Preliminary studies of a monoclonal antibody raised to the over expressed plasma membrane associated glycoprotein of a multidrug resistant cell line.

Elizabeth Moran, Alice Redmond and Martin Clunes.

National Cell and Tissue Culture Centre, Bioresource Ireland, Dublin City University, Dublin 9.

A murine monoclonal antibody against a multidrug resistant (MDR) variant of the CHOK1 cell line (which overexpresses the P170-180kD glycoprotein receptor) was produced by the fusion of NS0 myeloma cell and splenocytes from a Balb/c mouse immunised with the whole cell preparations and plasma membrane enriched microsomal fractions of the MDR variant cell line.

The fusion technique was a modification of the method described by Kohler and Milstein [1]. All tissue culture media and supplements were obtained from Flow laboratories, Herts, England. Instead of using mouse feeder cells (e.g.macrophages) Briclone was used. This is a conditioned medium containing high levels of human IL-6 and is produced at the National Cell and Tissue Culture Centre. This product increases the efficiency of both fusion and cloning steps in hybridoma formation.

The following cloning by limiting dilution (2x), one monoclonal antibody, designated P3A8/12H, was selected for further study. This antibody was also found to be an ascite tumor in Balb/c mice. The commercially available antibody (Centocor Diagnostics USA) was used concurrently with P3A8/12H during all screening procedures. C219 recognises specifically the F-glycoprotein (F-170) receptor on MDR variant cell lines and could be used as a positive control for the screening systems used.

Initial screening of hybridomas by ELISA was performed by growing the drug-sensitive parental cell line (CHOK1) and the MDR variant cell line on 96 well polyvinyl plates and fixing with 0.05% glutaraldehyde in PBS. Nitrocellulose bottomed 96 well plates (obtained from Amersham, U.K.) were also used for the ELISAs. These were coated with whole cells or plasma membrane enriched microsomal fractions prior to screening, no fixation of cells was required.

Preparation of plasma membrane enriched microsomal fractions from the CHOK1 cell line, the multidrug resistant variant and four other multidrug resistant variants (produced in our laboratories) and their drug sensitive counterparts were prepared by two methods. The method of Gerlach et al.[2] was used to produce relatively crude microsomal fractions. The method of Ronchi et al.[3] was used to produce a purer microsomal preparation. The total protein concentration of cell membrane preparations was determined by bicinchoninic acid (BCA) protein assay [4]. Dot blotting and Western blotting were performed to detect the presence and the molecular weight of the antigen recognised by the selected antibody. Prior to Western blotting, cell membrane proteins were separated on 7.5% SDS-polyacrylamide gels in a discontinuous buffer system according to the method of Laemmli [5]. Western blotting was performed by the method of Towbin et al.[6]. Both dot blotting and Western blotting were done using Hybond-C super nitrocellulose (Amersham, U.K.). Indirect immunofluorescence on fixed cells was performed on cells grown on 8 well slides (Munc) which were fixed for 10 min in ice cold acetone prior to incubation with neat supernatant from P3A8/12H. The conventional ELISA system, using glutaraldehyde-fixed, antigen coated, 96 well polyvinyl microtitre plates for screening hybridomas and the C219 antibody was necessary to determine positive antibody/antigen reactivity. Overnight incubation with test antibody was necessary to determine positive antibody/antigen reactivity. Further studies with this antibody and also production of more antibodies to the DLP-A cell line are presently underway.

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