scientist in Argentina, throughout his career Milstein devoted himself to assisting science and scientists in less developed countries. In March 2000, he wrote trenchantly, 'Science will only fulfil its promises when the benefits are equally shared by the REALLY poor of the world'.

In recognition of his legacy, the MRC later created a doctoral studentship in Milstein's name after his death to help support Argentinian scientists who wished to pursue their studies in Cambridge.

Personal life

Away from work, Milstein had a rich life. He enjoying cooking, eating, sailing, cycling, skiing and walking. He was also an enthusiastic supporter of charitable causes such as Shelter and Amnesty International.

Milstein died in 2002 from a heart attack, aged 74. First diagnosed with cardiovascular problems in the 1970s, he adhered, thereafter, to a strictly controlled diet and pursued a regular exercise regime. Colleagues recall seeing him taking his regular walk around Cambridge. Accompanied by his dog, Milstein would either take a portable radio to listen to the news or dictate letters into a tape-recorder while walking. Often he would invite colleagues to accompany him on his walks to discuss their work. Those who accepted frequently found it difficult to keep pace with his brisk step let alone keep up with the complex discussion that ensued.

<< The first clinical applications of monoclonal antibodies Glossary >>

what is biotechnology?

Glossary

Definitions of selected terms

Allele
One of two or more alternative forms of a gene, only one of which can be present in a chromosome.

Allelic exclusion
The process by which one allele of a gene is expressed while the other allele is silenced.

Amino acids
These are organic compounds that combine to form proteins.

Antibody
A type of protein made by the body's white blood cells (B lymphocytes) in response to a foreign substance (antigen). The production of antibodies is a major function of the immune system. Some antibodies destroy antigens directly while others make it easier for white blood cells to destroy the antigen. Each antibody is highly specific and will only bind to or destroy the antigen for which it was made.

Antibody specificity
The specificity of an antibody is defined as its capacity to target a specific antigen or foreign substance.

Antigen
Any substance that triggers an immune response, this includes pollen, micro-organisms such as bacteria, viruses, fungi or parasites and non-living substances such as toxins, chemicals, drugs, or foreign particles considered alien to the body.

Antiserum (plural: antisera)
This is serum that contains antibodies. It is obtained from an animal or human that has been exposed to a specific antigen.

B lymphocytes
A type of white blood cell made in the bone marrow that responds to an antigen by producing antibodies.

Bence-Jones proteins
A small protein made by plasma cells found in the urine of most people with multiple myeloma, a cancer that starts in plasma, a type of white blood cell.

Chimeric antibodies
Antibodies with a mixture of human and non-human components. Chimeric antibodies are approximately two-thirds human in form.

Chromatography
A chemical technique used for separating the components of a mixture. During the test a mixture dissolved in a liquid or gas is passed through a column, paper or glass support, where the elements of the mixture are either absorbed or hindered to varying degrees and thereby become separated. The technique is used both for the purification and collection of components as a means to quantify and measure component parts in a mixture.

Culture medium
A liquid or gel designed to aid the growth of micro-organisms or cells.

Disuplhide bridge
The bond that links atoms in a chemical compound.

DNA
This stands for DeoxyriboNucleic acid, and is a complex chemical located in the cell nucleus, specifically contained in chromosomes. It provides the genetic instructions needed for an organism to develop, survive and reproduce.

Enkephalin
Part of the family of opioid peptides produced by the body, enkephalin occurs in the brain, spinal cord and gastrointestinal tract. The peptide is inolved with pain perception, movement, mood, behaviour and neuroendocrine regulation.

Enzyme-linked immunosorbent assay (ELISA)
This is a test that uses antibodies conjugated with a colour enzyme (such as horseradish peroxide, alkaline phosphate or glucose oxidase) to identify a substance. ELISA tests are used as a diagnostic tool in medicine.

Epitope

Part of the target molecule, antigen, recognised by an antibody.

Flow cytometry
A laser based technique for counting and examining microscopic particles, such as cells and chromosomes. The particles are suspended in a stream of fluid which is passed through an electronic detection apparatus.

Fluorescence-activated cell sorting (FACS)
This is a specialised form of flow cytometry that allows for the sorting of a heterogeneous mixture of biological cells into two or more containers. The cells are sorted according to their specific light scattering and fluorescent characteristics.

Histocompatibility antigens
These are the many proteins (antigens) found on the surface of cell membranes that serve to identify a cell as self or non-self. They help determine tissue/organ compatability and rejection in transplantations or blood transfusions.

