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IN VITRO STIMULATION OF LYMPHOCYTES AS A SOURCE OF HUMAN MONOCLONAL ANTIBODIES USING THE EBV-HYBRIDOMA TECHNIQUE.

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We have previously shown that human hybridomas can be constructed with antigen-specific, Epstein-Barr virus transformed cell lines by fusion with SB-4, a 6-thioguanine and ouabain resistant lymphoblastoid cell line (Kosbor, D; Lagarde, A; and Roder, J.C., Proc. Natl. Acad. Sci. 79:6651-6655, 1982). The resulting hybrids were stable (>1 yr), have high cloning efficiencies (6%), divide rapidly (< 24 hr), and produce reasonable levels of specific antibody (5-7 ug/ml). In this study we compare the usefulness of specific antigens and polyclonal activators in stimulating cultures prior to fusion. Stimulation of peripheral blood lymphocyte (PBL) cultures from tetanus toxoid immunized donors with Epstein-Barr virus yielded cells with much higher frequencies of hybrid formation (36 x 10^{-3}) compared to unstimulated PBL or cells cultured with Pokeweed mitogen (PWM) or tetanus toxoid (TT) antigen. The proportion of hybridomas (approximately 1%) producing anti-TT antibody was similar in EBV and TT stimulated cultures. A marked increase in immunoglobulin secretion was observed after hybridization and pre-selection of EBV cocultures for high anti-TT production prior to fusion resulted in a five fold increase in TT specific hybridomas ($p < .001$). Most (20/21) specific hybrids produced IgM anti-TT whereas few (1/21) produced IgG anti-TT, possibly due to the immature stage of differentiation in EBV stimulated parental cells. The ability to choose an antigen, immunize a human subject and expand the rare antigen specific B cells from PBL, in vitro, with EBV, prior to fusion, should yield an increasing spectrum of human monoclonal antibodies for diagnostic, therapeutic or basic studies.

This work was supported by the National Science and Engineering Research Council of Canada. Donata Kosbor was a research student of the National Cancer Institute of Canada.

CELL SURFACE ANTIGENS DEFINED BY FOUR MONOCLONAL ANTIBODIES RAISED AGAINST KG-1a CELLS. C.I. Civin, C. Brovall, L.C. Strauss, J.F. Schwartz and J.H. Shaper. The Johns Hopkins Oncology Center, Baltimore, MD 21205

Libraries of monoclonal antibodies (McAb) specific for immature as well as mature human lymphoid cells have been described. We and several others have developed McAb against mature human granulocytic and monocytic cells. In addition, we have recently characterized 4 McAb, anti-My-10-13, raised against the immature (pre-myeloblast) granulocytic cell line KG-1a. Anti-My-10 reacts with protein with an apparent molecular weight of about 110 kilodalton, and anti-My-11 about 230 kilodalton. We have not yet been able to detect the My-12 or My-13 antigens using KG-1a cell surface radioiodination and immunoprecipitation. The cell surface expression of these 4 antigens and the My-1 antigen, as determined by indirect immunofluorescence, on several cell types is shown below:

Cell type	My-1	My-10	My-11	My-12	My-13
KG-1a	-	+	+	+	+
KG-1	-	+	+	+	+
U-937	+	-	+	+	+
Daudi	-	-	+	-	+
K-562	+	-	-	+	+
HEL	-	-	-	+	-
ML-1	+	-	-	-	-
ML-60	+	-	-	-	-
Granulocytes	+	-	-	-	-
Red Cells	-	-	-	-	-
Platelets	-	-	-	-	-
Monocytes	-	-	+	-	-
Lymphocytes (B,T)	-	-	+	-	-
Marrow Leukocytes	>50%	<5%	25%	<5%	<10%
AMLL Patients	50%	30%	60%	20%	5%
ALL Patients	0%	25%	40%	5%	10%

My-1 is not expressed by granulocyte-macrophage colony-forming cells (CFC-GM). Our recent data indicate, however, that My-10, -11, and -13 are expressed on CFC-GM. Thus, these novel antigens are expressed on very early marrow cells and are components of the program of human granulocyte differentiation.

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