MONOCLONAL ANTIBODIES AND CANCER THERAPY

Ralph A. Reisfeld and Stewart Sell, Organizers

January 26 — February 2, 1985

January 27:

Monoclonal Antibodies to Human Cancer (02) 35-37

Poster Abstracts 0077—0103: Monoclonal Antibodies to Tumor Antigens and their Use in Diagnosis (04) 37-46

Human-Human Hybridomas (05) 46-48

January 28:

Monoclonal Antibody Therapy in Patients with Leukemia and Lymphoma (06) 48-49

Workshop: Monoclonal Antibodies to Premalignant Cells (07) 49-51

Poster Abstracts 0112—0118: Monoclonal Antibodies to Premalignant Cells (08) 50-51

Modulation of Effector Cells by Monoclonal Antibodies (09) 51-52

January 29:

Drug-Antibody Conjugates (10) 53

Workshop: Monoclonal Antibody Conjugates with Drugs or Toxins (11) 54

Poster Abstracts 0125—0136: Monoclonal Antibody Conjugates (12) 54-58

Toxin-Antibody Conjugates (13) 58-59

January 30:

Monoclonal Antibodies To Cell Surface Receptors (14) 59-61, 74

Workshop: Monoclonal Antibodies to Glycolipids and their Applications (15) 61-62

Poster Abstracts 0145—0160: Monoclonal Antibodies to Cell Surface Receptors, Glycolipids, Lymphokines, Matrix and Cytoskeletal Components (16) 62-67

Monoclonal Antibodies to Lymphokines (17) 67

January 31:

Monoclonal Antibody-Radionuclide Conjugates (18) 68

Cancer Vaccines (21) 69-71

February 1:

Monoclonal Antibodies to Oncogene Products (22) 71-73

Special Lecture (23) 73

Monoclonal Antibodies to Human Cancer

0072 CLINICAL APPLICATION OF MONOCLONAL ANTIBODIES REACTIVE WITH EPITHELIAL OVARIAN CANCER. R.C. Bast, Jr., V.R. Zurwaski, Jr., and R.C. Knapp. Duke University Medical Center, Durham, N.C. 27710; Centocor, Malvern, Pa. 19355; and the Dana Farber Cancer Institute, Boston, Ma. 02115.

Several monoclonal reagents have been prepared in different laboratories which react with epithelial ovarian carcinomas (1). The murine IgG1 immunoglobulin OC125 reacts with CA125 determinants expressed by >80% of nonmucinous epithelial ovarian carcinomas. Traces of CA125 can be detected in amnion and in tissues derived from the coelomic epithelium, but CA125 is not found in fetal or adult ovary. Using a radioimmunometric sandwich assay, elevated serum CA125 levels have been found in >80% of patients with surgically demonstrable ovarian cancer (2). Doubling or halving of abnormal antigen levels has correlated with increasing or decreasing tumor burden in >90% of instances studied. Elevation of CA125 preceeded clinical recurrence of disease by 1-14 (mean=5) months in 19 of 22 patients. In one fortuitous case CA125 levels were elevated 10 months prior to the primary diagnosis of ovarian cancer (3). Among 988 non-pregnant patients with benign gynecological disease CA125 levels were comparably elevated in 1% on a single determination and in 0.5% on two determinations (4). In patients undergoing diagnostic laparotomy for pelvic masses, CA125 was elevated in 13 of 14 with frankly malignant gynecologic tumors, but in only 1 of 78 with benign disease. CA125 deserves further evaluation as a test for early detection of ovarian neoplasms. Different groups are currently evaluating monoclonal antibodies for radionuclide imaging and for treatment of ovarian cancer.

1. Bast, R.C. and R.C. Knapp, Seminars Oncol, 11:264-274, 1984.
2. Bast, R.C., et al, New England J. Med. 309: 883-887, 1983
3. Bast, R.C., etal, Gynecol Oncol, in press.
4. Niloff, J., et al, Am. J. Obstet Gynecol, in press.

0073 MONOCLONAL ANTIBODIES FOR HISTIOCYTE/INTERDIGITATIN RETICULUM CELL-RELATED LYMPHOMAS. Su-Ming Hsu and Pei-Ling Hsu, University of Texas, Houston, Tx. 77030

Recent development in monoclonal antibodies has improved significantly the understanding of T or B cell lymphomas. However, a third group of human lymphoma derived from monocyte/histiocyte/interdigitating reticulum cell (IRC) has not been yet studied due to the lack of specific antibodies. We have produced three MoAb, 2H9, 1E9 and 1A2 that may facilitate the diagnosis of true histiocytic lymphoma (THL), malignant histiocytosis (MH) and Hodgkin's disease (HD). These antibodies were made by immunizing mice with the SU-DHL-1 cells that lack Ig gene rearrangements and do not express B or T cell markers, but share features of monocyte/histiocyte. All three antibodies do not react with B or T lymphocytes. Monoclonal 1A2 react with rare cells scattered in the normal lymphoid tissues. Both 2H9 and 1E9 stain histiocyte and IRC. The finding suggests a possible relationship between histiocyte and IRC. In frozen sections of lymphomas, the reactivity to these three antibodies are restricted to only THL, MH and HD (See Table).

	2H9	1E9	1A2
THL	+	+	+
MH	-	+	-
HD	+	-	+

All 37 cases of B or T cell lymphomas are consistently negative. In order to clarify the relationship between THL, MH or HD and monocyte, histiocyte or IRC, the phenotypes of THL, MH and HD are also determined by other monoclonal antibodies, including Leu M1, Mo2, OK M1, HeFi-1 and Tac. The results are then compared with the phenotypes of SU-DHL-1, U-937 histiocytic cell lines and H-RS cells in short term culture with or without phorbol ester induction. U-937 cells show a phenotype similar to that of three cases of MH tested. After induction, U-937 cells present a phenotype resembling that of free histiocyte. SU-DHL-1 and all THL have a similar expression which resembles that of fixed histiocyte. H-RS cells have markers similar to that of IRC.

It is concluded that THL, MH and HD are most likely the neoplasms of fixed histiocyte, free histiocyte and interdigitating reticulum cell, respectively.

0074 CLINICAL STUDIES OF AN ANTIGEN (DU-PAN-2) DEFINED BY A MONOCLONAL ANTIBODY TO PANCREATIC ADENOCARCINOMA CELLS, Richard S. Metzgar, David M. Mahvi, Michael S. Lan, Olivera J. Finn, Robert C. Bast, William C. Meyers, Michael J. Borowitz and H. F. Seigler, Departments of Microbiology and Immunology, Medicine, Surgery and Pathology, Duke University Medical Center, Durham, NC 27710

DU-PAN-2 is a heavily glycosylated antigen defined by a monoclonal antibody elicited to a pancreatic adenocarcinoma cell line (1). Soluble DU-PAN-2 antigen is readily detected in body fluids by a competition radioimmune assay (RIA). Details on the isolation and properties of the mucin-like DU-PAN-2 antigen are in press (2) and will be summarized in the presentation. The antigen is expressed on a variety of neoplastic and non-neoplastic tissues (3) and appears to be an oncofetal or differentiation type antigen. Data will be presented on the oncofetal nature of the antigen as reflected in a difference in the tissue and cell distribution of the antigen between the fetus and the adult. Moreover, cord blood levels of the DU-PAN-2 antigen as determined by the competition RIA are high before falling to normal adult levels in the post natal period. The antigen is also expressed at high levels in the serum of patients with adenocarcinomas, especially those with tumors of the pancreas, stomach and gall bladder (4). Serial studies of DU-PAN-2 levels in the sera of patients with pancreatic cancer undergoing various forms of therapy will be presented. Antigen levels can accurately reflect the clinical course of the disease and may be a valuable adjunct to predicting recurrent or progressive disease. The data also suggests that decreases in DU-PAN-2 antigen levels may be a way of evaluating therapy in these patients.

1. Metzgar, R.S., Gaillard, M.T., Levine, S.J. et.al. Cancer Research 42: 601-608, 1982.

2. Lan, M.S., Finn, O.J., Fernsten, P.D. and Metzgar, R.S. Cancer Research (In Press).

3. Borowitz, M.J., Tuck, F.L., Sindelar, W.F., Fernsten, P.D., and Metzgar, R.S. J.N.C.I. 72: 999-1005, 1984.

4. Metzgar, R.S., Rodriguez, N., Finn, O.J. et.al. Proc. Natl. Acad. Sci. USA 81: 5242-5246, 1984.

0075 MONOCLONAL ANTIBODIES, ONCOGENES, AND HUMAN CARCINOMAS, J. Schlom, P. Horan Hand, D. Colcher, A. Thor, D. Wunderlich, R. Muraro, and M.O. Weeks, Laboratory of Tumor Immunology and Biology, NCI, Bethesda, MD 20205

Monoclonal antibody (MAb) B72.3 is directed against a 200,000 to 400,000-d tumor-associated glycoprotein (termed TAG-72) which is found on the surface of human colon carcinoma and breast carcinoma cells and not to any appreciable extent in normal adult tissues examined (1). This MAb has been used successfully to detect occult adenocarcinoma cells. Retrospective studies revealed that greater than 95% of effusions from patients with confirmed adenocarcinoma of the breast, ovary, or colon contained cells expressing TAG-72, while the various cell types in benign effusions were negative (2). The TAG-72 antigen has also been found in the blood of approximately half of colon carcinoma patients tested; the competition RIA developed has been shown to be useful in detecting tumor associated antigen in bloods of certain carcinoma patients scoring negative for other tumor associated antigens. Radiolabeled MAb B72.3 is also currently being evaluated for its ability to detect metastatic lesions in colon carcinoma patients via gamma scanning. In parallel studies, human recombinant interferon has been shown to enhance the cell surface expression of TAG-72 as well as other carcinoma-associated antigens (3).

MAbs of predefined specificity have been generated by utilizing a synthetic peptide reflecting amino acid positions 10-17 of the Hu-*ras*^{T24} gene product as immunogen (4). Since the Hu-*ras* reactive determinants (positions 10-17) have been predicted to be within the tertiary structure of the p21 molecule, it was not unexpected that denaturation of cell extracts or tissue sections with formalin or glutaraldehyde enhanced binding of the MAbs. When paraffinembedded formalin-fixed tissue sections and the avidin-biotin complex immunoperoxidase methods were used, the MAbs clearly defined enhanced *ras* p21 expression in the majority of human colon and mammary carcinomas. The majority of all abnormal ducts and lobules from fibroadenoma and fibrocystic disease patients were negative, as were all normal mammary and colonic epithelia examined. These studies thus provide a means to evaluate *ras* p21 expression within individual cells of normal tissues and benign, "premalignant," and malignant lesions.

1. Stramignoni, D., Bowen, R., Atkinson, B., and Schlom, J. Intl. J. Cancer 31: 543-552, 1983.
2. Szpak, C.A., Johnston, W.W., Lottich, S.C., Kufe, D., Thor, A., and Schlom, J. Acta Cytologica 28: 356-367, 1984.
3. Greiner, J.W., Horan Hand, P., Noguchi, P., Fisher, P., Pestka, S., and Schlom, J. Cancer Res. 44: 3208-3214, 1984.
4. Horan Hand, P., Thor, A., Wunderlich, D., Muraro, R., Caruso, A., and Schlom, J. Proc. Natl. Acad. Sci. 81: 5227-5231, 1984.

0076 CANCER MARKERS: PAST, PRESENT AND FUTURE. Sell, S., University of Texas Medical School, Houston, Texas 77005, USA

The diagnosis and prognosis of cancer diploids primarily on identification of cancerous tissue by a pathologist. Pathologist recognize morphologic differences between cancer and normal tissue essentially by their degree of differentiation. Less differentiated cells also produce substances reflecting their state of differentiation that may be identified by biochemical immunological methods. So called cancer markers include secretial proteins, cell surface markers, hormones, enzymes, isozymes, and cytoplasmic constituants. The modern era of cancer markers began in 1962 with the discovery of alphafetoprotein by C.I. Abelev of the Soviet Union. Since the availability of monoclonal antibodies to cancer cells in the late 1970's, there has been an experimental increase in the number of new cancer markers identified. This presentation will cover the historical development of cancer markers with a level prediction of things to come.

Monoclonal Antibodies to Tumor Antigens and Their Use in Diagnosis

0077 INTERACTIONS OF HUMAN OVARIAN TUMOR CELLS WITH HUMAN MESOTHELIAL CELLS GROWN ON EXTRACELLULAR MATRIX: CHARACTERIZATION WITH HUMAN TUMOR SPECIFIC MONOCLONAL ANTIBODY F36/22. Ralph J. Bernacki, Gary A. Croghan, Kent Crickard[1], and Michael J. Niedbala, Roswell Park Mem. Inst. and Buffalo General Hospital[1], Buffalo, NY 14263.
Human ovarian tumor cells generally metastastize by direct extension within the peritoneal cavity and this phenomena is associated with tumor cell implantation onto peritoneal surfaces lined with mesothelial cells. We report here on the use of bovine corneal endothelial cell extracellular matrix (ECM) coated tissue culture dishes, as a growth substrate for cell lines established from human ascites ovarian tumors including both an ovarian tumor cell line A-1 and a mesothelial cell line HMC-70, and these cells' reactivity with murine monoclonal antibody (McAb) F36/22. Direct immunofluorescence staining using fluorescein conjugated McAb F36/22 and indirect immunoperoxidase staining of A-1 tumor cells plated onto a monolayer of highly flattened, closely opposed, human mesothelial cells HMC-70 grown on ECM demonstrated antibody reactivity specifically with only the A-1 human ovarian tumor cells. In an immunoperoxidase study performed at various times after seeding A-1 tumor cells onto normal mesothelial cells grown on ECM we observed that the tumor cells caused a retraction of the mesothelial cells allowing for the preferential attachment of A-1 tumor cells to the exposed ECM. These studies demonstrate that monoclonal antibodies can distinguish tumor from normal cells in mixed cultures and that tumor cells have a direct effect on normal cell behavior and a preference for attachment to ECM. (Supported by NCI Program Grant CA-13038).

0078 BLOOD-GROUP RELATED TUMOR ASSOCIATED ANTIGENS RECOGNIZED BY MONOCLONAL ANTIBODIES. Magdalena Blaszczyk, Carolyn Ernst, Jan Thurin, Zenon Steplewski, Hilary Koprowski, The Wistar Institute, Philadelphia, PA 19104
Monoclonal antibody PrD8 detects type-2 chain structures- paragloboside: Galβ1→4 GlcNAcβ1→3 LacCer and H Group: Fucα1→2 Galβ1→4 GlcNAcβ1→3 LacCer but not other isomeric structures of type 2 and 1. It detects antigen(s) present in normal gastric mucosa (8/14) and pancreatic accini (4/4) and adenocarcinomas derived from these sites (4/14) and (1/4) respectively, independently of blood group status. The antigen detected by monoclonal antibody PrD8 is not present in normal proximal or distal colon tissue (0/15) but was seen in carcinomas derived from the proximal (3/7) but not distal colon. BR-55-2 belongs to the series of monoclonal antibodies which react with X structure, however the final specificity is not determined. The differences in specificity for X structure of BR-55-2 and the antibody WGHS 29-1, which recognizes terminal X structure, are reflected in immunohistochemical reactivity. The antigen 55-2, although present in normal tissues, is present with lower frequency than 29-1. The most striking difference in binding of those monoclonal antibodies is seen in colon and breast tissues. Whereas BR-55-2 MCF-7 was not seen in normal colon (0/4) it is present in colon carcinomas (4/4). On the other hand, 29-1 is detected in both normal (4/4) and malignant tissues (4/4). Presented examples of the antigens which are defined by monoclonal antibodies PrD8 and BR-55-2 might be considered as tumor asssociated markers for proximal and distal colon adenocarcinomas, respectively.

