

CELL BIOLOGY

Production of monoclonal antibodies

During the year efforts on the generation of monoclonal antibodies catering to the needs of the institute was continued. This year we have raised a murine monoclonal antibody against A red cells using a conventional protocol. The antibody thus produced will be used as a blend with the anti – A murine monoclonal previously generated. The titre of the present antibody is 1:64 with A1 cells and 1:8 with A2 cells.

Year of commencement : 2005

Year of completion : 2008

Monoclonal antibody against Rh antigens:

The Rh blood group system is one of the most polymorphic and immunogenic systems known in human and forms a most important and significant system in Transfusion medicine next to ABO. Till date there are nearly 37 well-defined independent epitopes. Hence the aim of the present study is to generate monoclonal antibodies against Rh antigen indigenously by EBV transformation of B lymphocytes obtained from immunized cases, followed by fusion with SP2/0 Ag.14 mouse myeloma cells.

EBV transformation of B lymphocytes :

B95.8 marmoset cell line was obtained from ACTREC. This was incubated for a week in RPMI – 1640 culture medium in 5% CO₂ and 37⁰ C at 1 X 10⁵ cells / ml. The EBV culture supernatants were harvested by centrifugation and stored at – 80⁰ C until use.

Mononuclear cells was obtained from hyper immunized donor having a titre of 1:1024 with Rh positive cell by Ficoll hypaque density gradient. They were treated with sheep erythrocytes to obtain B cells. The separated B lymphocytes were incubated at 37⁰ C with 1 ml of EBV culture supernatant / 1 X 10⁶ cells for 2 hours. This was washed and distributed in 96 well plates at 100 ul/ well and further incubated at 37⁰ C, 5% CO₂ and 95% humidity for 2 weeks. Positive clones were identified. Further work on fusion with SP2/0 Ag.14 for heterohybridoma is in progress.