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Set up of an easy selective method for the isolation of hybrid hybridomas. Evidence for the production of heterobifunctional monoclonal antibodies.

For classical enzyme immunoassays, it is necessary to conjugate specific immunoglobulins to an enzyme molecule. In order to avoid the purification of the antibody, the purification of the enzyme and the conjugation steps, heterobifunctional monoclonal antibodies can be obtained from hybrid hybridomas. The molecular chimeras are capable to bind to both antigen and enzyme (MILSTEIN & CUELLO, 1983).

Hybrid hybridomas are derived by fusing two hybridoma cells and are recovered in a medium containing two selective drugs (one for each parental cell).

146.BR.OR is an 8-azaguanine-resistant hybridoma which secretes an IgG1 (Kappa) against alkaline phosphatase. This cell line was infected by SVX, a retroviral vector containing the neo gene which confers resistance to geneticin to eukaryotic cells (CEPKO *et al.*, 1984). Infected cells were found to be able to grow in a medium containing 2 mg/ml geneticin. They were fused with several hybridomas and the hybrid hybridomas were recovered in HAT medium supplemented with geneticin.

Heterobifunctional antibodies were identified by the following ELISA : 1) Coating with the antigen corresponding to the HAT-resistant hybridoma. 2) Saturation of substrate with foetal calf serum. 3) Washing, and incubation with culture supernatants. 4) Washing, and incubation with free alkaline phosphatase. 5) Washing, and incubation with the enzyme substrate. A mixture of both parental immunoglobulins was used as a negative control.

We have produced by this method several hybrid hybridomas secreting bispecific antibodies to phosphatase and human IgG, to phosphatase and human IgG3, and to phosphatase and human serum albumin.

In conclusion, acquisition of resistance to geneticin by use of infection with a retroviral vector appears to be a process which is easy, fast and harmless for hybridomas.

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References

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