0079 MONOCLONAL ANTIBODIES TO TUMOR SPECIFIC PROTEINS IN COLON ADENOCARCINOMAS.
Lidia C. Boffa and Stefania Sciallero, Istiuto Nazionale per la Ricerca sul Cancro, IST, Viale Benedetto XV n. 10, 16132 Genova ,Italy.

We have biochemically characterized and electrophoretically purified tumor specific nuclear protein markers (similar in size and isoelectric point) in carcinogen induced (DMH:1,2-dimethylhydrazine) murine and in spontaneous human colon carcinomas. We were able to obtain two tumor specific monoclonal antibodies from these "tumor specific" human adenocarcinoma purified non histone nuclear protein fractions. These antibodies react with not only nuclear proteins and chromatin but also ,to some extent, with the correspondent cytoplasmic fraction of all the human colon carcinoma analyzed so far. They react also with most of the available colon carcinoma derived established cell lines. These human antibodies present a certain degree of crossreactivity with the monoclonal antibodies obtained from "tumor specific" nuclear proteins derived from the murine (carcinogen induced) colon carcinomas. The human antibodies do not recognize the above mentioned cellular components from: normal adiacent colon mucosa of adenocarcinoma bearing patients, ulcerative colitis or familial polypiosis patients at a non degenerative stage and normal rat colon.
Their degree of specificity makes these antibodies suitable for clinical diagnostic and possibly chemiotherapeutic applications

0080 CHARACTERIZATION OF A FORMALDEHYDE FIXATION RESISTANT EPITOPE SELECTIVE FOR HIGHLY DIFFERENTIATED ADENOCARCINOMA OF THE LUNG,
K. Bosslet*, K. Kayser°, G. Lüben*, H.H. Sedlacek*. *) Research Laboratories of Behringwerke AG, P.O.-Box 1140, D-3550 Marburg/Lahn, FRG; °) Institute of Pathology, University of Heidelberg, D-6900 Heidelberg, FRG.

The specificity of the murine monoclonal antibody (moab) BW 436/15 of IgG3, κ-isotype, was evaluated using the indirect immunoperoxidase technique on formaldehyde fixed paraffin-embedded tissue sections. Highly differentiated human adenocarcinomas of the lung were strongly stained (4/4), whereas a variety of undifferentiated adenocarcinomas (0/7), squamous carcinomas (0/12), large cell carcinomas (0/10) and small cell lung carcinomas (0/7) did not react. Furtheron, the moab defined epitope was strongly expressed on dysplastic epithelium of the lung and the interlobular ducts of the pancreas. Antigens bearing this epitope were immunoprecipitated from extracts of a xenotransplanted tumor of the pancreas (PaTuI) and found to have an approximate molecular weight of 200 kDa in SDS-PAGE under reducing conditions. Experiments to find out the biochemical nature of the epitope are underway.

0081 MONOCLONAL ANTIBODIES DIRECTED AGAINST HUMAN SARCOMAS.
Ø. Bruland, Ø. Fodstad, S. Funderud, A. Pihl. NHIK, The Norwegian Radium Hospital, Oslo 3, Norway.

Monoclonal antibodies directed against human sarcomas were prepared in an effort to define sarcoma-associated antigens. Cells from a xenografted human osteosarcoma were used for immunization and 230 hybridomas were obtained. 7 secreted ab capable of binding to 2 different osteosarcomas, but not to fibroblasts from several sarcoma patients as demonstrated by indirect immunofluorescence on unfixed cells. Of the 7 ab, TP-1 (isotype Ig-2a), did not cross-react with non-sarcoma tumor cells or normal hematopoietic cells. Binding of TP-1 was not affected by methanol or acetone fixation. Further specificity was tested on acetone fixed frozen sections using 3-step peroxidase staining. TP-1 showed no significant binding to a wide range of normal adult tissue, but weak binding was seen with kidney tubules and striated muscle from fetal tissue (28 weeks). Of the 26 sarcomas tested 5/5 osteosarcomas, 5/5 malignant fibrous histiocytomas, 1/1 chondrosarcoma, 2/2 synovialsarcomas as well as 2/2 malignant hemangiopericytomas and 3/4 unclassified sarcomas stained positive. 3 rhabdomyo-, 2 leiomyo-, and 2 liposarcoma were all negative. 41 non-sarcoma malignancies of 12 different histological types were also negative. This specificity profile indicates that the TP-1 monoclonal may prove valuable in immunohistological classification of tumors.

0082 ANALYSIS OF RAT MONOCLONAL ANTIBODIES DIRECTED AGAINST IDIOTYPE RESCUED FROM MALIGNANT HUMAN B CELLS HYBRIDIZED TO A NOVEL HETEROMYELOMA, Michael Campbell, Marcia Bieber, Ronald Levy and Nelson N.H. Teng, Stanford University, Stanford, CA 94305

Since the initial report of the therapeutic benefit of murine monoclonal anti-idiotype antibody therapy of a human B cell lymphoma, such treatment modalities have met with varying degrees of success. We chose to analyze a panel of monoclonal antibodies directed against a single idiotype in an attempt to define which properties of these antibodies might be important in improving therapeutic efficacy. Tumor cells from four patients with B cell malignancies were hybridized to a human-mouse heteromyeloma cell line. Hybridoma yields of 21 to 62% were obtained from these rescue fusions; 35 to 100% of these hybrids secreted human immunoglobulin. Several subclones from these have been stable for growth and production over a one year period. One rescue in which the majority of hybrids secreted IgM molecules containing κ light chains was chosen for further study. For the production of monoclonal antibodies against this rescued protein, a rat hybridoma system was chosen as several advantages of this system, compared to the murine system, have been reported in the literature. A panel of rat monoclonal antibodies specific for the immunizing IgM, κ idiotype was produced. FACS analysis demonstrated binding to tumor cells. Further characterization was performed with regard to isotype, avidity, idiotope recognition, and ability to induce antigenic modulation. (Supported in part by the Robert L. Seidner Research Fund and N.I.H. Grants CA-36422 and CA-09302.)

0083 TREATMENT OF CHRONIC LYMPHATIC LEUKEMIA WITH MONOCLONAL ANTI-IDIOTYPIC ANTIBODIES Peter J.A.Capel, Frank W.M.B.Preijers and Wil Allebes, Dept.Nephrology, Radboud Hospital, Nijmegen, The Netherlands

A monoclonal antibody of the IgG1 subclass reactive with the idiotype of the leukemic B-cells of a B-CLL patient was raised. This antibody did not show any cross reactivity with other cells or tissues, and in total 40 grams of anti-idiotypic antibody was purified from ascites. Because human Fc receptors are polymorphic (only 70% of normal individuals can react with murine IgG1), the patient's Fc receptors were typed and showed to be reactive with murine IgG1. The patient had a high level of free circulating idiotype (150 μg/ml) and upon in vivo treatment the free idiotype was cleared without any signs of serum sickness or other side effects. After the removal of free idiotype a strong tumor reduction was observed. The swollen lymph nodes decreased to a normal size and the spleen, with a weight of about 4 kg, reduced 20% in size. In vivo a moderate modulation of the antigen was present and the modulated idiotype was reexpressed within 16 hours. During repeating treatments the in vivo modulation became more pronounced and the therapy lost its effectiveness.

The anti-idiotype was conjugated to Ricin A chain and tested in vitro for its capacity to induce specific toxicity. The protein syntheses of the B-CLL cells was inhibited for 50% at a concentration of 5.10^{-8}M immunotoxin after a 24 hrs incubation. This low specific toxicity was most likely due to a low idiotype expression on the B-CLL cells.

We may conclude that murine IgG1 anti idiotypic antibodies are effective in vivo without any side-effects even in the presence of high levels of free idiotype, but due to antigen modulation this type of therapy was only partially effective.

0084 BIOCHEMICAL CHARACTERIZATION OF A PROSTATE TISSUE SURFACE ANTIGEN, Ann M. Carroll and Mark I. Greene, Tufts Medical School and Harvard Medical School, Boston, MA.

We have previously described a monoclonal antibody, F77-129, which binds to a tissue-specific antibody that is selectively expressed by human prostate and breast cells. Preliminary specificity screening and imaging analyses using iodinated antibody in xenograft-bearing nude mice indicated a potential role of this antibody in diagnostic scanning and therapy. Recently we have studied the biochemical nature of this surface antigen by immunoprecipitation of radiolabeled prostate and breast cell lysates and SDS-PAGE. Using Sepharose protein-A coupled F77-129, a 32-35 Kd structure is precipitated from lysates of surface iodinated PC-3 human prostate and BT-20 human breast adenocarcinoma cell lines; this band is not precipitated from lysates of G-361 human melanoma cells(negative control). A similar band (31-33 Kd) is also reproducibly precipitated from BT-20 breast but not G-361 or T cell lysates prepared from cells internally labeled by culture with ^{35}S-cysteine. Preliminary analysis of supernates of ^{35}S-cysteine labeled BT-20 cultures show that this antigen is not secreted. Unlabeled supernates also failed to block binding of F77-129 to surface antigen positive cells in RIA and FACS analysis. The surface structure identified by F77-129 is similar to described intracytoplasmic human prostate antigen (PA) in molecular weight, but it shows distinctive subcellular localization. (This work is supported by Grant 3P01 CA 14723-11 and a grant from the Council for Tobacco Research).

0085 A Radioimmunoassay to Detect a Tumor-Associated Glycoprotein (TAG-72) in the Serum of Carcinoma Patients using Monoclonal Antibody B72.3, David Colcher, Andrew J. Paterson, Henry F. Sears, and Jeffrey Schlom, Laboratory of Tumor Immunology and Biology, NCI, Bethesda, MD 20205 and Fox Chase Cancer Center, Philadelphia, PA 19111

Monoclonal antibody (MAb) B72.3 was generated against a carcinoma metastasis and has been shown to bind with a high degree of selectivity to a tumor associated 200-400 Kdal glycoprotein (TAG-72) found in human colon and breast carcinomas versus normal adult tissues. A competition radioimmunoassay has been developed for the detection of TAG-72 in the blood of patients with carcinomas based on the inhibition of binding of MAb B72.3 to a target preparation of carcinoma cell extract. A standard competition curve was established and the results that were obtained with serum samples were converted into units per ml, with 1 unit of activity being equivalent to competition observed with one microgram of the tumor cell extract. Sera from apparently normal patients (n=12) were tested and a mean of 2.2 units/ml was detected. None of the 19 sera tested from patients with benign colon tumors or inflammatory colon diseases contained antigen above the normal cut-off level. Seven out of 20 (35%) of the advanced colon carcinoma patients had greater than 5.6 units/ml (mean + 3 standard deviations obtained for the normal sera), with values ranging from 6.7 to 73 units/ml. Elevated levels of TAG-72 were found in the sera of 7 of 20 patients with other carcinomas including those of the lung, pancreas and stomach. Several sera from colon cancer patients were identified which were negative for other antigens recognized by other monoclonal antibodies (CEA, GICA, and OC125), but which scored positive for TAG-72. These studies thus show the potential utility of the detection of TAG-72 in sera in the management of human carcinomas.

0086 EXPRESSION OF BLOOD GROUP ANTIGENS AND IMMUNOGLOBULINS BY HEAD AND NECK SQUAMOUS CARCINOMA TISSUES. Lyman E. Davis, Paul Lyons, Annette Barnes and Byron Anderson Northwestern University, Chicago, IL 60611

The blood group antigen (Ag) composition of head and neck squamous carcinoma tissues was compared to that of normal control tissues using the immunohistochemical procedure of Pankow _et al._ (_J. Histochem. Cytochem._ 32, 771, 1984). Ags examined included A,B,H,I, i,Le^a,Le^b,Le^x, Le^y, sialyated Le^a,P,P_1,P^K, SSEAIII, type 1 chain, M, N,T,T_n, mannose and sialic acid containing structures. While ABH expression for normal epithelia was that expected based upon ABO blood type, loss of these Ags was observed in a majority of tumor tissues examined. Le^x and T structures were detected on selected tumor tissues, but were not observed on normal tissues. Le^Y and various sialyated structures, while present on normal epithelia, were found in elevated amounts on certain carcinoma tissues. Since immunoglobulins may bind to tissues and mask blood group Ag expression, methodologies were developed for the removal of tumor associated antibody which maintained immunoreactivity of the eluted fraction. Such eluted antibodies were quantitated by a sandwich enzyme-linked fluorescence assay system which allowed detection of as little as 1ng of immunoglobulin.

0087 ANTIGENS OF HUMAN SQUAMOUS LUNG CARCINOMA DEFINED BY MURINE MONOCLONAL ANTIBODIES, Philip D. Fernsten, Ralph A. Reisfeld, and Leslie E. Walker, Scripps Clinic and Research Foundation, La Jolla, CA 92037

A panel of ten murine monoclonal antibodies has been produced against cultured human squamous lung carcinoma cells. With one exception, all react with cell surface components. These antibodies can be subdivided into three groups based upon their reactivity. All react with fresh frozen sections of squamous lung carcinoma tissues in immunoperoxidase assays and all are unreactive with normal liver, lung, kidney, colon, spleen, and pancreas and with cultured lymphoblastoid cells in ELISA. Group 1 (PF2/2 and PF2/9) detect antigens which are shed from the surface of cultured cells and react also with breast, gastric, and colonic adenocarcinomas and some tumors of neuroectodermal origin. Group 1, which are both IgG3, immunoprecipitate non-glycosylated polypeptide components of Mr 24K and 46K, and a minor component of Mr 125K. Group 2 (PF2/1, PF2/5, PF2/7, PF2/8, and PF2/12) immunoprecipitate non-sulfated glycoprotein components of Mr 90K and 180K and a non-glycosylated component of Mr 38K. With the exception of PF2/12, Group 2, which are also all IgG3, react also with some lung adenocarcinomas and with certain differentiated strata within normal adult and fetal epidermis. Group 3 (PF2/4,an IgG1; PF2/6,an IgM; and PF2/11,an IgG2a) all additionally react with certain other tumors, as well as with normal adult and fetal epidermis. By western immunoblotting of cell extracts, PF2/4 detects an Mr 70K component and PF2/6 detects components of Mr 50K and 70K and a high molecular weight component. PF2/11 detects an antigen which is not expressed on the cell surface.

