Generation of the first monoclonal antibody using mouse hybridomas

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TITLE	Continuous cultures of fused cells secreting antibody of predefined specificity.
AUTHORS	Köhler G, Milstein C.
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Monoclonal antibodies have revolutionized several fields of medicine, especially hematology. The game started in 1975 when Köhler and Milstein, in a Letter to *Nature*,[1] reported that it was possible to extract spleen B cells and fuse them with a mouse myeloma cell line to create hybrid cells (hybridomas) producing antibodies specific to the inoculated antigen and to immortalize them (Figure 1). This goal had been the source of frustration for scientists for decades and achieving it was the result of many efforts in the field of biochemistry, cell culture, immunology, and somatic cell genetics. The authors concluded in their Letter that "*Such cultures could be valuable for medical and industrial use*".

The link between monoclonal antibodies and hematology has been very tight since the beginning. Incidentally, the antigen used to generate the first monoclonal antibodies was sheep red blood cells. Köhler and Milstein stated: "*It remains to be seen whether similar results can be obtained using other antigens*".[1] Fortunately, this was the case. In the mid 1980s, the number of monoclonal antibodies directed against lympho-hematopoietic antigens and applicable for diagnostic purposes in hematology ex-

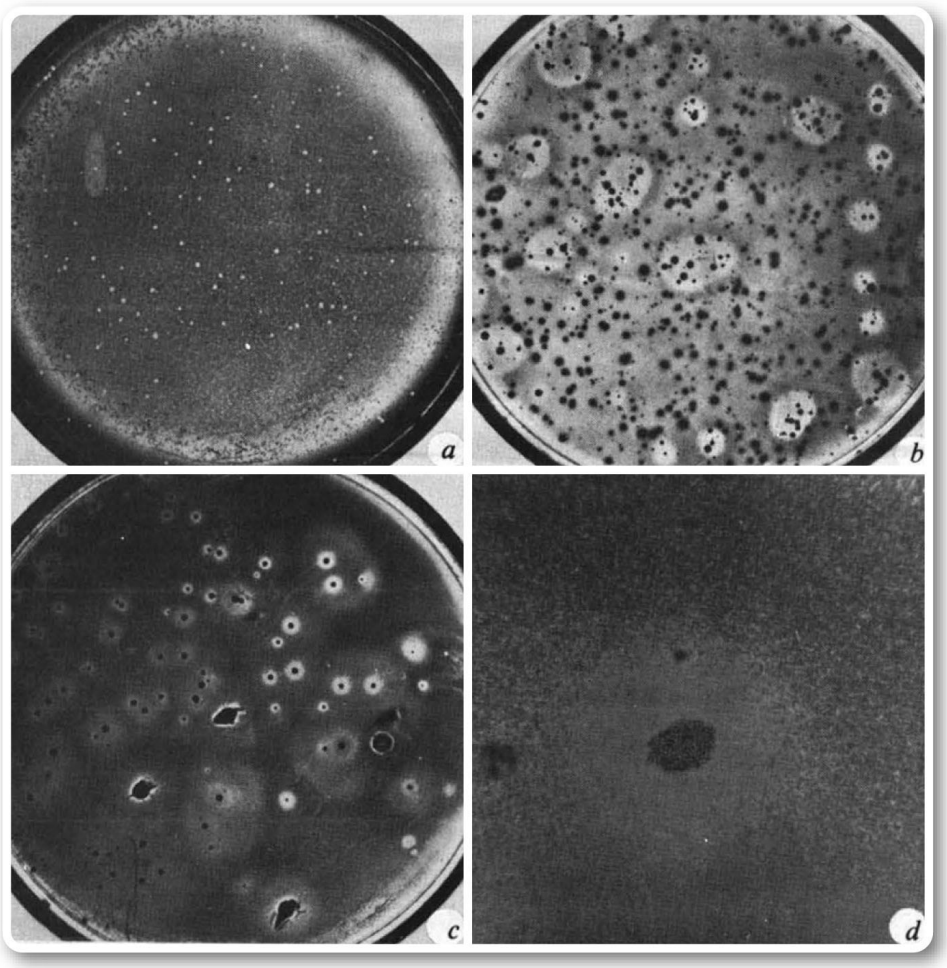

Figure 1. *"Isolation of an anti-SRBC antibody-secreting cell clone. Activity was revealed by a halo of haemolysed SRBC"* (Figure 2 from Köhler G, Milstein C. Continuous cultures of fused cells secreting antibody of predefined specificity. Nature. 1975 Aug 7;256(5517):495-497. doi:10.1038/256495a0. PMID 1172191, with permission). To achieve continuous cultures of fused cells secreting antibodies, Köhler and Milstein used the selective culture medium HAT (hypoxanthine, aminopterine, thymidine) that, in the mixture of fused and unfused cells, allowed only the growth of hybrid cells but not mouse myeloma cells (because they lacked hypoxanthine-guanine-phosphoribosyl transferase-HGPRT) and spleen B cells (because of their limited life span). Thus, hybridomas could grow indefinitely in HAT medium because the spleen cell partner supplied HGPRT and the myeloma partner (being a cancer cell) made the hybrid immortal and furnished the machinery required to produce antibodies at high rate. The monoclonal antibody of interest was then selected with appropriate screenings, as shown in the Figure.

Surrozen 2023
Merck v. Surrozen PGR2026-00027
Page 1

