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Use of Fluorescence-Activated Cell Sorting to Select Hybrid Hybridomas Producing Bispecific Monoclonal Antibodies

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A. Introduction

Antibodies containing two different antigen-binding sites were prepared for the first time in 1961 by Nisonoff and Rivers [1] by dissociating two different antibodies and reassociating the mixture of half molecules. But this method of chemical recombination has some disadvantages because it results in protein denaturation and therefore loss of antibody activity.

The production of bispecific antibodies by hybrid cells containing the antibody genes for expressing two different antibodies is, therefore, regarded as a more reliable method. Such hybrid cells are produced by fusing selection medium-sensitive antibody-producing parental cell lines and isolating the fused cells by means of the corresponding selection media [2, 3].

This paper describes a method of selecting bispecific antibody-producing hybrid hybridomas by using a fluorescence-activated cell sorter. This method avoids the labor-intensive production of selection medium-sensitive mutants of the parental cell lines which is substituted by labeling the cell lines by two different fluorescent markers [4].

B. Experimental Set-up and Results

Two mouse hybridoma cell lines producing monoclonal antibodies to human alpha-fetoprotein (AFP; [5]) and horseradish peroxidase (HRP; [6]) were used for the experiments. Cells of these lines were labeled by fluorescein isothiocyanate (FITC; 0.5 μg/ml) or tetramethyl rhodamin isothiocyanate (TRITC; 1.5 μg/ml) and then fused by using polyethylene glycol (PEG) according to the standard fusion method [7]. After cultivating the mixture for 4 h the cells were analyzed by a fluorescence-activated cell sorter (FACS III, Becton Dickinson, Sunnyvale, USA), and the fused hybrid hybridomas were sorted out which gave a double (green and red) fluorescence.

The sorted cells were immediately cloned in microtitration plates and 2 weeks later grown clones were checked for the production of bispecific antibodies with anti-AFP/anti-HRP activity. A solid-phase immunoassay was used with the following incubation steps: purified AFP, phosphate-buffered saline containing 10% calf serum, culture fluid to be tested for antibody activity, HRP followed by the substrate O-phenylene diamine. Several stable hybrid hybridoma clones were obtained and one clone was transplanted in mice for the production of ascitic fluid.

The ascitic fluid was fractionated by hydroxylapatite column chromatography [8] which resulted in five peaks in contrast to three peaks when the ascitic fluids of the parental lines were fractionated (Table 1). Peak 4 from the ascitic fluid of the hybrid hybridoma contained the bispecific antibodies. SDS-polyacrylamide gel electrophoresis showed that this fraction contained the heavy chains of both anti-AFP and anti-HRP antibodies [4].

The bispecific anti-AFP/anti-HRP antibodies could be used to build up an enzyme

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Table 1. Antibody activity in ascitic fluid from hybrid hybridomas after hydroxylapatite column chromatography

Fractions	Antibody activity		
	Anti-HRP[a]	(Anti-AFP/ anti HRP)	Anti-AFP[a]
1	–	–	–
2	–	–	–
3	+++	(+)	(+)
4	++	+++	++
5	(+)	(+)	+++

[a] Tested by a solid-phase immunoassay with the incubation sequence: purified goat anti-mouse immunoglobulin, monoclonal antibody, HRP followed by the substrate or ^{125}J-labeled AFP.

immunoassay for the demonstration of AFP with the incubation sequence: monoclonal anti-AFP antibody with different epitope specificity at the solid phase, solution containing AFP, bispecific antibody, and HRP followed by the substrate.

C. Conclusion

These experiments demonstrate that hybrid hybridomas secreting bispecific monoclonal antibodies can be produced by fusing hybridoma cell lines and selecting hybrid hybridomas by using a FACS. For this purpose the parental cell lines are labeled by two different fluorescence markers. It is not necessary to convert the parental lines into selection medium-sensitive mutants and hybrid hybridomas can, therefore, be obtained in a relatively short time compared with the methods used so far [2, 3]. The technique presented here is a simple and effective method for the production of bispecific antibodies and can be applied to different systems. The bispecific monoclonal antibodies produced should be useful reagents to build up sensitive immunoassays and other immunological tests.

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