0088 Two Murine Monoclonal Antibodies That Bind To Human Neuroblastoma Cell Surface Antigens. Christopher N. Frantz, Reggie E. Duerst, Daniel H. Ryan and Louis S. Constine, University of Rochester School of Medicine, Rochester, NY 14642.

Two murine IgG2a monoclonal antibodies are characterized in terms of binding to human cell lines and tissues. They were selected by ELISA for binding to cell surface antigens on multiple human neuroblastoma cultured cell lines but not to peripheral blood mononuclear cells. Both antibodies bind to all human cultured non-hematopoietic tumor cell lines tested and to cultured human fibroblasts. In spite of negative binding by ELISA to blood and bone marrow mononuclear cells, one antibody (6-3) binds specifically to monocytes but not granulocytes, and to 5 of 5 human leukemia cell lines tested. The other (6-19) does not bind detectably to any hematopoietic cells. Both bind to non-hematopoietic tumor cells of many types, and to fibrous stroma of tumors. In the presence of complement, they are cytotoxic to cultured human neuroblastoma cells but not to bone marrow granulocyte-macrophage colony forming cells. When assayed by both Hoechst 33342 dye/trypan blue quenching and clonogenic assay, each antibody kills 99-99.9% of cultured neuroblastoma cells. These antibodies may prove useful to identify stromal cells and tumor cells in bone marrow. Similarly, they may prove useful for killing tumor cells by antibody dependent, complement mediated cytotoxicity in bone marrow harvested for autologous transplantation.

0089 TREATMENT OF ADVANCED B CELL LYMPHOMA WITH MONOCLONAL ANTI-IDIOTYPE ANTIBODIES, Annemarie Hekman, Elaine M. Rankin and Anke M. Honselaar, The Netherlands Cancer Institute, Amsterdam, The Netherlands.

Three patients with non-Hodgkin's lymphoma have been treated with mouse monoclonal antibodies (moab) directed against the idiotype of the tumor immunoglobulin. Two moabs were IgG2a and one IgG1. The patients, all with massive disease, received total amounts of 3.8, 5.8 and 5.2 gr of moab over periods of 3 weeks to 2 months. Patients were monitored for binding of moab to malignant cells in different sites, serum moab level, level of serum idiotypic Ig if present, clinical response, side effects and anti-mouse antibodies. Infusion of anti-idiotype resulted in transient falls in lymphocyte counts and/or temporary removal of circulating idiotype. Thereafter free moab became detectable in the serum. Binding to peripheral tumor cells was seen before complete clearance of free idiotype. Binding of moab in vivo to tumor cells in lymph nodes, bone marrow and ascites could be demonstrated. There was no evidence of modulation in 2 patients, in the 3rd case the circulating tumor cells showed a reversible decrease in antigen density. By reinjecting In^{111}-labeled cells with and without simultaneous moab infusion, it could be shown that the moab rapidly and permanently cleared malignant cells from the circulation. There was no increase of cell division in the peripheral cells. Treatment was free of side effects in 2 patients, the 3rd had an episode of acute hypotension and suffered from urticarial reactions. None of the patients made anti-mouse antibodies. Two patients had a minimal response to the treatment, with ca. 10% decrease in size of the lymph nodes. In the 3rd patient the tumor was unaffected.

0090 PRODUCTION OF ANTI-TUMOR ANTIBODIES BY IN VITRO IMMUNIZATION. May-kin Ho, and Paul J. Durda. E.I. DuPont de Nemours & Co., Billerica, MA 01862.

Anti-tumor monoclonal antibodies were generated by in vitro immunization of mouse spleen cells against a human breast tumor line, MCF-7. The sensitization procedure involved culturing spleen cells with 33% allogeneic thymocyte conditioned medium, and a monolayer of MCF-7 for five days. The sensitized cells were fused with P3.Ag8.653. After two weeks, 67% of the wells showed hybrids and 10% of the hybrids were reactive with MCF-7 as determined by cellular ELISA. One of the antibodies, MC1, recognises a tumor-associated antigen which is present on MCF-7 cells, but not on fourteen other tumor or normal cell lines. In contrast, another antibody, MC2, binds to all cell lines tested. The MC1 antigen is surface-associated and periodate-sensitive. Immunohistochemical studies of paraffin-embedded human tissues show that $MC1^+$ cells are found in all colon and pancreatic tumors examined. However, there is great heterogeneity in the percentages of positive cells and the intensity of staining. Some breast and lung tumors also weakly express MC1. Normal liver, heart, colon, intestines, and bone marrow are not stained. Interestingly, four out of six cases of morphologically-normal colon tissues adjacent to tumor sites are $MC1^+$ whereas normal colon is consistently negative. Therefore, the appearance of $MC1^+$ in colon tumors may preceed morphological changes associated with neoplasia.

0091 PRODUCTION OF IGM IN MICROCAPSULES: GENERATION OF MURINE ANTI-IDIOTYPE MONOCLONAL ANTIBODY FROM ENCAPSULATED HUMAN-HUMAN HYBRIDOMAS. A.P.Jarvis, P.Abrams, S.Littlefield, A.Buczynski, T.Grdina, C.Williams, S.Giardina, T.Gregorio, R.Klein, R.Schroff, and G.Koch. National Cancer Institute, Frederick, MD 21701, and Damon Biotech, Needham Heights, MA 02194.

A significant obstacle in the treatment of B-cell lymphoma with anti-idiotypic monoclonal antibodies is the isolation, in sufficient quantities of patient idiotypic IgM for immunization of mice. Our experiments used the human-human hybrid 7D7-G9, which was derived from the fusion of UC729-HF_2 and PBL from a patient with chronic lymphocytic leukemia. The IgM/k produced by this hybrid was harvested from microcapsule cultures at a concentration of 0.9-1.1 mg/ml and an initial purity of 26-52%. The IgM was purified further using conventional procedures and used to immunize BALB/c mice. Four IgG_{2a}/λ and two IgG_1/λ anti-idiotype clones were generated. Cytofluorographic analysis demonstrated that the two IgG_1 monoclonals reacted specifically with the patient tumor cells, while not reacting with tumor cells from either of two other patients. All of the anti-idiotype clones reacted with the 7D7-G9 hybridoma IgM as determined by ELISA and immunofluorescence, while none reacted with the HF_2 human parental line.

0092 AR-3: A MONOCLONAL ANTIBODY REACTIVE WITH HUMAN CARCINOMAS, M. Prat, I. Morra, G. Bussolati, G. Cortesina and P. Comoglio, Univ. of Torino, Med. School, 10126 Torino, Italy
2400 monoclonal antibodies (MAbs) were raised against the human epidermoid carcinoma line A431. The antibody produced by clone AR-3, when tested in ELISA, reacted with the cells used as immunogen and with lines established from human gastric, colon, pancreatic and ovarian carcinomas. This MAb was unreactive with human peripheral blood leukocytes or with a number of normal or neoplastic cell lines. The antibody precipitated a high molecular weight glycosylated component. When tested on paraffin sections, by the avidin-biotin peroxidase method (ABP), the AR3 antibody stained pancreatic (6:7), gastric (11:14), ovarian (5:6) and colon (4:8) carcinomas. A small minority of carcinomas of other organs was also stained; sarcomas, lymphomas and other tumors of non-epithelial origin were constantly negative. Staining of some normal epithelial cells was also observed. The MAb recognized metastatic carcinoma cells in peritoneal effusions. The monoclonal AR-3 was not inhibited by partially purified preparations of Carcino Embryonic Antigen (CEA) or Gastro Intestinal Carcinoma Antigen (GICA). Moreover, comparative analysis performed by the ABP method on tissue sections and MAbs against CEA or GICA showed lack of cross-reaction with the AR-3 defined antigenic determinant (CAR-3). The AR-3 MAb appears, thus, to broaden the number of available reagents for histopathological diagnosis of carcinomas, mainly of gastro-intestinal, pancreatic and ovarian origin (Supported by CNR, PFTB and PFCCN).

0093 DEVELOPMENT OF MOUSE MONOCLONAL ANTIBODIES REACTIVE AGAINST HUMAN SQUAMOUS CELL CARCINOMA. Raymond Ranken, Carmen White, Shirlee Yonkovich, Toby Gottfried and Emanuel Calenoff, Carcinex, Burlingame, California 94010

Balb/c mice were hyperimmunized with either solid squamous cell tumor pieces, or combinations of cell lines derived from squamous cell tumors. Supernates from the hybridomas derived from fusion with SP2/0 myeloma cells were extensively screened on frozen sections of different tumor types as well as normal tissues of squamous cell and non-squamous cell origin. The degree of tumor association with normal components has been assessed. The antibodies have been characterized as to potential clinical use - diagnostics, imaging, or therapy. Biochemical characterization is in progress as well as generation of preliminary data for their use in the clinical setting.

0094 MONOCLONAL ANTIBODIES DISTINGUISHING HUMAN MELANOMA CELLS AND NEVUS CELLS IN SITU
Bernhard Holzmann, Judith P. Johnson, Peter Kaudewitz and Gert Riethmüller. Institut für Immunologie der Universität München, Schillerstr. 2, 8000 München 2
The malignant melanoma of man provides a unique opportunity to study the natural history of a spontaneous solid tumor in situ. Normal melanocytes and several forms of nevi with activated melanocytes in various states are easily accessible for analysis of antigen expression. Monoclonal antibodies were induced by immunization with fresh tumors or melanoma cell lines and screening of hybridomas was performed by a binding test on sections of freshly frozen melanomas and nevi. Both melanomas and nevi were shown to express human Ia-like antigens, transferrin receptor and the transferrin related molecule p97. In contrast only 1 nevus out of 21 tested expressed both the glycoprotein gp75 defined by MoAb 15.75 and the protein p89 defined by MoAb P3.58, whereas 12 out of 15 melanomas tested expressed both antigens. MoAb P3.58 reacted with one additional melanoma and one nevus. The expression of these two molecules therefore appears to be correlated with the appearence of the malignant phenotype of melanocytes.

0095 HUMAN MONOCLONAL ANTIBODIES PRODUCED BY EPSTEIN BARR VIRUS CELL LINES BIND PROTEIN A, Michael Steinitz and Sara Tamir, Department of Hematology, Hadassah University Hospital, POB 24035, Jerusalem, Israel

Epstein Barr virus (EBV) is a polyclonal T-independent activator of viral-receptor-positive human B lymphocytes. Lymphocytes infected in-vitro with the virus are transformed into immortalized cell lines. Based on this phenomenon human cell lines that secrete specific IgM,IgA,IgG monoclonal antibodies are established. The targets of protein A (which is also a polyclonal T-independent B cell activator) are surface immunoglobulin and C3d receptor positive cells, as are the targets of EBV. We found that almost all (15 out of 16) of the tested specific monoclonal antibodies (IgM, IgG and IgA) produced in-vitro by EBV cell lines bind protein A. Unlike these in-vitro produced antibodies, a substantial fraction of the immunoglobulins in human sera does not bind protein A. Thus, those plasma cells which in-vivo secrete protein A non-binding immunoglobulins originate from B cell precursors that were EBV non infectible. Alternatively, binding of the human monoclonal antibodies might be related to the fact that they are produced in-vitro and secreted into tissue culture medium.

0096 Immunocytodiagnosis on cytospin preparations from fine needle tumor biopsies and malignant effusions using MoAb D6.1. Gary M. Stuhlmiller, Michael J. Borowitz, William W. Johnston and H. F. Seigler. Durham VA Hospital and Duke Univ. Med. Ctr., Durham, N.C. 27710.

A murine monoclonal antibody, D6.1, has been derived from the fusion of murine myeloma X63Ag8.653 with splenic lymphocytes from mice immunized with lectin-bound tumor associated antigen (TAA) partially purified from fresh melanoma tissue. The TAA defined by D6.1 is present on the melanoma cell membrane and appears not to be shed into tissue culture medium. Using D6.1 in both cell binding RIA and immunoperoxidase assays on fresh frozen tissue sections, the TAA was demonstrable on the majority of melanomas, 78% and 100%, respectively, as well as on certain other tumor types, most notable colon carcinomas. However, strong quantitative differences in TAA expression by melanoma and non-melanoma cell types readily permitted their differentiation. Based upon this observation, we have evaluated the diagnostic utility of D6.1 for solid tumor diagnosis on 53 cytospin preparations of fine needle tumor biopsies and malignant effusions by immunoperoxidase techniques. All melanomas tested, 10/10, reacted with D6.1, as did 4/5 colon carcinomas, 5/8 breast carcinomas and 5/7 lung carcinomas. Our previous studies failed to demonstrate appreciable reactivity of D6.1 with these latter two cell types, suggesting that visualization of weak membrane binding of MoAb's may be enhanced on cytospins compared to tissue sections. In spite of the apparently broader cross reactivity of D6.1 with non-melanoma tumor types, the strong quantitative differences in TAA expression on melanomas compared to non-melanomas were again evident. These findings suggest a potential diagnostic application for D6.1.

0097 INDUCTION OF SYNGENEIC ANTI-IDIOTYPIC IMMUNE RESPONSE BY HYPERVARIABLE REGION PEPTIDES TO A B CELL LYMPHOMA, Kristiaan Thielemans, Jonathan B. Rothbard, Shoshana Levy and Ronald Levy, Stanford University, Stanford, CA, 94305

The nucleic acid sequence of the heavy chain variable region expressed by 38C13, a B cell tumor of C3H origin, was determined by a combination of direct mRNA sequencing by primer extension and cDNA isolation and sequencing in M13. The V_H amino acid sequence was deduced and hypervariable regions were identified. From an analysis of predicted secondary structure and hydrophilicity, regions of predicted antigenicity were chosen and a series of synthetic peptides corresponding to CDR2 and CDR3 were produced. These peptides were coupled to protein carriers and used to immunize syngeneic C3H mice. All peptides gave rise to a vigorous antibody response, however, only the CDR3 peptides induced antibodies which cross-reacted with the isolated heavy chain protein. One CDR3 peptide induced a low frequency of antibody clones, isolated as hybridomas, which reacted with the intact IgM protein. All antibodies reacting with the heavy chain or the intact IgM protein were idiotypically specific for 38C13. These monoclonal anti-idiotype antibodies, raised against CDR3 peptides, gave strong reactions in ELISA and "Western" blots. However, they were of low affinity compared to "conventional" syngeneic anti-idiotype antibodies raised against the intact IgM protein.