Humanised antibodies
Antibodies from non-human species whose protein sequences have been re-engineered to increase their similarity to antibody variants produced naturally in humans.

Hybrid cell
A cell formed by fusion of two cells of different origin.

Hybridoma
A hybrid cell made in the laboratory through the fusion of an antibody producing lymphocyte with a non-antibody producing cancer cell, usually myeloma or lymphoma. The hybridoma proliferates and produces a continuous supply of a specific monoclonal antibody.

Immune system
A biological defence system in humans and other mammals that protects the body against the invasion of foreign material (such as pollen, or invading micro-organisms) and helps prevent cancer.

Immunoassay
This is a biochemical test that measures the concentration of a specific substance in blood or a fluid sample which takes advantage of the binding mechanism of an antibody with an antigen.

Immunoglobulin (Ig)
Also known as an antibody, this is a protein produced by the immune system to fight infection.

Immunohistochemistry
A laboratory technique that uses antibodies to detect and visualise antigens in cells and tissues.

Immunology
The investigation of all phenomena connected with the defence mechanism of the body.

Interferon
A small protein messenger produced by the immune system in response to the presence of pathogens such as viruses, bacteria, parasites or tumour cells. Interferon has two functions. It sends signals to neighbouring cells to trigger their resistance mechanisms, and it activates other immune cells that kill invading pathogens.

Lymphocytes
A type of white blood cell which plays an important role in the body's defence mechanism. The two primary types of lymphocytes are B lymphocytes (B-cells) and T lymphocytes (T cells). Both originate from stem cells in the bone marrow. Those that migrate to the thymus mature into T cells, while those that remain in the bone marrow develop into B cells. Each lymphocyte has a receptor molecule on its surface which it uses to bind antigens (foreign substances) and help to remove them from the body. In the presence of an antigen, B cells can differentiate into plasma cells which secrete large quantities of antibodies.

Monoclonal
Derived from a single cell.

Monoclonal antibody
This is an antibody produced from a single clone of cells in a laboratory. The advantage of monoclonal antibodies is that they can be made on a large-scale and each one is identical to the other. Highly specific in their target, monoclonal antibodies are today used as reagents for basic research tools, and as diagnostic tools and therapeutics. In the context of therapy monoclonal antibodies can be used alone, or they can be used to deliver drugs, toxins, or, in the case of cancer, radioactive material directly to a tumour.

Myeloma
A cancer of plasma cells, a type of white blood cell found in the bone marrow.

Myeloma cells
Plasma cells that have become cancerous. Myeloma cells can spread throughout the bone marrow and into the bone, causing thinning of the bone, pain and sometimes fractures. Such cells produce a large amount of a single type of abnormal antibody. Myeloma cells are an essential tool for monoclonal antibody production.

Neuropeptide
Small, protein-like substance produced and released by neurons which helps neurons communicate with each other.

Neurotransmitter
A brain chemical that relays signals between nerve cells called neurons. Neurotransmitters tell the heart to beat, lungs to breathe and the stomach to digest. They can also affect mood, sleep, concentration, weight and can cause adverse reactions when imbalanced.

Patent
A patent is a form of intellectual property rights granted by a government to an inventor or their assignee for a limited amount of time in exchange for the public disclosure of the invention. A patent provides the right to exclude all others from making, using, or selling an invention or products made by an invented process. Like any other property right, it may be sold, licensed, assigned or transferred, given away or simply abandoned.

Polyclonal

Mixed pool of antibodies produced in an animal by a number of different white blood cells in response to an antigen. Each antibody has different binding specificities.

Polyethylene glycol (PEG)
A petroleum derived compound with many applications from industrial manufacturing to medicine.

Radioimmunoassay
This is an immunoassay test which makes use of radioactively labelled antibodies and antigens to detect and quantify important substances, such as hormone levels in the blood.

Recombinant DNA
Also known as gene cloning or splicing, recombinant DNA is a technique that produces identical copies (clones) of a gene.

Restriction enzymes
These are DNA-cutting enzymes found in and harvested from bacteria. The advantage of a DNA-digesting enzyme is that it can cleave the DNA molecule at precisely-located sites. Each enzyme recognises and cuts DNA at a particular sequence of nucleotides.

Sendai virus
A virus that affects mice, hamsters, guinea pigs, rats and sometimes pigs which is used research laboratories for its ability to induce genetically different cells to fuse.

