This Week's Citation Classic®

Galfrè G, Howe S C, Milstein C, Butcher G W & Howard J C. Antibodies to major histocompatibility antigens produced by hybrid cell lines. *Nature* 266:550-2, 1977. [MRC, Lab. Molecular Biology, and ARC, Inst. Animal Physiology, Cambridge, England]

Describing the derivation of hybrid myelomas secreting monoclonal anitbody to rat major histocompatibility antigens, this paper claimed to "bring the goal of producing standard permanent supplies of monoclonal antibodies for...clinical use one step nearer." [The *SCI*® indicates that this paper has been cited in more than 1,430 publications.]

The First "Useful" Hybridoma

César Milstein
Laboratory of Molecular Biology
Medical Research Council
Hills Road
Cambridge CB2 2QH
England

The derivation of the first hybrid myeloma cell line (or hybridoma, as we now know it) against a predefined antigen[1] was not received with wild enthusiasm by the scientific community at largeor even by immunologists. But, there were important exceptions. Among them, the late Ruggero Cepellini was enthusiastic enough to persuade one of his brightest young postdoctoral fellows, Giovanni Galfrè, to join me. Human histocompatibility antigens were in his mind, and Giovanni was well trained in histocompatibility testing. Unfortunately, when he arrived we were bedeviled for six months with a toxic batch of reagent. During this depressing period, a chance conversation among the authors took place.

As a result, we decided that the (at that time virtually uncharted) rat major histocompatibility complex might be more interesting to tackle, since we would not have to fight with established HLA serology based on polyclonal antisera. The tissue culture work was done in the Laboratory of Molecular Biology, mostly by Galfrè, with the technical assistance of S.C. Howe, and the serology and genetics at Babraham, by G.W. Butcher and J.C. Howard. We expected the so far untested mouse-rat hybrids to work, based on our experience using mouse/rat myeloma fusions.[2] We initially detected positive wells using a visual complement-dependent hemolytic assay (rat class I MHC antigens are well expressed on red cells). But, we could never directly detect positive clones in agar using a complement-dependent hemolytic plaque overlay, a technique we had used earlier to identify sheep red cell and DNP-specific clones.[1] We eventually isolated a positive clone by assaying the supernatants for hemolytic activity, and this clone, R3/13, is what the paper describes. It was never a good hemolysin. We also refer to several other lytic lines that we could not clone. In fact, when "negative" clone supernatants from these "unclonable" active cultures were pooled, the lytic activity returned.[3] So our unwise choice of assay helped us to define "synergistic" lysis, whereby two IgG antibodies directed against different epitopes on the same molecule have the remarkable ability to fix two molecules of C1q, giving exceptionally efficient lysis.[4,5] Even so, it would have been better to have used a simple binding assay for the initial screen, as was used subsequently![6]

The paper describes some technological innovations, like the use of interspecific hybrids and of polyethylene glycol to derive hybridomas. But, what made it popular was that it represented the first attempt to solve a biological problem by hybridoma technology. These were also, of course, the first monoclonal antibodies against the products of the major histocompatibility complex. For the first time, hybridomas were being used to produce "useful" monoclonal antibodies.[3]

1. **Köhler G & Milstein C.** Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature* 256:495-7, 1975. (Cited 6,905 times.)
2. **Cotton R G H & Milstein C.** Fusion of two immunoglobulin-producing myeloma cells. *Nature* 244:42-3, 1973. (Cited 120 times.)
3. **Howard J C, Butcher G W, Galfrè G, Milstein C & Milstein C P.** Monoclonal anitbodies as tools to analyse the serological and genetic complexities of major transplantation antigens. *Immunol. Rev.* 47:139-74, 1979. (Cited 125 times.)
4. **Hughes-Jones N C, Gorick B, Miller N G A & Howard J C.** IgG pair formation on one antigenic molecule is the main mechanism of synergy between antibodies in complement-mediated lysis. *Eur. J. Immunol.* 14:974-8, 1984.
5. **Hughes-Jones N C & Howard J C.** Complement-mediated lysis with monoclonal antibodies. (Waldmann H, ed.) *Monoclonal antibody therapy.* Basel, Switzerland: Karger, 1988. p. 1-15.
6. Williams A F, Galfrè G & Milstein C. Analysis of cell surfaces by xenogenic myeloma-hybrid antibodies. Differentiation antigens of rat lymphocytes. *Cell* 12:663-73, 1977. (Cited 590 times.)

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