0098 RADIOIMMUNOSCINTIGRAPHY AND RADIOIMMUNOTHERAPY OF RENAL CELL CARCINOMA XENOGRAFTS USING MONOCLONAL ANTIBODIES, Robert L. Vessella, Rei K. Chiou, Michael K. Elson Timothy D. Moon, Donald F. Palme, Rex B. Shafer and Paul H. Lange, University of Minnesota and VA Medical Centers, Minneapolis, MN 55417

Previous presentations from this laboratory have described the generation of monoclonal antibodies (Mabs) to human renal cell carcinoma (RCC) and the selection of three IgG1 Mabs (A6H, C5H and D5D) for radioimmunoscintigraphy (RIS) studies in nude mice (N > 180). Using 131-I as a radiolabel, RCC xenografts >40 mg were clearly imaged without the need for background subtraction. Biodistribution studies showed excellent localization within the RCC xenografts, greatly exceeding that found in the blood on a per weight basis. Preliminary radioimmunotherapy studies were initiated and have now been completed. Mice were either untreated, received unlabeled A6H, 100 uCi of 131-I radiolabeled A6H or a similar dose of a control IgG1 Mab. RCC xenografts (TK-177G or TK-82) were well established (approximately 100 mg) at the time the studies were commenced (day 0). The mice receiving radiolabeled A6H exhibited no progression in tumor size (now at day 105) and some tumors have regressed significantly. Those mice receiving no treatment, non-labeled A6H or the radiolabeled control Mab exhibited rapid progression and most had expired by day 60. At the time of death these tumors had increased >50 fold. Some animals receiving non-labeled A6H had a faster rate of progression than the untreated animals. The radiolabeled A6H mice received a total body dose of 300 rads and an RCC xenograft dose of approximately 5500 rads (single injection on day 0). These investigations demonstrate that a prolonged, and possibly permanent, arrest in RCC xenograft growth can be achieved by radioimmunotherapy when utilizing Mabs that perform well in RIS.

0099 DETECTION OF RAS GENE EXPRESSION USING MONOCLONAL ANTIBODIES GENERATED TO RAS GENE PRODUCTS, M.O. Weeks, P. Horan Hand, A. Caruso, A.D. Thor, D. Wunderlich, R. Muraro, and J. Schlom, Laboratory of Tumor Immunology and Biology, NCI, Bethesda, MD 20205

A variety of monoclonal antibodies (MAbs) have been generated to viral p21 (Furth et al., 1982, J. Virol., 43: 294) or to peptides representing the putative GTPase/GTP binding site in the normal vs. onc gene products. These antibodies have been used to detect: 1. the expression of ras related proteins in a variety normal and neoplastic human tissues through immunoperoxidase techniques, and 2. to examine the levels of ras gene expression in virally transformed or DNA transfected cells, transformation-defective viral mutants, and control cell lines.

Immunohistochemical analyses using MAbs generated to the synthetic peptide characteristic of the T24 ras gene product indicate that enhanced ras gene expression exists in colon carcinomas vs. normal colonic epithelium, benign colon tumors, or inflammatory lesions. Results also imply that expression level is related to local tumor invasiveness. Similar studies have revealed enhanced expression in mammary carcinomas when compared to fibroadenoma, fibrocystic disease, or normal breast epithelium.

Studies will be reported on a competition radioimmunoassay based on reactivity to bacterially synthesized, substantially pure ras p21 that will permit determination of p21 distribution in a variety of cells and an accurate quantitation of the levels of ras related protein expressed in normal, benign, "premalignant," and malignant tissues. A recently developed tranformation defective mutant synthesizing a pro-p21 will also be discussed.

0100 USE OF ^{75}Se-METHIONINE TO METABOLICALLY LABEL AN IGG3 MONOCLONAL ANTIBODY RECOGNIZING NORMAL AND MALIGNANT GLANDULAR EPITHELIUM, Tien-wen Tao, Hung Pham, Biru Zheng, Shao-ling Leu and Joseph P. Kriss, Stanford University, Stanford, Ca. 94305
A hybridoma producing a monoclonal antibody reacting with normal human thyroid was isolated from fusion between FO myeloma and spleen cells from mice immunized with human thyroid membranes. The antibody is of the IgG3 isotype and was purified on an anti-IgG3 affinity column. Immunohistochemical staining showed that the antibody reacted with normal and malignant glandular epithelium staining most strongly the outer membrane of glandular or secretory cells and the ductal lining. These included normal and malignant cells from such organs as thyroid, breast, colon and prostate. No reaction was observed with such tissues as liver, spleen, muscle and such malignant tumors as lymphoma, lung carconoma and rhabdomyosarcoma.

The antibody was metabolically labelled with ^{75}Se by incubating the hybridoma cells with ^{75}Selenomethionine in tissue culture for 2 days. The monoclonal antibody was the predominant labelled protein secreted into the culture medium which was passed through an anti-IgG3 affinity column to yield a purified monoclonal antibody preparation. The ability of the ^{75}Se-antibody to bind specifically to reactive cells in vitro was superior to that of antibody preparations externally labelled with ^{131}I using chloramine T oxidation or with ^{111}In using a bifunctional chelating agent. Thus, the strategy of internally labelling the antibody with radionuclide for tumor localization in vivo warrants further investigation.

0101 A NEW PROSTATE TUMOR MARKER IDENTIFIED BY MONOCLONAL ANTIBODY TURP-27, George L. Wright, Jr., James J. Starling, Susan M. Sieg, Mary L. Beckett, Patricia R. Wirth, Solobodan Poleksic, Leopoldo E. Ladaga, Ann E. Campbell, and Paul F. Schellhammer, Eastern Virginia Medical School, Norfolk, VA 23501.
Monoclonal antibody (Mab) TURP-27 (IgG3) was isolated from a fusion in which mice were immunized with membrane extracts from benign (BPH) and malignant prostate (CaP) tumors. Radioimmunoassay, quantitative absorption, and immunohistochemical analyses demonstrated that TURP-27 has a restricted pattern of reactivity, binding strongly to BPH and CaP tumors and weakly to normal prostate and breast tissues and membrane extracts. TURP-27 failed to bind to the prostate organ specific markers, prostatic acid phosphatase (PAP) and prostate-specific antigen (PA), as measured by direct and blocking assays. One primary and three metastatic CaP tumors negative for the PAP or PA marker stained positive with TURP-27. The TURP-27 antigen was detected in prostatic fluid and seminal plasma obtained from healthy donors but at a concentration three to five times lower than PAP and PA, respectively. The data derived from this study suggests that Mab TURP-27 identifies a new prostate tumor marker that may have potential for diagnosis and therapy of prostate cancer. Supported by NIH grants CA-26659 and CA-27623 and a grant from the Elsa U. Pardee Foundation.

0102 MONOCLONAL ANTI-IDIOTYPIC ANTIBODIES FOR HAIRY CELL LEUKEMIA, Willem van Ewijk, Dept. Cell Biology & Genetics, Erasmus University, Rotterdam, The Netherlands.
Monoclonal anti-idiotypic antibodies were raised to the idiotype of a patient with Hairy Cell Leukemia. These antibodies were raised in a two step procedure. The first step involves rescue of the idiotype by somatic cell fusion of the patient's Hairy cells with the mouse myeloma P3-X63-Ag.8653 cell line, followed by affinity purification of the secreted human immunoglobulin (Ig). The second step involves the production of mouse monoclonals directed to the idiotype of the rescued Ig. Monoclonal anti-idiotypic antibodies were selected on the basis of comparative ELISA's and comparative flow cytometric analyses (Fig. 1), using purified idiotypes, sera and cells from several patients and healthy donors. The reactivity of these antibodies with the patients' Hairy cells will be presented.

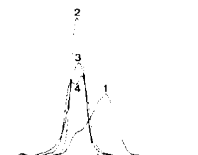

Fig. 1. Flowcytometric analysis of the reactivity of subclone nr. 33-65 with purified Hairy cells (1) and three individual mixtures of control cells (2,3,4).

0103 A MONOCLONAL ANTIBODY (NKI/C-3) REACTING PREFERENTIALLY WITH HUMAN MELANOMA AND NEVOMELANOCYTIC LESIONS IN FORMALIN-FIXED TISSUE SECTIONS, J.G. van den Tweel, Ph.C. Hageman, C. Vennegoor, M.A.van der Valk, E.P.van der Esch, D.J. Ruiter, S.G. van Duinen and Ph. Rümke, The Netherlands Cancer Institute, Amsterdam, De Wever Hospital, Heerlen, University Medical Center, Leiden, The Netherlands

NKI/C-3 is a monoclonal antibody (IgG1), prepared in BALB/c mice after immunisation with partly purified membranes of the human melanoma cell line MeWo. It reacts in the indirect radioimmunoassay with living cells, but also with formalin-fixed and paraffin-embedded tissues in the indirect immunoperoxidase test. In the latter technique the NKI/C-3 antibody showed a strong preference for malignant melanoma, nevocellular nevi, carcinoids and medullary carcinoma of the thyroid. Other tumors (breast, lung, ovary) showed only occasional positive reactions and these were usually weakly positive compared to melanocytic lesions. Normal melanocytes were negative. The antibody was also tested on nearly one hundred tumors of the nervous system, and no positive reactions were observed. Using frozen sections, the NKI/C-3 antibody showed less selectivity. Also in the indirect radioimmunoassay with living cells, positive reactions were observed with cell lines obtained from other types of tumors and normal cells, in addition to melanoma cell lines. This discrepancy in the reaction pattern of the NKI/C-3 antibody in different techniques stresses the importance of early screening of monoclonal antibodies on formalin-fixed tissue sections. The antibody NKI/C-3 has proven to be usefull in the diagnosis of malignant melanoma in formalin-fixed specimens especially in the case of a metastasis of an unknown primary tumor, despite the fact of some cross-reactions.

Human—Human Hybridomas

0104 HUMAN MONOCLONAL ANTIBODIES DERIVED FROM PATIENTS WITH CANCER, Richard J. Cote, Alan N. Houghton, Timothy M. Thomson, Donna M. Morrissey, Herbert F. Oettgen and Lloyd J. Old, Memorial Sloan-Kettering Cancer Center, New York, New York 10021

Hybridomas secreting human monoclonal antibody (hmAb) have been derived from patients with epithelial tumors (breast, lung, renal and colon carcinoma) and melanoma. Over 7000 hybrids have been produced through fusions of human lymphocytes with human drug-marked cell lines (LICR-LON-HMy2, SKO-007, UC729-6, GM4672) or the mouse myeloma NS-1. Approximately 50% of the hybrids secrete detectable levels of immunoglobulin (>500 ng/ml). 3-5% (150) of these produce hmAb reactive with cultured human tumor cell lines, indicating that a substantial proportion of the B cell repertoire is directed to the production of antibodies which recognize cellular antigens (Ag). 5 hmAb detecting cell surface Ag have been studied in detail (Ri37, Ma4, Ev248, Gr169, Sp909). Ri37 reacts with an Ag showing a restricted distribution, being present on 7/60 different cell types tested. Ri37 identifies a B cell differentiation Ag, as it is expressed by normal, mature B cells but not by any other normal, malignant or transformed hematopoietic cells. Ma4 reacts with a glycolipid Ag present on a variety of cultured cells, including melanoma, astrocytoma, breast and colon cancer. Ma4 is not expressed by any normal cultured cells tested so far. Ev248 detects a glycolipid Ag expressed by malignant epithelial cells, but not by neuroectodermally derived cells, or any normal cells. Gr169 and Sp909 react with broad (nondifferentiationrelated) patterns. 145 hmAb reactive with intracellular(IC) Ag, including cytoplasmic, cytoskeletal, nuclear and nucleolar components, have been characterized. While many of the antibodies (50%) are directed against broadly distributed IC Ag (present in all or most cell types tested), a substantial number of hmAb detect IC Ag which show tissue specificity or are highly restricted. A series of hmAb directed against intermediate filament proteins have been generated, including antibodies that detect vimentin (M307), several of the low molecular weight cytokeratins (Pa24, Hu11, M54, Hu22) and a hmAb (De8) which reacts with an epitope common to all classes of intermediate filament proteins (vimentin, cytokeratins, GFAP, neurofilaments and desmin). HmAb which react with IC Ag expressed by only a few cell types have been found, including 7 which have not reacted with any normal cells. These antibodies were derived from patients with lung, colon and breast cancer and have only reacted with epithelial tumors.

In summary, hmAb have been generated that define a) lineage-specific Ag, b) stage-specific Ag within a given lineage and c) apparently tumor-restricted Ag. With the development of these reagents we are gaining a deeper insight into the human B cell repertoire in general, and can now approach some of the fundamental questions in human tumor immunology.

Supported in part by grants from the American Cancer Society (IM-333) and National Cancer Institute (CA-34709). R.C. is a recipient of a National Research Service Award from the National Institutes of Health (IF32 CA-7325).

0105 HUMAN MONOCLONAL ANTIBODY TO GANGLIOSIDE GD2: POTENTIAL FOR CLINICAL APPLICATION, Reiko F. Irie, M.D., Division of Surgical Oncology, John Wayne Cancer Clinic, Jonsson Comprehensive Cancer Center, UCLA School of Medicine, Los Angeles, CA

The method developed for production of animal-derived monoclonal antibody does not have an immediate application for human monoclonal antibody for two major reasons—the lack of a consistent supply of human B-lymphocytes immunized against desired antigens, and the lack of human myeloma cell lines. In animal systems, the immunized recipients are splenectomized and the sensitized B-lymphocytes are collected from the spleen. The scarcity of human myeloma cell lines that produce appreciable amounts of antibody will eventually be overcome in the future. At present, many investigators are attempting to fuse human B-lymphocytes with mouse myeloma cells. The major problem in this instance is to prevent the loss or expulsion of the human chromosomes. An alternative technique, the EBV-transformation method is being used more frequently because it produces a relatively high yield of antibody-producing cells in vitro even though this production is extremely unstable. Recently, we used this technique to establish a long term human B-cell line (L72) that secretes human IgM κ monoclonal antibody to a tumor-associated antigen (Proc. Natl. Acad. Sci. 79:5666, 1982). The chemical nature of the antigen was identified as ganglioside GD2,GalNAcβ1→4 (NeuAcα2→8NeuAcα 2→3)Galβ1→4Glc→ Cer, (Proc. Natl. Acd. Sci. 79:7629, 1982). GD2 is expressed on membranes of human tumors of neuroectodermal origin, such as melanoma, neuroblastoma and glioma. L72 has produced 5-10 μg/ml antibody for the past 4 years. To provide a basis for future clinical application of the antibody, four important aspects of its nature were investigated: 1) The establishment of a method to purify the antibody from the FCS-containing spent culture medium. Twenty-seven mg of pure human IgM were obtained from 10 liters of spent cutlure medium (J. Clin. Lab. Immunol. in press). 2) Demonstration of binding capacity of the antibody to tumor cells in vivo. The maximum binding was obtained 2-4 hours after systemic injection of the antibody into nude mice-bearing human melanoma. 3) Demonstration of the anti-tumor effect of the antibody in vivo. Systemically injected antibody (single injection of 200 μg/mouse) suppressed the growth of established subcutaneous nodules of human melanoma in nude mice (Immunol. Letters 8:169, 1984). 4) Possible contamination of the purified antibody preparation by EBV was excluded (J. Clin. Lab. Immunol. in press). Our successful production of human monoclonal antibody to tumor antigens was largely dependent upon our strategy in which we first searched for tumor-associated antigens that were capable of inducing immune responses in man. Thus, we believe the identification of tumor antigens that are immunogenic in cancer patients is the most important first step for the successful production of human monoclonal antibodies.