panded dramatically thanks to screening on lymphoid tissue sections, which enabled the detection of even those cells difficult to bring into suspension, e.g. endothelial cells, macrophages and follicular dendritic cells within B-cell follicles. This strategy also allowed the type of labeled cells to be recognized by their topographic distribution (e.g., mantle *vs*. germinal center B cells). Another major step forward in improving the diagnosis of lymphomas and leukemias occurred in the early 1990s with the demonstration that monoclonal antibodies could recognize antigen epitopes resistant to fixation and paraffin-embedding procedures. The number of monoclonal antibodies with this property increased hugely over the years, allowing routine paraffin-embedded biopsy samples to be investigated by immunohistochemistry, contributing to the development of modern classifications of lympho-hematopoietic tumors. Finally, monoclonal antibodies recognizing tumor-specific antigens (e.g. ALK) or atypical distribution of mutated proteins (e.g. cytoplasmic NPM1)[2] led to the identification of specific genetic entities.

In 1986 the Food and Drug Administration approved the first therapeutic anti-CD3 monoclonal antibody (muromonab) for the prevention of kidney transplant rejection. After that, the field moved very slowly and a third therapeutic monoclonal antibody (rituximab, anti-CD20) was approved by the Food and Drug Administration only in 1997 for the treatment of B-cell lymphomas. The era of chemo-immunotherapy had started, with monoclonal antibodies directed against many lymphoid and myeloid-associated antigens or aimed at blocking signaling pathways (e.g. PD-1/PDL-1) increasing over the years. In April 2021, the monoclonal antibodies approved for clinical use had reached 100.

Antibody engineering, including humanization, construction of immunotoxins and generation of bispecific antibodies to recruit immune cells to cancer cells further contributed to the success of monoclonal antibodies in treating hematologic malignancies. The most recent and revolutionary impact of monoclonal antibodies was in constructing chimeric antigen receptor (CAR) T cells engineered to express on the cell surface a single-chain fragment variable domain (a monoclonal antibody portion) able to recognize a given target molecule on tumor cells.[3] CAR T cells have revolutionized the therapy of several hematologic neoplasms, including refractory/resistant large B-cell lymphomas and lymphoblastic leukemia.[3]

In 1984, Köhler and Milstein shared the Nobel Prize in Physiology or Medicine with Niels Jerne. Notably, Milstein never patented his extraordinary discovery on monoclonal antibodies since he believed that it was mankind's intellectual property.

Disclosures

No conflicts of interest to disclose.

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Surrozen 2023
Merck v. Surrozen PGR2026-00027
Page 2