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Ten-Minute Presentations from  
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IN VITRO STIMULATION OF LYMPHOCYTES AS A SOURCE OF HUMAN MONOCLONAL ANTIBODIES USING THE EBV-HYBRIDOMA TECHNIQUE. Denise Kozler and John C. Roder, Department of Microbiology & Immunology, Queen's University, Kingston, Canada. K7L 3N6.

We have previously shown that human hybridomas can be constructed with antigen-specific, Epstein-Barr virus transformed cell lines by fusion with SP-4, a 6-thioguanine and ouabain resistant lymphoblastoid cell line (Kozler, D; Lagarde, A; and Roder, J.C., Proc. Natl. Acad. Sci. 79:6451-6455, 1982). The resulting hybrids were stable (51 yr), have high cloning efficiency (64%), divide rapidly (< 24 hr), and produce reasonable levels of specific antibody (3-7 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 tumor toxoid immunized donors with Epstein-Barr virus yielded cells with much higher frequencies of hybrid formation ( $36 \times 10^{-6}$ ) compared to unstimulated PBL or cells cultured with Pokeweed mitogen (PWM) or tumor toxoid (TT) antigen. The proportion of hybridomas (approximately 12) 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 subcultures for high anti-TT production prior to fusion resulted in a five fold increase in TT specific hybridomas (p < .001). Most (10/21) 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. Denise Kozler 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. Drovall, 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 radiolodination 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	-	-	-	+	-
HL-60	+	-	-	-	-
Granulocytes	+	-	-	-	-
Red Cells	-	-	-	-	-
Platelets	-	-	-	-	-
Monocytes	-	-	+	-	-
Lymphocytes (0.1)	-	-	+	-	-
Marrow Leukocytes	+50%	+5%	25%	+5%	+10%
AHL 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.

Supported in part by NIN grants CA 32310, CA 06973, CA 090-7104, and grant #1418 from the Council for Tobacco Research USA, Inc. C.I. Civin is the recipient of an ACS Junior Faculty Clinical Fellowship.

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# Hybridoma

edited by ZENON STEPLEWSKI  
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NUMBER 1

1983

Hypothesis: Macrophages as Effector Cells for Human Tumor Destruction Mediated by Monoclonal Antibody. By Z. STEPLEWSKI, D. HERLYN, G. MAUL, and H. KOPROWSKI.	1
A Comparative Analysis of the Phenotypic Characteristics of Available Fusion Partners for the Construction of Human Hybridomas. By D. KOZBOR, D. DEXTER, and J.C. RODER.	7
Further Biochemical Studies of the Human B-Cell Differentiation Antigens B1 and B2. By H.W. OETTGEN, P.J. BAYARD, W. VAN EWIK, L.M. NADLER, and C.P. TERHORST.	17
Mouse Alloantigen System Ly-m22 Predominantly Expressed on T Lymphocytes and Controlled by a Gene Linked to Mls Region on Chromosome 1. By N. TADA, S. KIMURA, Y. LIU-LAM, and U. HAMMERLING.	29
Identification of Human Hepatoma-Defined Cell Surface Molecules. By D.H. MORIARITY, N. FOX, D.P. ADEN, J.R. HOYER, AND B.B. KNOWLES.	39
Monoclonal Antibodies to Murine Immunoglobulin Isotopes. By S. WEISS, K. LEHMANN, and M. COHN.	49
Characterization of Murine Hemopoietic Cells Using Rat Anti-Mouse Monoclonal Antibodies. By M.R. LOKEN, D.S. DESSNER-DE JOSE, G. VAN ZANT, and E. GOLDWASSER.	55
In Vivo and In Vitro Binding of Iodinated Monoclonal Antibody A2B5 to RIN Insulinoma Cells. By K. SHIMIZU, D. REINTGEN, R. ROWLEY, W. COLEMAN, W. BRINER, H. SEIGLER, and G.S. EISENBARTH.	69
Monoclonal Antibodies Directed Against Human Fibroblast Interferon: Characterization and Functional Studies. By L.J. NYARI, Y.H. TAN, and H.A. ERLICH.	79
Contamination of Polyethylene Glycol with Aldehydes: Implications for Hybridoma. By J.L. KADISH and K.M. WENC.	87
Statistical Analysis of Repetitive Subcloning by the Limiting Dilution Technique with a View Toward Ensuring Hybridoma Monoclonality. By H.A. COLLER and B.S. COLLER.	91 ✓
Monoclonal Antibodies Specific for the b5 Allotype of Rabbit Kappa Light Chains. by W.T. McCORMACK and K.H. ROUX.	97
Invited Speakers' Abstracts from the Second Annual Congress for Hybridoma Research	109
Ten-Minute Presentations from the Second Annual Congress for Hybridoma Research	119

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