0106 RECENT ADVANCES IN THE EBV-HYBRIDOMA TECHNIQUE, John C. Roder, Tsehay Atlaw, Susan P.C. Cole, Department of Microbiology & Immunology, Queen's University, Kingston, Ontario, Canada K7L 3N6; Danuta Kozbor, Wistar Institute of Anatomy and Biology, 36th Street at Spruce, Philadelphia, PA 19104

In our laboratory we have developed a system whereby human lymphocytes are transformed with Epstein-Barr virus (EBV) and subsequently hybridized to a Oua^R, Thg^R human fusion partner called KR-4 (Kozbor, Lagarde and Roder, PNAS 79:6651-6655, 1982). The use of EBV allows (i) immortalization of donor lymphocytes for repeated fusions, (ii) expansion of rare, antigen-specific B cells prior to fusion, and (iii) 10 fold higher rates of fusion (fusion frequency = 10^{-5}). Hybridomas developed in this manner have been grown as ascites tumors in pristane treated nude mice following a prior in vivo passage as a subcutaneous tumor in x-irradiated nudes. Specific human antibody concentrations were 1000 fold higher (5mg/ml) in ascites fluid than in tissue culture supernatants (0.005 mg/ml) and ascites passage of hybrids enhanced their tumorigenicity. New fusion partners with more myeloma-like characteristics have been generated by hybridizating KR-4 to RPMI 8226 (Kozbor, Tripputi, Roder and Croce, C. Immunol. in press). In addition, we have developed systems for in vitro immunization of human peripheral blood lymphocytes followed by EBV transformation and fusion to KR-4 (Kozbor and Roder, Eur. J. Immunol. 14:23-27, 1984). In applying the technology, we have EBV transformed lymphocytes from the draining lymph nodes of small cell lung carcinoma patients, fused with KR-4 and generated many hybridomas with tumor reactivity (Cole et al., Cancer Res. 44: 2750-2753, 1984). Further screening is necessary to assess the level of tumor specificity in these monoclonal antibodies. In another project we have hybridized EBV lines from lepromatous leprosy patients and generated human hybridomas specific for soluble proteins and glycolipids from Mycobacterium leprae (Atlaw et al., submitted). We feel that human monoclonal antibodies may eventually offer effective immunotherapy in cancer, autoimmunity and acute viral infections.

0107 HUMAN MONOCLONAL ANTIBODIES TO HUMAN CANCER CELLS, Ivor Royston, Mark C. Glassy, Robert E. Peters, S.A. Gafar, Cancer Center and Department of Medicine, University of California, San Diego, CA 92103.

The study of human monoclonal antibodies (MoAbs) to human cancer cells is important for[1] two reasons: (1) Human MoAbs used clinically in man should be less immunogenic than mouse or rat MoAbs and (2) the human immune response to human tumors may yield antibodies more specific for tumor cells than normal cells. For these reasons, we have developed a 6-thioguanine resistant B cell line, termed UC 729-6, which fuses with human B cells to produce stable hybridomas secreting human MoAbs[1]. We have shown that the UC 729-6 human hybridoma system compares favorably with the murine hybridoma system. Since cancer patients can make antibodies reactive with their own tumors[2,3] we have used UC 729-6 to fuse with lymphocytes from lymph nodes draining tumors and thereby immortalize B cells making putative anti-tumor antibodies. Using lymph nodes from patients with cancer of the cervix, prostate, vulva, and kidney we have made a series of human-human hybridomas producing both IgM and IgG antibodies which have broad reactivity profiles with human carcinoma cells but do not react with hematopoetic or fibroblast cell lines and normal peripheral blood leukocytes. These human hybridomas grow in serum free media with enhanced production of Ig synthesis (1-9 μg/ml). One IgG MoAb, termed VLN3G2, precipitated a 2 subunit glycoprotein of 60K and 18K M.W. from membranes of A431 human epidermoid carcinoma cells. Our studies to date have shown that (1) UC 729-6 is a suitable cell line for producing human-human hybridomas and immortalizing human B cells and (2) regional draining lymph nodes of cancer patients contain B cells whose Ig is reactive with tumor-associated antigens.

1. Glassy MC, Handley HH, Hagiwara H, Royston I. Proc. Natl Acad Sci USA 80:6327-6331, 1983.

2. Schlom J, Wunderluk D, Teramoto YA. Proc. Natl Acad Sci USA 77:6841-6845, 1980.

3. Sikora K, Wright R. Br. J. Cancer 43:696-700, 1981.

Monoclonal Antibody Therapy in Patients with Leukemia and Lymphoma

0108 MONOCLONAL ANTIBODIES IN PATIENTS WITH CLL AND CTCL, Robert O. Dillman, Daniel L. Shawler, Jacquelyn B. Dillman, Maureen Clutter, Department of Medicine, University of California, San Diego Cancer Center and VA Medical Center, San Diego, CA 92161

Because of their specificity, monoclonal antibodies (MoAbs) may provide an important alternative approach to cancer therapy. Pilot studies with murine anti-human T cell MoAbs showed some evidence of an anti-tumor effect in CLL and CTCL. Because of toxicity associated with rapid infusions, we conducted a study of 24 hour infusions of MoAb T101 in these patients at doses of 10, 50, 100, or 500 mg. Ten patients had cutaneous T cell lymphoma (CTCL) and six chronic lymphocytic leukemia (CLL). Side effects of therapy included fever, flushing, sweats, and/or chilling in patients in whom a substantial number of circulating target cells were removed during therapy, but there was also a 15% frequency of allergic manifestations including pruritis and urticaria, and rarely, bronchospasm or anaphylaxis. In vivo binding of T101 to circulating target cells, skin infiltrates, and bone marrow was demonstrated using immunofluorescence techniques, while lymph node uptake was demonstrated using 111-Indium-T101. Antigenic modulation occurred rapidly and was directly associated with saturation of circulating cells and serum T101 levels. These variables were all related to tumor burden and dose as well as to the presence or absence of endogenous anti-mouse antibodies. Such antibodies were demonstrated by ELISA in 5/10 CTCL and 0/6 CLL patients. Although in vivo binding and clinical effects were seen in the presence of anti-mouse antibodies, these effects were less striking and of shorter duration. All patients appeared to have an anti-idiotype component to their anti-mouse response. One patient with high anti-mouse levels developed fever, chilling, an erythematous rash, joint tenderness, and synovial effusion shortly after receiving 500 mg T101. There were no long term consequences associated with anti-mouse levels. Transient clinical benefit was observed in 6/10 CTCL and 6/6 CLL patients, but sustained objective responses were seen in only 3/10 CTCL and 0/6 CLL. No additional clinical improvement was observed in the presence of antigenic modulation or anti-mouse antibodies. The anti-tumor effects of T101 alone appear limited and useful anti-tumor effects may be achievable only with cytotoxic immunoconjugates. The kinetics of antigenic modulation and the anti-mouse response need to be taken into account in scheduling such therapy.

0110 APPLICATIONS OF MONOCLONAL ANTIBODIES (McAB) IN BONE MARROW TRANSPLANTATION, Paul J. Martin, John A. Hansen, and E. Donnall Thomas, Fred Hutchinson Cancer Research Center and the Department of Medicine, University of Washington, Seattle, WA

Observations in rodent models that graft-versus-host disease (GVHD) can be prevented by removing mature T cells from the donor graft have prompted similar investigations in human bone marrow transplantation. Successful transfer of this technology from rodent models to the human setting has required the development of methods by which human T cells could be distinguished from stem cells and which would allow efficient, specific removal of T cells from the graft. In the scale-up and transfer of this technology from experimental models it must be established in humans whether GVHD can be prevented altogether or whether removal of mature T cells will only diminish the severity of the disease or delay its onset. If the disease can be prevented in man, then it will become necessary to determine the degree of depletion required in order to assure the prevention of GVHD in different populations of patients. This number may be variable, depending on the degree of genetic disparity between the donor and recipient. Finally, it has become imperative to achieve an understanding of any beneficial functions that might be served by T lymphocytes in the donor marrow. We have carried out two pilot studies designed to explore the feasibility of using murine McAb to achieve depletion of T cells in donor marrow as a method of preventing GVHD in man. In the first trial, it was found that treatment of donor marrow with a combination of 8 McAb alone did not prevent GVHD. Results of the second trial suggest that 2-3 log depletion of T cells in donor marrow achieved with the use of the same McAb and rabbit serum complement can decrease the incidence and severity of GVHD after genotypically HLA identical bone marrow transplantation in patients with hematologic malignancies. Removal of T cells, however, was associated with an increased incidence of marrow graft failure. This was not related to the dose of nucleated cells, BFU-E or CFU-C, and did not appear to correlate with whether the donor was sex matched, whether the recipient had received transfusions before transplantation, or whether residual T cells were detected in the treated marrow. It was noteworthy that graft failure occurred in patients conditioned for transplantation with 12.0 Gy of fractionated total body irradiation, but not in patients conditioned with 15.75 Gy of fractionated total body irradiation. The results of our studies have demonstrated the feasibility of preventing acute GVHD after HLA identical allogeneic bone marrow transplantation by removing mature T cells from the graft. They further emphasize the role played by T cells in helping to maintain sustained hematopoietic function after allogeneic marrow transplantation. The optimal application of in vitro T cell depletion for preventing acute GVHD will require further trials to elucidate the factors that govern engraftment after transplantation.

Monoclonal Antibodies to Premalignant Cells

0111 PRODUCTION OF MONOCLONAL ANTIBODIES TO OVAL CELLS AND PRENEOPLASTIC HEPATOCYTES FRACTIONATED FROM LIVERS OF RATS EXPOSED TO HEPATOCARCINOGENIC REGIMENS, Dunsford, H.A. & Sell, S. University of Texas Medical School, Houston, TX 77025, USA.

Monoclonal antibodies have been produced to oval cells induced in male Fischer rats fed N-2-acetylaminofluorene in a choline deficient diet, and to preneoplastic gamma glutamyl transpeptidase (GGT) positive hepatocytes induced by I.P. injection of diethylnitrosamine into male Fischer rats (Solt-Farber Model). Partially purified fractions containing approximately 70% oval cells, and unpurified fractions containing approximately 20% GGT positive hepatocytes were obtained by enzymatic perfusion and used to immunize Balb-C female mice. Spleen cells from the immunized mice were fused with mouse SP/2/0-M5 myeloma cells. The supernates were screened either by an ELISA assay or by indirect immunofluorescence on cryostat sections of rat liver containing preneoplastic cell populations (Solt-Farber model). Positive clones were limit diluted, and further characterized by immunofluorescent staining of normal liver, preneoplastic liver (nodules) and transplantable Morris hepatoma 7777. Of the many monoclonal antibodies produced, two react with oval cells and bile duct cells, but not liver or tumor; two react with normal liver and nodules but not tumor; one reacts variably with normal hepatocytes, strongly with nodular hepatocytes, but not tumor; and one reacts with oval cells and other non-parenchymal cells and tumor, but not normal liver or nodules. These and other monoclonal antibodies being developed will be useful in the study of the lineage of hepatocellular carcinoma. Supported by NIH grant R01-CA-34635-02.

0112 IMMUNOTHERAPY OF A DRUG RESISTANT PHENOTYPE IN BN RAT LEUKEMIA, A.G. Brox, A.K. Sullivan, G. Price, Division of Hematology, Royal Victoria Hospital, McGill University, Montreal, Quebec, Canada.

Resistance to chemotherapeutic agents often limits successful treatment in acute leukemia. New modalities including autologous transplantation and immunotherapy are currently being developed in an attempt to overcome this problem. To this end, we have developed a resistant cell line in the BN rat leukemia that expresses a unique surface determinant the "p" glycoprotein. The parental leukemia is promyelocytic with reproducable kinetics. The resistant cell line was obtained by intravenous passage in vivo with repeated exposure to daunarubicin. This line designated P_8D_9 expresses a 180,000 mw glycoprotein on its cell surface the "p" or permeability glycoprotein. This glycoprotein modulates resistance to daunarubicin. A murine monoclonal antibody designated 3E9 an IgG^2 reacted positively with the P_8D_9 cells by immunofluorescence and chromium release. After incubation of P_8D_9 and control with antibody in vitro without complement these cells were reinjected at a concentration of 10^5 cells/rat. The parental BN cells incubated with 3E9 or control, as well as P_8D_9 and control all gave similar results death between days 26 and 27. P_8D_9 incubated with 3E9 survived an average of 35 days. In view of the rapid doubling time of less than 24 hours the results are significant. One intravenous boost of antibody rise to two long term survivors. Our results demonstrate the efficacy of immunotherapy in BN rat leukemia. Appropriate chemotherapy with the addition of immunotherapy may be curative in this leukemia. Further studies are underway to better define these therapy modalities.

0115 CELL SURFACE-DIRECTED MONOCLONAL ANTIBODIES FOR ANALYSIS OF CELLULAR TRANSFORMATION RISK AND TUMOR CELL SURFACE PHENOTYPES IN ONCOGENESIS BY ETHYLNITROSOUREA IN THE DEVELOPING RAT NERVOUS SYSTEM, Andrea Kindler-Röhrborn, Ursula Langenberg, Romano Minwegen, Sigrid Reinhardt and Manfred F. Rajewsky, Institut für Zellbiologie (Tumorforschung), Universität Essen, Hufelandstrasse 55, D-4300 Essen 1, Federal Republic of Germany

A single dose of ethylnitrosourea (EtNU) applied to rats causes a similar degree of ethylation in the cellular macromolecules of all tissues; however, malignant tumors arise predominantly in the brain and peripheral nervous system. The neuro-oncogenic effect of EtNU is strongly dependent on the stage of rat brain development at the time of the carcinogen-pulse (maximum: late pre- and early postnatal period). Fetal brain cells transferred to long-term culture after transplacental exposure to EtNU *in vivo* give rise to multiclonal tumorigenic cell lines. To analyze the neural target cell system in terms of cell surface phenotypes, we have raised monoclonal antibodies (Mab) directed against neural cell surface determinants (NCSD), using BDIX-rat brain cells (11th-21st day of prenatal development) as immunogens. Development stage-dependent expression of NCSD recognized by these Mab was analyzed both on rat brain plasma membranes and on intact brain cells (prenatal days 13-22 and adult) using solid-phase radioimmunoassays and a FACS. Four types of developmental NCSD expression profiles were obtained: NCSD whose expression increases, decreases, or remains unchanged, and NCSD whose expression changes transiently during development. Expression of Mab-defined NCSD was also detected on premalignant and malignant neural rat cell lines induced by EtNU during prenatal development, and attempts were made to correlate the NCSD patterns of the malignant cells with those of their normal counterparts at the time of the EtNU-pulse.