Serotonin
A compound widely distributed in the tissues, particularly in blood platelets, intestinal wall and central nervous system. It is thought to play a part in transmitting nerve impulses and regulating moods, temper, anxiety, depression, sleep, aggression, appetite and sexuality. Serotonin is also considered instrumental in regulating body temperature and metabolism.

Serum
The straw coloured liquid component of blood from which blood cells and the chemicals which cause clotting have been taken out.

Somatic cell
Any cell type in the mammalian body apart from the sperm and ova.

Somatic mutation
The alteration of DNA that occurs after conception. Such changes can happen in any of the cells of the body except germline cells (sperm and ova) and so cannot be passed on to offspring. The alteration can cause various diseases including cancer.

Spleen
An organ that plays an important role in the immune system and helps in the creation of red blood cells. The spleen removes old red blood cells and recycles iron. It also synthesises antibodies and removes from circulation antibody-coated bacteria and antibody-coated blood cells.

Substance P (SP)
A small peptide found in the spinal cord and brain that transmits pain signals from the sensory nerves to the central nervous system. It is also associated with the regulation of stress and anxiety.

Supernatant
This is the upper layer of fluid found after a mixture has been centrifuged.

Tissue culture
A technique used to keep tissues or cells alive separate from an organism.

<< The legacy of Milstein's work

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what is biotechnology?

Timeline of key events in Milstein's life

Date	Event	People	Places
October 8, 1927	Cesar Milstein was born in Bahia Blanca, Argentina	Milstein	Bahia Blanca, Argentina
1945 - 1952	Cesar Milstein studies for a chemistry degree	Milstein	University of Buenos Aires
1954 - 1958	Cesar Milstein pursues his first doctorate in biochemistry	Milstein, Stoppani	University of Buenos Aires
1957	The Associacion Quimica Argentina awards Milstein a prize for the best doctoral thesis in chemistry that year	University of Buenos Aires	
1957 - 1959	Cesar Milstein publishes papers from his doctorate with his supervisor Andres Stoppani	Milstein	University of Buenos Aires
1958 - 1961	Cesar Milstein takes up a British Council Scholarship at Cambridge University	Milstein, Dixon, Webb	Sir William Dunn School of Pathology
1961	Cesar Milstein is awarded a second doctorate in biochemistry at Cambridge University	Milstein	Sir William Dunn School of Pathology
1961	Cesar Milstein takes up a position at the Instituto Malbran, Buenos Aires	Milstein	Instituto Malbran
1962	An Argentinian military coup throws Cesar Milstein's academic work into disarray	Milstein	Instituto Malbran
1963	Cesar Milstein returns to Cambridge and begins researching the structure and diversity of antibodies	Milstein	Laboratory of Molecular Biology
1964	Cesar Milstein publishes his first paper on antibodies	Milstein	Laboratory of Molecular Biology
July 1966	Cesar Milstein and Sydney Brenner publish theory attributing antibody diversity to somatic mutation	Brenner, Milstein	Laboratory of Molecular Biology
July 1970	Cesar Milstein launches experiments to determine whether somatic mutation underlies antibody diversity	Cotton, Milstein, Secher	Laboratory of Molecular Biology
1973	Cesar Milstein meets Georges Kohler at the Basel Institute of Immunology	Kohler, Milstein	Basel Institute of Immunology
July 1973	Cesar Milstein and Dick Cotton report the successful fusion of two different myeloma cell lines, one from a mouse and the other from a rat	Cotton, Milstein	Laboratory of Molecular Biology
June 1974	Georges Kohler joins Cesar Milstein's research team to investigate somatic mutation and antibody diversity	Kohler, Milstein	Laboratory of Molecular Biology
January 1975	Unlimited long-surviving monoclonal antibodies created	Milstein, Kohler	Laboratory of Molecular Biology
1975 - 1979	First monoclonal antibody created to target a neurotransmitter peptide	Milstein, Cuello	Laboratory of Molecular Biology, MRC Neurochemical Pharmacology Unit, Oxford University
August 1975	First step taken to patent Kohler and Milstein's monoclonal antibodies	Milstein	Laboratory of Molecular Biology
August 7, 1975	Cesar Milstein and Georges Kohler publish a paper outlining a technique for producing limitless monoclonal antibodies	Kohler, Milstein	Laboratory of Molecular Biology

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The exhibition has been constructed using a sample of curated letters, notebooks and other papers from César Milstein's archive held by the Churchill Archives Centre, Churchill College, Cambridge. Permission to use these papers for the website was kindly granted by Celia Milstein and the Churchill Archives Centre. Photographs were also kindly provided by the MRC Laboratory of Molecular Biology.

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