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Review

From the discovery of monoclonal antibodies to their therapeutic application: An historical reappraisal

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ABSTRACT

Vertebrate make billions of different antibodies, each with a binding site that recognizes a specific region of a macromolecule. The hybridoma technique allows monoclonal antibodies, highly specific antibodies produced in the laboratory by a variety of methods. In the last 35 years since the first process for creating monoclonal antibodies was introduced, their application have improved the growing biotechnology industry, but the most important application concerns the therapy of human malignancies.

1. The discovery

The development by the Argentinian biochemist César Milstein (1927–2002) and his German post-doctoral fellow Georges Köhler (1946–1995) (Figs. 1 and 2) in Milstein's lab in the "Molecular Research Council" (MRC) "Laboratory of Molecular Biology" in Cambridge of the hybridoma technology has disclosed a new era in basic and applied oncology. For this discovery, in 1984 Milstein and Köhler shared the Nobel Prize for Medicine or Physiology together with Niels K. Jerne (1911–1994) (Fig. 3) for "theories concerning the specificity in development and control of the immune system and discovery of the principle for production of monoclonal antibodies".

Milstein was interested in the mechanism of antibody diversity and he believed that by determining the chemical structure of different antibodies, the antibody diversity problem could be solved. Milstein's lab was experienced in studying myeloma in mice. Using a technique developed earlier by Potter, they had established a culture of rapidly proliferating tumor cells that produced immunoglobulins or antibodies.

Milstein suggested that Köhler investigated the antigen specificity of myeloma P3 antibodies. Instead of fusing two myelomas, Köhler decided to fuse one with mouse spleen cells, antibody producing B-cells. Spleen cells from immunized donors were fused with myeloma cells bearing a selection marker and the fused cells were then cultured in a selective medium until visible colonies grew, and their supernatants were then screened for antibody production. For the first time, large quantities of a pure antibody, specific for a single agent determinant, could be produced.

The key to success was the development of a selective technique to recover only fused cells, employing a mutant myeloma cell line deficient the enzyme hypoxanthine phosphoribosyl transferase. Without this enzyme, the cells would die in a medium containing hypoxanthine, aminopterine, and thymidine (HAT), but the hybrid cells would survive and could be selected, since the normal antibody-forming cell component of the hybrid would contribute the enzyme required [1,2].

The technique proposed by Köhler and Milstein is founded on three key principles: (i) each B cell produces only one antibody; (ii) the lymphocytes used for the fusion are derived from donors that were sensitized with specific immunogens; (iii) B cells can be immortalized into immunoglobulin-secreting *in vitro* cell lines.

Köhler and Milstein explained their collaboration in these extremely essential terms: "We agree that both conception and execution of the work was the result of close collaboration between us, with the skilled technical assistance of Shirley Howe. We are further convinced that the combined effect which results from such close collaboration was of a synergistic nature, synergistic effects taken to mean, as with monoclonal antibodies, effects which result

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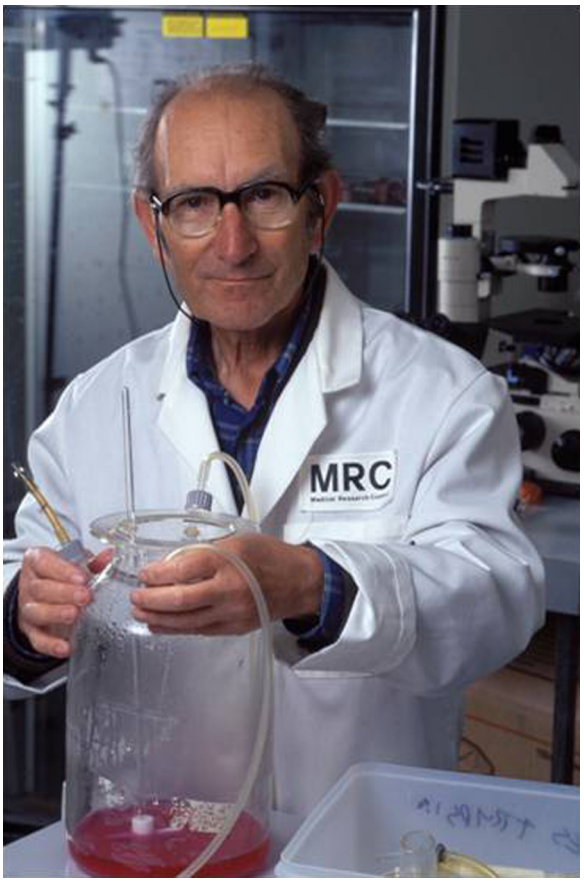