0116 MONOCLONAL ANTIBODIES PRODUCED THROUGH IN VITRO IMMUNIZATION SPECIFICITY AGAINST MCF-7 CELLULAR ANTIGENS, K. John Morrow, Jr.[1], Dean P. Edwards[2], David J. Adams[2], George Sledge[2], Lynn Dressler[2], Robert J. Bjercke[2] and William L. McGuire[2], Texas Tech University Health Sciences Center, Lubbock, TX 79430[1] and University of Texas Health Sciences Center, San Antonio, TX 78284.[2]

We have used in vitro immunization procedures to produce monoclonal antibodies against cellular proteins from the human breast cancer cell line, MCF-7. Mouse spleen cells were exposed for four to five days in vitro to a membrane extract from the MCF-7 line. The splenic lymphocytes were fused with NS-1 myeloma cells and the resulting hybridomas were screened for antibodies that would bind to surface membrane antigens of the MCF-7 cell line. Four independently derived hybridomas were isolated and cloned; all were IgM producers. Antibodies were evaluated for tissue distribution of binding by immunohistochemical staining procedures. Three of the antibodies recognized antigens widely distributed on normal human tissues, however, one of these, designated 102A11, reacted against a number of malignancies derived from mammary gland and other tissues, but only weakly against normal tissues. Treatment with trypsin completely abolished the ELISA reaction in which supernatant from 102A11 cultures were reacted against the antigen, while neither treatment with neuraminidase nor with methanol had any effect. The 102A11 antibody was further characterized by immunoblotting and was found to react against a single protein band (m.w.=50,000) on SDS polyacrylamide gel electrophoresis. The native 102A11 antigen, however, appears to be a large molecular weight protein or aggregate of m.w.=400,000 based on HPLC separation.

0117 MAGNETIC MONOCLONAL ANTIBODY-MEDIATED REMOVAL OF LEUKEMIC CELLS FROM BONE MARROW USED FOR AUTOLOGOUS TRANSPLANTATION, Christopher L. Reading and Karel A. Dicke, The University of Texas M.D. Anderson Hospital, Houston, TX 77030

Autologous bone marrow transplantation is currently used as supportive therapy after high dose cytoreduction in acute leukemias and in solid tumors. Relapses in acute leukemia after autologous bone marrow transplantation are due in part to leukemic cells contaminating the "remission" bone marrow autografts. We have devised a monoclonal antibody-mediated magnetic separation procedure to remove leukemic cells from bone marrow autografts. This procedure utilizes an immunomagnetic fluid comprised of magnetite nuclei coated with cobalt boride, crosslinked human serum albumin, and benzoquinone-linked IgG fraction of goat-anti mouse immunoglobulins. The cells are incubated with monoclonal antibodies, with the immunomagnetic colloidal fluid, and then subjected to high gradient magnetic separation. We have studied the efficiency of this procedure using monoclonal antibody CF-1 and K562 cells and CT-2 with peripheral blood mononuclear cells. The effect on normal hematopoietic progenitors has been tested using anti leukemic monoclonals J5, and MDA 001, followed by progenitor assays in vitro. We have also used this procedure clinically to remove CALLA positive leukemic cells from bone marrow autografts. The procedure is nontoxic, allowing rapid reengraftment of the treated bone marrow. In vitro experiments indicate that it is possible to remove 4-5 logs of contaminating leukemic cells with this procedure.

0118 INHIBITION OF HUMAN HEPATOMA CELL GROWTH BY MONOCLONAL ANTI-5'-NUCLEOTIDE PHOSPHODIESTERASE ANTIBODY, K. C. Tsou and Z. K. Pan, University of Pennsylvania School of Medicine, Philadelphia, Pa. 19104

Serum diagnosis of human hepatoma has been developed in these laboratories with the aid of an isozyme of 5'-nucleotide phosphodiesterase. The inhibition of human hepatoma cell growth _in vitro_ has now been demonstrated by the treatment of Hep-G2, Hep-3B, and NPLC-KC cells _with two_ anti-5'-nucleotide phosphodiesterase monoclonal antibodies TA8 and TB7. Plating efficiency study and cell-cycle analysis by flow cytometry both suggest that these monoclonal antibodies affect cells in the blocking of late S phase of their cell-cycle. Since this enzyme is a proliferative marker for liver growth, the use of these new monoclonal anti-enzyme antibodies may therefore offer new enzyme modulation strategies for the treatment of liver cancer in man.

Modulation of Effector Cells by Monoclonal Antibodies

0119 IgG3 ANTIBODIES TO A HUMAN MELANOMA-ASSOCIATED GANGLIOSIDE ANTIGEN WITH STRONG ANTI-TUMOR ACTIVITIES, Karl Erik Hellström, Vera Brankovan, Gary E. Goodman, and Ingegerd Hellström, Oncogen, 3005 First Avenue, Seattle, Washington 98121, Tumor Institute, Swedish Hospital Medical Center, Seattle, Washington, and Departments of Microbiology/Immunology and Pathology, University of Washington, Seattle, Washington 98195.

There are many approaches to the therapeutic application of anti-tumor antibodies. One of the most straightforward of these is to use the antibodies alone, without further modification. However, this requires antibodies that have strong anti-tumor effects either by themselves, or in the presence of complement or effector cells such as K cells or macrophages.

We have produced monoclonal mouse antibodies to several cell surface antigens which are primarily expressed in human melanomas. One of the most specific of these is a GD3 ganglioside, which was first defined by Dippold et al. using their antibody R_{24}, and subsequently by Yeh et al. using an IgM antibody, 4.2. We have recently obtained three additional antibodies to this antigen, 2B2, IF4, and MG-21, which, in difference to antibody 4.2, are of the IgG3 subclass. All three antibodies mediate antibody-dependent cellular cytotoxicity (ADCC) in vitro when tested with human lymphocytes as effector cells, using a 4-hr ^{51}Cr-release test, and one antibody, MG-21, also gives strong complement-depdendent cytotoxicity with human serum. Antibody 2B2, which gives ADCC also in the presence of mouse lymphocytes, inhibited the outgrowth of a human melanoma in nude mice, while antibody IF4, which showed no ADCC with mouse lymphocytes, did not. Lymphocytes from healthy human subjects consistently give high ADCC with the three antibodies, while lymphocytes from most cancer patients tested have proven ineffective.

We have started to assess the localization and possible in vivo effects, of anti-GD3 IgG3 antibodies in patients with metastatic melanoma.

0120 ADCC WITH HUMAN EFFECTOR CELLS AND MONOCLONAL ANTIBODIES. Ronald B. Herberman, John R. Ortaldo and A. Charles Morgan. Biological Response Modifiers Program, National Cancer Institute, Frederick, MD 21701

Phase I clinical studies in patients with malignant melanoma have been performed with the anti-melanoma monoclonal antibody 9.2.27. The emphasis has been on determining the dose and schedule resulting in optimal localization of the antibody in malignant lesions. Upon administration of 200-500 mg of 9.2.27, coating of most tumor cells with high levels of antibody could be consistently achieved, as demonstrated by immunoperoxidase staining or flow cytometry. Radioimaging studies also showed good localization in known lesions. However, administration of antibody alone has had no detectable effect on tumor size. In view of ADCC observed with 9.2.27 and mouse NK cells and the potential anti-tumor benefits to be achieved from such interactions, we have explored the conditions required for optimal ADCC with human effector cells and human melanoma cell lines coated with 9.2.27. Purified large granular lymphocytes, with high levels of NK activity, and purified monocytes were used as effector cells, with or without pretreatment with interferon or interleukin 2. Using a variety of experimental conditions and the M14 cell line, which has been shown to be susceptible to ADCC by mouse NK cells and 9.2.27, very low or negative levels of ADCC have been observed. Thus, it appears that the species of effector cells as well as the particular monoclonal antibody and target cell line have a major influence on the level of ADCC, and such parameters need to be carefully evaluated before the promising results from experimental tumor models in mice can be translated into clinical applicable approaches.

0121 DESTRUCTION OF HUMAN TUMORS BY IgG2a MONOCLONAL ANTIBODIES (MAbs) AND MACROPHAGES, Dorothee Herlyn, Michael Lubeck, Zenon Steplewski, and Hilary Koprowski, The Wistar Institute, Philadelphia, PA 19104

Murine MAbs of IgG2a isotype inhibited growth of human tumors implanted in nude mice whereas MAbs of IgG1, IgG2b, IgG3, IgM, and IgA isotypes were inefficient (1). The role of complement, killer, or T cells in MAb-dependent tumor growth inhibition could be virtually excluded. Macrophages, on the other hand, were strongly implicated as effector cells _in vivo_ (1, 2). The ability of IgG2a MAbs to inhibit tumor growth in nude mice strongly correlated with their reactivity in antibody-dependent macrophage-mediated cytotoxicity (ADMC) assays. IgG2a MAbs that were inactive _in vivo_ also did not react in these assays _in vitro_ (3). The number of antibody binding sites per tumor cell was significantly higher for tumoricidal MAbs as compared to unreactive MAbs (3). In light of the correlation between tumoricidal activities of MAbs _in vivo_ and MAb isotype and reactivity in ADMC assays _in vitro_, we have developed procedures for selection early after fusion of hybridomas secreting MAbs with tumoricidal potential _in vivo_ (4). In another approach to enhance the availability of tumoricidal MAbs, we isolated hybridoma variant cells that had switched from IgG1 to IgG2a MAb production but that had retained the same idiotype. The IgG2a variant MAbs differed from the IgG1 MAbs in their reactivities with macrophages _in vitro_ and in their tumor growth-inhibiting capacities _in vivo_.

Analogous to murine macrophages, human macrophages were shown to lyse tumor targets in the presence of mouse IgG2a MAbs (5). The isolation of macrophages with Fc receptors for mouse IgG2a MAbs from human tumors (5) further implicated these cells as effector cells that mediated immunotherapeutic effects of IgG2a MAb 17-1A administered to gastrointestinal cancer patients (6). ADMC-reactivity of fresh human peripheral blood monocytes was significantly lower as compared to macrophages (5) but could be enhanced by treatment of monocytes with γ-interferon. The concomitant increase in MAb binding to Fc receptors on these cells was accompanied by quantitative and qualitative changes in the receptor proteins.

1. D. Herlyn and H. Koprowski, Proc. Natl. Acad. Sci. USA 79, 4761 (1982).
2. D. Adams, et al., Proc. Natl. Acad. Sci. USA 81, 3506 (1984).
3. D. Herlyn, et al., J. Immunol., in press.
4. D. Herlyn, et al., J. Immunol. Methods, in press.
5. Z. Steplewski, et al., Science 221, 865 (1983).
6. H. Sears, et al., J. Biol. Resp. Modif. 3, 138 (1984).

Drug—Antibody Conjugates

0122 ANTI-CANCER DRUG CONJUGATES WITH THE ANTIBODY TO α-FETOPROTEIN

Hidematsu Hirai
Tumour Laboratory, Kokubunji-shi, Tokyo

The anti-cancer effect of the antibody to α-fetoprotein (AFP) upon AFP producing tumors, e.g. hepatoma and yolk sac tumors has been demonstrated either in vitro or in vivo. A highly purified antibody preparation was administered to 30 hepatoma patients and 2 AFP producing ovarial tumor patients. The amounts of the antibody administered were 300~1,000mg per patient depending on serum AFP levels of the patients.

It was not clear whether the treatment was effective or not, because most of the cases were in advanced stages and it was difficult to take a control group in clinical study. However, a marked change in serum AFP levels were observed. In all cases AFP levels fell down to zero level immediately after administration of the antibody. This decrease was most likely caused by neutralization of serum AFP with the antibody. After the administration, however, in about 1/3 of patients AFP levels increased rapidly within 2 weeks and in other 1/3 AFP levels came back slowly to original levels. In other 1/3, however, the lowered levels were maintained for long period over 20 weeks. This long term suppression of AFP levels were understood only by suppression of AFP production by the tumor cells.

A specific accumulation of the antibody in these tumor tissues was clearly demonstrated by the technique of radioimmunodetection.

Anti-cancer drugs, such as daunomycin, Adriamycin and mitomycinc were covalently coupled to dextran or polyglutamic acid chains (M.W. ca 10K) followed by conjugation to the antibody. Moles of the drugs were approximately 20~40 per mole of the antibody. Anti-cancer effect of the conjugates was remarkable in vitro as well as in vivo. The life span of the tumor bearing rats was obviously elongated.

These studies above mentioned have mostly been carried out with purified polyclonal horse antibody to rat or human AFP. Since this couple of years monoclonal atibody (mAb) to AFP were produced by conventional technique of hybridization of the mouse cells. Several monoclonal antibodies obtained showed different cross-reactivity with AFPs of different mammalian species.

These mAb and drug conjugates showed anti-cancer effect of the same degree as of horse polyclonal antibody. As far as the cytotoxic effect of the AFP antibody concerns the difference between polyclonal and mononoclonal antibodies may not easily be detected. Because AFP is a completely purified soluble protein molecule and its antigenic structure is relatively simple compared to the complicated structure of tumor cell surface.

The mAb will give full play when tumor cell itself is used as an immunogen.

0123 ASSESSMENT OF DRUG AND TOXIN CONJUGATES OF MONOCLONAL ANTIBODIES TO HUMAN MELANOMA AND LINE 10 HEPATOCELLULAR CARCINOMA,

A. C. Morgan, Jr., G. Pavanasasivam, W. Bonn, A. Alarif, J. Pearson. Monoclonal Antibody/Hybridoma Section, Biological Response Modifiers Program, National Cancer Institute, Frederick, MD 21701

We have conjugated two monoclonal antibodies, 9.2.27 (γ2a;k) to a human melanoma associated 250 kd glycoprotein/proteoglycan (1) and D3 (γ1, K) to guinea pig line 10 hepatocellular carcinoma (2) with drug, toxins, and radioisotopes. A summary is given in the Table.

Antibody	Drug	Toxin	Isotope
9.2.27	Methotrexate	A/abrin	I^{125}(6)
	Adriamycin	A/ricin	I^{131}
	Bleomycin	Abrin	In^{111}
		Gelonin	
		Pokeweed antiviral protein	
D3	Vindesine(3)	A/Diptheria(4)	^{111}In(7)
		A/Abrin(5)	
		Gelonin	
		Pokeweed antiviral protein	
		Abrin	

Conjugates were characterized in the following manner 1) immunoreactivity, 2) in vitro potency and selectivity, 3) kinetics of internalization, 4) molecular nature, 5) biodistribution and tumor localization and with several conjugates, (6) therapeutic evaluation.

