Abstract

A procedure for in vitro immunization of splenic lymphocytes with a glycolipid antigen is described. Culture medium supernatant of ConA- and PHA-stimulated spleen cells and that of Con A-stimulated human Jurkat T cell line (IL-2-rich medium) were used as sources of cytokines to support T and B cell stimulation, and anti-mu was used to support B cell differentiation. Unprimed rat spleen cells (2 x 10(6)/ml) were stimulated with 2 micrograms/ml Forssman glycolipid antigen coupled to Sepharose for 4 days. The cells were fused with a mouse myeloma cell line P3-X63-Ag8-U1. At initial screening, 12% of the colony forming wells were secreting specific antibody. After cloning, a stable hybridoma cell line (designated 4C3) was established which secreted a monoclonal IgM antibody directed against the carbohydrate moiety of Forssman glycosphingolipid (GalNAc alpha 1-3GalNAc beta 1-3Gal alpha 1-4Gal beta 1-4Glc-ceramide).