NOTE: Please see the erratum on p. 7.

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This Week's Citation Classic®

Galfré G, Howe S C, Milstein C, Butcher G W & Howard J C. Antibodies lo major hisiocompalibililj 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 antibody to rat major histooompatibility 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 1,490 publications.]

The First "Useful" Hybridoma

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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 large—or 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 Galfre, 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 Galfre, 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 hemo-lytic assay (rat class IMHC 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 C1 q, 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**. Cntinuous cultures of fused cells secret ing antibody of predefined specificity. *Nature* 256:495-7, 1975. (Cited 6,940 times)

2. **Cotton K G H & Milstein C**. Hision of two immumiglobulin-producing myeloma cells. *Nature* 244:42-3. 1973. (Cited 125 times,)

3. **Howard C. Butcher G W. Galfre G. Milstein C & Milstein C P**. Moinoclonal anitbodies as tools to analyse the serological and genetic complexties of major minsplamulion antigens, *himnnmi Her* 47:l39-74. 1979. (Ciied 130 times.)

4 **Huyhvs-.Jones N C. Gorick B. Miller N G A & Howard J C**. IgG pair formution on one aniipenic molecule is the mjin mechanism of synergy between antibodies m complement-mediuted lysis, Eu*r. J. Immanol.* 14:974-8. 1984.

5 **Hughes-.Jones N C & Howard J C**. Complement-mediated lysis with monoclonal antibodies. (Waldmann H, ed.) *Mntunlomltimihmh therapy* Basel. Switzerland: Karger. 1988 p. 1-15.

6. **Williams A K. Galfre G & Milstein C**. Analysts oi tell surfaces by xeinogenic myeloma-hybrid antibodies. Differentiation antigens of rat lymphocytes, *Cell* l2:663-73. 1977. (Cited 660 times,)

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