Potency of 9.2.27 drug conjugates varied between 1×10^{-8} and 1×10^{-10}M (ID_{50}, antibody concentration). D3-vindesine had a 10^{-7} ID_{50}. Toxin conjugates of 9.2.27 varied between 10^{-11} to 10^{-14}M ID_{50}. D3-toxin conjugates varied between 10^{-9} to 10^{-12}M potency. Biodistribution studies with a number of drug and toxin conjugates indicate they localize less well to tumor than unconjugated antibody. Toxin conjugates have shown efficacy against established palpable tumors. Reduction of tumor burden by treatment with drugs has led to greatly enhanced anti-tumor effects of toxin conjugates.

1. Morgan, Jr. A.C., Galloway, D.R., R.A. Reisfeld. (1981) Hybridoma 1:27-36.
2. Key, M.E., Bernhard, M.I., Hoyer, L.C., et. al. (1983) J. Immunol 130:1451-1457.
3. Embleton, M.J., Rowland, G.F., Simmonds, R.G., et. al. (1983) Br. J. Cancer 47:43-50.
4. Bernhard, M.I., Foon, K.A., Oeltmann, T.N., et. al. (1983) Cancer Res. 43:4420-4428.
5. Hwang, K.M., Foon, K.A., Cheung, P.T., Pearson, J.W., Oldham, R.K. Cancer Res., in press.
6. Hwang, K.M., Morgan, Jr., A.C., Fodstad, O., Oldham, R.K. Cancer Res., in press.
7. Bernhard, M.I., Hwang, K.M., Foon, K.A., et. al. (1983) Cancer Res. 43:4429-4433.

Monoclonal Antibody Conjugates with Drugs or Toxins

0124 IMMUNOTHERAPY OF HUMAN SOLID TUMOR XENOGRAFTS WITH VINCA ALKALOID-MONOCLONAL ANTIBODY CONJUGATES, Thomas F. Bumol, Robin G. Simmonds, George B. Boder, George Cullinan, Philip Marder and Lynn D. Apelgren, Lilly Research Laboratories, Indianapolis, IN 46285 and Lilly Research Centre Ltd., Windlesham, Surrey GU20 6 PH, U.K.
Monoclonal antibody KS1/4 defining a 40kd adenocarcinoma associated antigen (described by Varkki et al., Cancer Research 44:681, 1984) was examined as a drug targeting vehicle for vinblastine sulfate to the human lung adenocarcinoma cell line P3-UCLA. The N-hydroxysuccinimide ester of vinblastine succinate was utilized to substitute vinca alkaloid moieties on the antibody. Conjugation conditions were established for an optimal drug/moab ratio by close monitoring of serological activity of the conjugates by ELISA, indirect immunoflourescence and flow cytometry techniques. Pharmacological activity of the conjugate *in vitro* were determined by kinetic analyses of vinca alkaloid associated cell cycle effects by flow cytometry indicating that KS1/4-VLB could induce a specific antibody dependent block of P3UCLA cells in G_2+M. *In vivo* treatment of P3-UCLA xenografts in athymic mice with KS1/4-VLB by i.p. administration demonstrated a dose dependent suppression in xenograft growth. Comparable treatments of an irrelevant antigen negative human melanoma xenograft demonstrated no tumor suppression. Free drug control groups demonstrate that toxic or lethal doses of vinca alkaloid can be administered safely to nude mice in conjugate form while demonstrating good efficacy toward the target tumor. Additional results examining alternative routes of administration of KS1/4-VLB conjugates and the *in vitro* and *in vivo* activity of Fab-KS1/4-VLB conjugates will be discussed.

Monoclonal Antibody Conjugates

0125 IN VIVO LOCALIZATION OF SITE-SPECIFICALLY MODIFIED MONOCLONAL ANTIBODIES. Vernon L. Alvarez, John D. Rodwell, Chyi Lee, and Thomas J. McKearn. Cytogen Corporation, 201 College Road East, Princeton, NJ 08540.
Carbohydrate-mediated site-specific modifications can be effected without loss of the homogeneous binding which is characteristic of monoclonal antibodies (Rodwell, J.D., et al, 1984, Fed.Proc.Abstr. 32:2084). In order to evaluate the *in vivo* behavior of antibodies modified in this way, a rat monoclonal IgG specific for a major histocompatibility (MHC) antigen of Brown Norway (BN) rats was modified at the carbohydrate residues, using derivatives of the chelator diethylenetriaminepentaacetic acid (DTPA). Conjugates of either specific anti-MHC or an irrelevant IgG, chelated with ^{111}Indium, were injected into nude mice which carried a subcutaneously implanted BN tumor (~5 mm in diameter at the time of injection). Tumor-bearing animals receiving the specific antibody showed excellent localization of radioactivity after twenty-four hours. Tumor-bearing animals injected with irrelevant IgG, and non-tumor-bearing animals injected with anti-MHC IgG all showed no localization. In all cases, little accumulation appeared in the liver, lungs or spleen. Organ counting data confirm these results and show 15-25% of the injected dose localizes into the tumor. In a few cases, tumor-bearing animals injected with be MHC-specific IgG showed a secondary site of localization in the left axillary lymph node. Dissection showed enlargement of the node, and subsequent staining of cells confirmed the presence of a secondary or metastic site of tumor growth. We conclude that vascular accessibility is an important parameter affecting the rate of radio-nuclide accumulation, and that site-specifically modified monoclonal antibodies yield superior *in vivo* imaging results.

0126

ELIMINATION OF IMMUNOGENECITY AND Fc BINDING OF ANTIBODIES.
William L. Anderson and Thomas B. Tomasi. Department of Cell Biology and Cancer Center, University of New Mexico Medical School, Albuquerque, NM 87131
Affinity purified rabbit, goat and rat antibodies as well as a mouse monoclonal antibody were modified with the active ester of monomethoxy polyethylene glycol succinate (mPEG) (average molecular weight = 5000). These chemically modified antibodies show no change in affinity and also retain their ability to specifically bind to target cells. PEG-modified antibodies and immunecomplexes prepared from these antibodies exhibit little or no binding to cell surface Fc receptors on either mouse splenocytes or P388/D1 cells. Rabbit antibody with between 13 and 18% of the surface lysine residues modified with mPEG fail to elicit an immune response in Swiss mice following primary, secondary or tertiary exposure to antigen. PEG also provides an ideal "spacer arm" for the attachment of chemotherapeutic, radioimaging or NMR imaging agents to antibody molecules. This possibility has been evaluated by preparing monofluorescein-PEG, monorhodamine-PEG, monoprotophorphyrin IX-PEG and monoperfluorohexane-PEG and then attaching these derivatized PEG molecules to rabbit and mouse monoclonal antibodies. The ability of the modified antibody to specifically deliver the attached moiety to a target cell has been shown. PEG-modified antibodies exhibit excellent stability, low Fc binding obviating the necessity of preparing F(ab')$_2$ fragments as well as providing a means of attaching a variety of therapeutic or imaging agents to antibody molecules. The results suggest that PEG-modification of antibodies may provide advantages for use in *in vitro* diagnostic procedures and warrant further studies for possible use *in vivo* in both diagnostic imaging and therapy with antibodies.

0127 IMPROVED TOXICITY OF ANTI-T CELL IMMUNOTOXINS IN THE PRESENCE OF COMPLEMENT.
Peter J.A.Capel, Frank W.M.B.Preijers and Theo de Witte, Dept.Nephrology and Haematology, Radboud Hospital, Nijmegen, The Netherlands
We have studied the effect of complement on the toxicity of WT1-RicinA chain immunotoxin . WT1 recognizes a T-cell specific 40 Kd glycoprotein present on all thymocytes, T lymphoblastic leukemias (T-ALL) and almost all peripheral T cells (1). WT1 RicinA chain is a very potent immunotoxin (2), and treatment of autologous bone marrow with WT1 immunotoxin prior to grafting, resulted in the case of a T-ALL patient in a normal take of the graft and a complete functional restoration of the bone marrow including T cell functions. To improve the in vitro effects of immunotoxins rabbit complement was added during a 16 hrs incubation period with immunotoxin. When T cell lines or T-ALL cells were used, in all cases a strong increase in toxicity was observed. The increased toxicity of immunotoxins in the presence of complement required the formation of the lytic C5-C9 complex because C6 deficient rabbit complement lacked this potentiating effect, which after reconstitution with C6 was detectable again. As complement source four weeks old rabbits were used and with these baby-rabbits no unwanted toxicity was observed in different batches tested. When T101-RicinA immunotoxin was used, complement also increased the toxicity in this case. The combined incubation of complement and immunotoxin did not effect the progenitor cells in bone marrow as tested in clonogenic assays (CFU-c).

1. L.Vodinelich et al: Blood 1983; 62, 1108.
2. C. Myers et al: Blood 1984; 63, 1178.

0128 BREAST CANCER IMMUNOTOXINS,
A.E. Frankel, D. Ring, M. Bjorn, J. Raymond, W. Bloch, W. Laird, K. Chong, K. Hwang, J. Winkelhake, R. Ferris; Cetus Corporation, 1400 Fifty-third Street, Emeryville, CA 94608
Mouse monoclonal antibodies were purified from hybridomas generated in 75 fusions using Balb/C female mice immunized and boosted twice with either live breast cancer cells or breast cancer membrane extract. The immunized splenocytes were fused with SP2/0 myeloma cells. After positive selection on breast cancer cell lines and membrane extracts and breast cancer frozen sections and negative selection on 8 normal tissue membrane extracts and a fibroblast cell line, 124 hybridomas were cloned. 117 monoclonal antibodies were purified, titrated on breast cancer sections, and tested on 17 normal tissue sections and 5 blood cell components. 50 antibodies showed breast cancer selectivity. These were tested on 21 breast cancer sections and 14 breast cancer cell lines. Antigen molecular weight, antigen density, antibody KA were determined where possible. These antibodies were conjugated using SPDP and 2-IT to ricin toxin A chain (RTA). One third of these were active *in vitro* (TCID50 $\leq$ 10nM) on 1-4 breast cancer cell lines and not toxic to control cell lines (TCID50$\geq$ 50nM). Antibodies with a high KA and antigen copy number make better RTA conjugates. A number of these have been scaled up and tested in nude mice bearing MX-1 or MCF7 human breast tumor xenografts. LD_{50} single dose and cumulative dose was 200-400 ug/mouse. At doses near the LD_{50}, 50-80% tumor growth inhibition was observed relative to RTA or antibody alone or a control conjugate-MOPC21-IT-RTA. Preliminary *in vivo* pharmacokinetics suggest very little RTA conjugate localized to the tumor and if we can improve tumor localization *in vivo*, better therapeutic index may be achieved.

0129 EFFECT OF ANTIBODY VALENCY AND ANTIBODY-TOXIN LINKAGE ON THE SYNERGY BETWEEN RICIN-A AND RICIN-B IMMUNOTOXINS.
R. Jerrold Fulton, Jonathan W. Uhr, and Ellen S. Vitetta, The University of Texas Health Science Center, Dallas, Texas 75235.

Recent studies from this laboratory have demonstrated that the specific toxicity of ricin A chain-containing immunotoxins (IT-A) can be enhanced by the separate delivery of a ricin B chain-containing immunotoxin (IT-B) which is reactive with the antibody portion of a cell-reactive IT-A. It may be necessary to use Fab immunotoxins to adapt this delivery system for *in vivo* use in order for the primary IT-A to be cleared from the circulation rapidly enough to allow delivery of the secondary IT-B while target cells remain "tagged" with the primary IT-A. Therefore, we have examined the effect of using Fab fragments of IgG versus intact IgG for both IT-A and IT-B on the *in vitro* synergy between these immunotoxins. Since SPDP conjugates have been reported to be unstable *in vivo*, two distinct types of conjugates, both containing internal disulfide bonds, were used: 1) SPDP conjugates; and, 2) Ellman's conjugates in which antibody and toxin are linked via natural cysteine residues of the two proteins. The specific toxicity of IT-A and the enhancement of IT-A toxicity by IT-B (10-fold) was found to be independent of antibody valency or interchain linkage. In the presence of ammonium chloride, the synergy between IT-A and IT-B was enhanced an additional 10-20 fold. This increase in toxicity was also unaffected by valency or interchain linkage in both IT-A and IT-B. These results indicate that the use of Fab fragments coupled to ricin A or ricin B by the Ellman's procedure does not alter the synergy between these immunotoxins, but may increase their usefulness *in vivo*.

0130 NEW ANTITUMOR CHIMERIC PROTEIN: CON A-SOYBEAN TRYPSIN INHIBITOR CONJUGATE, Jung-Yaw Lin and Mon-Hwei Hu, Institute of Biochemistry, College of Medicine, National Taiwan University , Taipei, Taiwan, Republic of China
Soybean trypsin inhibitor (SBTI) was cross-linked to concanavalin A (Con A) with a bifunctional cross-linker: N-succinimidyl-3-(2-pyridyl dithio) propianate (SPDP). The chimeric protein was purified by gel filtration with a Bio-gel P-200 column. The molar ratio of Con A monomer to SBTI in the chimeric protein was determined to be 1:1. The molecular weight of the conjugate was measured to be 200,000 $\pm$ 2,000 daltons by Sepharose CL-6B gel filtration. The Con A-SBTI conjugate retained 72% of the trypsin inhibitory activity of PDP (2-pyridyl disulphide)-SBTI and all the biological activities of native Con A tested by the agglutination of Sarcoma-180 and red blood cells. The binding properties of Con A-SBTI conjugate on the Sarcoma-180 cells was similar to those of Con A. After the conjugate had been bound on the cell surface, it entered the cells and exhibited a high trypsin inhibitory activity as demonstrated by measuring the trypsin inhibitory activity in the cell lysate, while SBTI alone or the mixture of Con A and SBTI did not show these properties. The chimeric protein, Con A-SBTI, was much more resistant to tryptic digestion than the mixture of Con A and SBTI and could remain intact in Sarcoma-180 cells up to above two hours. By using in vitro colony test, it was found that Con A-SBTI conjugate showed a higher toxic activity to Sarcoma-180, L and NIH 3T3 cells than the equivalent dose of the mixture of Con A and SBTI. The two other conjugates, BSA-Con A and BSA-SBTI, exhibited no such strong inhibitory activity. The Con A-SBTI conjugate was able to prolong the life span of the mice inoculated with Sarcoma-180 cells intraperitoneally. Equivalent doses of controls did not.