Fig. 1. A port trait of César Milstein.

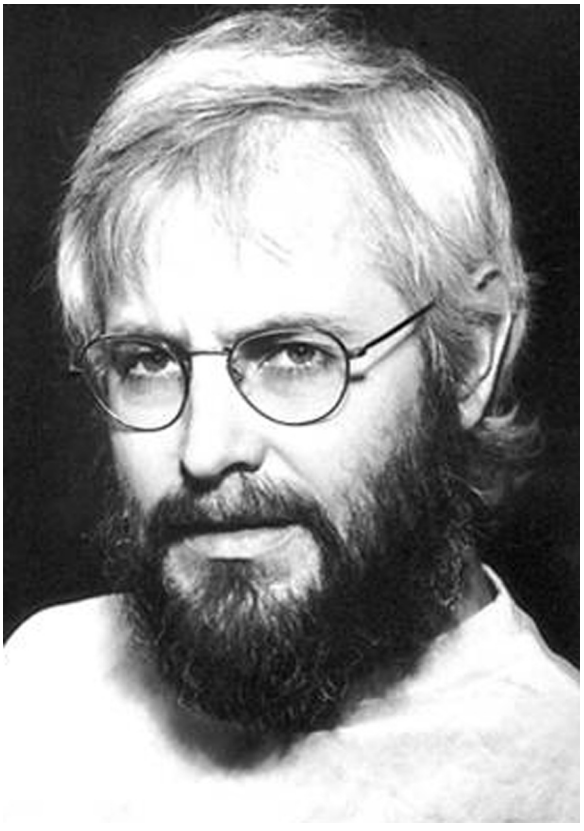

Fig. 2. A port trait of Georges Köhler.

Fig. 3. A port trait of Niels K. Jerne.

from the combined action of two but which cannot be produced by the two separately. We both have a most pleasant memory of an exciting period in which a word, a comment, or a passing remark made by one had a resonant effect on the other. We do not want such happy memories, which have sealed a close friendship, to be disturbed by superficial interpretations of our individual recollections. It was a collaborative work. It was a collaborative paper. We do not want to make further comments" [3].

Köhler and Milstein did not patent this method, allowing the use of the hybridoma technology to academics and pharmaceutical industry for generation of future potential therapy. Initially work used myeloma cells which retained the capacity to secrete their own immunoglobulin products. Later, such fusion was replaced by myeloma variants that express only one endogenous chain or that fail to express immunoglobulin so that the fused cells secreted primarily or exclusively antibody of the desired specificity.

The monoclonal antibody technology has improved diagnostic applications including epitope specific immunoblotting, immunofluorescence, and immunohistochemistry.

2. Therapeutic applications

Monoclonal antibody-mediated therapy started with mouse monoclonal antibodies, mouse to mouse-human chimeras, and later to humanized monoclonal antibodies. Antibodies of all types (murine, chimeric and human) have been approved by Food and Drug Administration (FDA) and by other international agencies for the treatment of several pathologies.

In the late 1980s, murine monoclonal antibodies were in clinical development. The first monoclonal antibody approved by FDA for human use was a murine anti-CD3 monoclonal antibody, muromonab (OKT3), used for the treatment of organ transplant rejection [4].