0131 ANTIBODY-DIRECTED STAPHYLOCOCCUS AUREUS PROTEIN A-CONJUGATED LIPOSOMES FOR DRUG DELIVERY TO A T-CELL LEUKEMIA (AKR/J SL2), Katherine K. Matthay, Timothy D. Heath, and Demetrios Papahadjopoulos, University of California, San Francisco, CA 94143
Drug delivery to cancer cells with antibody-directed liposomes is a promising method to increase specificity and efficacy of cancer therapy. We describe the use of Staphylococcus aureus protein A (SPA) conjugated to liposomes for screening liposomal efficacy with various leukemic cell lines and monoclonal antibodies. The SPA-liposomes contained methotrexate-γ-aspartate, a transport negative antifolate which we have previously shown to be dependent on liposomes for intracellular delivery. In a 48 h growth-inhibition assay, the SPA-liposomes were 150-fold more effective than unencapsulated drug against anti-Thy 1.1-coated AKR/J SL2 cells, and 50-fold more effective than SPA-liposomes against a non-specific antibody with the cells. The SPA-liposomes were 15-fold more effective than the direct anti-Thy 1.1-liposome conjugate, and also more effective than another indirect system, anti-mouse antibody conjugated to liposomes. The SPA-liposomes were effective against the leukemia cells with different antibody classes and over a wide range of antibody concentration (0.1-100 μg/ml). The SPA-liposomes showed increased activity with a mixture of antibodies that react with three separate antigens of AKR/J SL2. The efficacy of the SPA-liposomes compared to unencapsulated drug was further enhanced when the exposure of the cells was limited to 4-12 h. The SPA-liposomes containing methotrexate-γ-aspartate may provide a versatile, potent and specific means of removal of tumor cells from bone marrow prior to autologous transplantation.

0132 IMMUNOTOXIN-MEDIATED CYTOTOXICITY OF HUMAN PERIPHERAL BLOOD T-CELLS AND INHIBITION OF CLONOGENIC PROLIFERATION OF T-CELL LINES, S. Ramakrishnan, F.M. Uckun, and L.L. Houston, University of Kansas, Lawrence, KS 66045 and University of Minnesota, Minneapolis, MN 55455
Monoclonal antibodies against human T-cell differentiation antigen 3A1 and transferrin receptors were linked to pokeweed antiviral protein (PAP). The antibody-toxin conjugate (immunotoxin, IT) was analyzed for its effect on peripheral blood T-cells in a PHA-induced proliferative assay. The conjugates were also checked for their ability to inhibit the clonogenic growth of T-lymphoblastic cell lines (HSB-2) in vitro. To determine the usefulness of this method to treat leukemic bone marrow preparations ex vivo, the extent of stem cell recovery from human bone marrow was analyzed in the presence of IT. The results indicate that IT treatment in very low concentrations almost completely abolished the mitogen-induced proliferation and this effect was found to be specific. A brief preincubation with IT was found to be sufficient to inhibit T-cell DNA synthesis. While the clonogenic growth of HSB-2 cells was inhibited by the antibody-toxin conjugate, at optimal concentrations the stem cell populations were not affected by the treatment as revealed by the analysis of CFU-C, CFU-E, and CFU-GEMM.

0133 DEPLETION OF NEUROBLASTOMA CELLS FROM BONE MARROW WITH MONOCLONAL ANTIBODIES AND MAGNETIC IMMUNOBEADS, Robert C. Seeger[1], C. Patrick Reynolds[2], Dai Dang Vo[1], John Ugelstad[3], and John Wells[4], [1]Dept. of Pediatrics, UCLA School of Medicine, Los Angeles, CA 90024; [2]Naval Medical Research Institute, Bethesda, MD 20814; [3]University of Trondheim, Trondheim, Norway; [4]Dept. of Medicine, UCLA School of Medicine, Los Angeles, CA 90024

Supralethal chemotherapy and total body irradiation followed by allogeneic or autologous bone marrow transplantation is a promising new therapy for patients with widespread neuroblastoma. Approximately 75% of patients do not have a histocompatible sibling and thus require autologous marrow for hematologic reconstitution. Since many marrows that are harvested for autologous reconstitution may contain sufficient neuroblastoma cells to reestablish tumors, it is crucial to have effective means of removing tumor cells in vitro and of demonstrating that treated marrow is tumor-free. In this pre-clinical study, we investigated removing tumor cells from bone marrow with monoclonal antibodies and magnetic immunobeads. A mixture of monoclonal antibodies optimally label neuroblastoma cell surfaces. Second, optimal conditions for coating magnetizeable beads with with anti-Ig, for forming bead-tumor cell conjugates, and for removing these conjugates with magnets were defined. Third, two sequential treatments of marrow with antibody and then magnetic immunobeads is more effective than a single treatment. Finally, using a sensitive and quantitative detection system, this method was shown to reproducibly remove three to four logs of tumor cells from bone marrow with good recovery of total marrow cells and CFU-G,M. This latter data suggests, but does not prove, that marrow treated in such a manner retains stem cells necessary for hematological and immunological reconstitution.

0134 ANALYSIS OF IMMUNOTOXINS FOR THE DEPLETION OF NEOPLASTIC AND NORMAL MURINE T LYMPHOCYTES, Nancy E. Street and Ellen S. Vitetta, University Of Texas Health Science Center, Dallas, Texas 75235.

We are currently studying a group of spontaneous, in vitro-adapted T cell leukemias from AKR/J mice. In order to target ricin A chain-containing immunotoxins to these cells their cell surface phenotypes were first analyzed using a panel of monoclonal antibodies directed against H-2, Ia, Lyt-1, Lyt-2, Thy 1.1, Thy 1.2, Qa, and TL. The data indicate that these T cells display considerable heterogeneity, both with respect to the markers they express and their density. However, several of the tumors can be effectively killed with immunotoxins directed against H-2K, Lyt 2, Thy 1.1, and Thy 1.2. The degree of toxicity of the immunotoxins is not affected by the addition of NH_4Cl. We are currently investigating the ability of ricin B chain-containing immunotoxins to potentiate the toxicity. Strategies include using two different target antigens on the same cell with ricin A and ricin B chain-containing immunotoxins or a "piggyback" approach where the B chain-containing immunotoxin is directed against the antibody of the ricin A chain-containing immunotoxin.

0135 APPLICATION OF IMMUNOTOXINS TO HUMAN BLADDER CELL LINES MANO AND 486P, Hans-TJ. Thiesen, Horst Durkop and Rudiger Arndt, Urologic University Clinic, Hamburg, FRG.
In treatment of bladder tumors instillation of immunotoxins into the urinary bladder is a feasible approach. In order to evaluate the specific killing factor of immunotoxins, antibody 486P 3-12-1 reacted with 17/18 bladder tumors, was coupled to ricin, ricin A-chain and ricin A/B-chain by the crosslinker SPDP and applied to cell lines 486P (reactive with the MAb) and Mano (non-reactive with the mAb). The following results were obtained: 1) Mano is 100-fold more sensitive than 486P cells to ricin. Addition of 100 nM lactose reduces the toxicity of ricin to Mano by 100-fold and to 486P by 10-fold. The sensitivity of 486P to ricin is increased after neuraminidase treatment, but the inhibitory effect of lactose is not altered. 2) p-Azidophenyl-D-lactoside labeled ricin (galactose binding site blocked) reduces the lactose sensitivity to Mano by 90%, but the inhibitory effect to 486P cells is not affected. 3) The antibody 486P 3-12-1 conjugated to ricin, ricin A-chain and ricin A/B-chain has a cytotoxic effect on 486P cells, but in comparison to cell line Mano, no specific killing factor could be demonstrated. These results demonstrate that binding of antibodies to cell surface determinants are not sufficient for tumor antigen-specific killing. Whether this effect is cell line-specific for this antigen or a general phenomenon has to be evaluated further.

0136 DELETION OF CTL PRECURSORS USING H-2 COUPLED TO RICIN, Ellen S. Vitetta, Nancy E. Street, Mark Soloski* and Jonathan W. Uhr, University Of Texas Health Science Center Dallas, Texas 75235.

We are currently using an H-$2K^k$-ricin immunotoxin to delete precursors of alloreactive cytotoxic T_k lymphocytes (CTLs) from a population of lymph node cells from unprimed mice. Murine H-$2K^k$ molecules are isolated from RDM-4 tumor cells and coupled, by an SPDP linkage, to ricin molecules. Unprimed lymph node cells from C57BL/6 (H-2^b) mice are treated with the immunotoxin in the presence of galactose. The H-2K-ricin molecules are then cross-linked with a monoclonal anti-H-2K antibody. The treated cells are plated at limiting dilution with irradiated B10.A (H-2^a) spleen cells. After 7 days, ^{51}Cr labeled targets are added. Results indicate that approximately 50% of the H-2K-specific CTLs from the unprimed lymph node population are deleted. The immunotoxin does not kill precursors of CTLs directed against other H-2 antigens. Experiments are in progress to increase the potency of the immunotoxin. Thus far, however, results suggest that the CTL precursors are heterogeneous with respect to either the density or affinity of their anti-H-$2K^k$ receptors.

*Present Address: John Hopkins Univ. School Of Medicine, Baltimore, Maryland 21205

Toxin—Antibody Conjugates

0137 POTENTIATION OF IMMUNOTOXIN-INDUCED CYTOTOXICITY Pierre Casellas, Hildur E. Blythman, Bernard J.P. Bourrié, Xavier Canat, Franz K. Jansen, Department of Immunology, Centre de Recherches Clin-Midy (Groupe Sanofi) 34082 Montpellier, Cedex, France.

The kinetics of cytotoxicity induced by ricin and a series of immunotoxins consisting of ricin A-chain coupled to antibodies against cell-surface antigenshas been studied. The rate of protein synthesis decreases according to a mono-exponential function, indicating a first-order process. The inactivation rate induced by immunotoxins was much slower than that achieved with ricin, even when products were compared on the basis of an identical number of molecules bound per cell, demonstrating the real higher efficacy of ricin. The time required to reduce protein synthesis by 90%, denoted T_{10}, was 1.4-1.6 h with ricin, 60 h with anti-T65 immunotoxin on CEM human T leukemia cells (T65 positive), 65 h with anti-p97 immunotoxin on SK-MEL 28 human melanoma cells (p97 positive), and 20 h with an IgM anti-Thy 1.2 immunotoxin on WEHI-7 mouse T leukemia cells (Thy 1.2 positive). In this latter case, when the IgM antibody was replaced by an IgG anti-Thy 1.2, a 5-fold increase in the inactivation rate was obtained, demonstrating the importance of the binding moiety for the immunotoxins. Lysosomotropic amines such as ammonium chloride, chloroquine, and methylamine and carboxylic ionophores such as monensin, strongly increased the rate of protein synthesis inhibition by all immunotoxins tested and increased 4-50,000-fold the sensitivity of cells to the immunotoxin. Enhancement in the inactivation rate was as much as 7-10-fold when either of these compounds was added, generating T_{10} values comparable to those of ricin (1, 2).
Such activation permits an utilization of IT for in vitro treatment of human bone marrow contaminated with leukemic cells prior to autologous bone marrow transplantation. Using a clonogenic assay, we determined the optimal conditions for the use of anti-T65 immunotoxin for in vivo treatment. The maximum nontoxic dose of 2ug/ml co-administered with with 10 mM NH4Cl allows a cytoreduction of more than 6 orders of magnitude of T65 positive clonogenic leukemic cells while the negative counterpart was spared. Such IT offers a new method which is simple, reliable and highly efficient to get rid of undesirable leukemic T cells from bone marrow (3).

1) Casellas P., Brown JP., Gros O., Gros P. et al., Int. J. Cancer (1982), 30, 437-443.
2) Casellas P., Bourrié B.J.P., Gros P. and F.K. Jansen. J. Biol. Chem. (1984), 259, 9359-9364.
3) Casellas P., Canat X., Fauser A.A., Gros O., Laurent G., Poncelet P. and F.K. Jansen (1984). Blood (in press).

0138 POKEWEED ANTIVIRAL PROTEIN-CONTAINING IMMUNOTOXINS. L.L. Houston and S. Ramakrishnan, University of Kansas, Lawrence, KS 66045.

Pokeweed contains several antiviral proteins that inhibit the action of eucaryotic ribosomes. At least three different species of pokeweed antiviral proteins (from spring leaves (PAP), late summer leaves (PAP-II), and seeds (PAP-S)) exist that differ in their amino terminal sequences and which do not cross-react with antibodies. The proteins are not glycosylated. Because PAP does not naturally have a B chain analog of ricin, it cannot easily cross the plasma membrane. When coupled covalently to ricin B chain, PAP forms a potent toxin similar to ricin. The various species of PAP have molecular weights of about 29,000 and are not cytotoxic to cells grown in culture except at high concentration. These proteins catalytically inhibit ribosomes with about the same efficiency as ricin A chain. Immunotoxins (directed against antigens found on the surface of mouse T-cells) containing PAP were nearly as effective as immunotoxins that contain ricin A chain in inhibiting protein synthesis. Protein synthesis by cultured cells was inhibited by 50% using 1-100 ng/ml depending on the antibody used in the immunotoxin. Immunotoxins containing PAP or PAP-S are equally efficient immunotoxins. PAP linked to antibody by a non-cleavable bond was cytotoxic, whereas, ricin A chain similarly linked is not cytotoxic. A PAP-containing immunotoxin was able to prevent the growth of Thy 1.1 positive leukemia cells in mice if the immunotoxin was directed against Thy 1.1 antigen, but protection was not afforded if the immunotoxin was directed against Thy 1.2 antigen. The divalent fragment of the antibody coupled to PAP was also effective. Agents, such as chloroquine, that perturb the pH of lysosomes potentiated the effect of these immunotoxins. Chloroquine reduced the amount of immunotoxin needed to inhibit protein synthesis and it increased the rate at which protein synthesis was inhibited.

Monoclonal Antibodies to Cell Surface Receptors

0139 IDIOTYPIC ROUTES TO MONOCLONAL ANTI-RECEPTOR ANTIBODIES, W.L. Cleveland, N.H. Wassermann, B.L. Hill, A.S. Penn, H.H. Ku, R. Sarangarajan and B.F. Erlanger, Department of Microbiology, Columbia University, New York, N.Y. 10032

A procedure has been developed for the preparation of antibodies to the acetylcholine receptor (AChR). This procedure is based on an idiotypic mechanism and gives antibodies that are inherently specific for the receptor combining site. In one form of the procedure, antibodies were first raised to a structurally constrained agonist of the AChR (BisQ) using a bovine albumin conjugate of BisQ (BisQ-BSA). The specificity of these antibodies was similar to the specificity of the AChR in its excited state, i.e. agonists were bound by the antibodies in a rank order similar to that obtained with receptor, and antagonists were bound poorly. In the second step of the procedure, rabbits were immunized with anti-BisQ antibodies in order to raise anti-idiotypic antibodies. The anti-idiotypic antibodies reacted with cross-reactive determinants found on AChR preparations from *Electrophorus electricus*, *Torpedo californica*, and rat muscle. In some of the rabbits, signs of transient experimental myasthenia gravis were seen.

In a second, more direct form of the procedure, mice were immunized with BisQ-BSA and splenocytes were immortalized by fusion with myeloma cells. Screening of the fusion revealed a substantial number of clones secreting auto-anti-idiotypic antibodies, some of which reacted with receptor. One clone, F8-D5, was selected for further analysis. It bound to both anti-BisQ and AChR, and binding to one was inhibited by the other. Binding to both was also inhibited by BisQ, decamethonium, and α-bungarotoxin. In immunofluorescence with *Torpedo* tissue, staining patterns were identical to that produced by conventional anti-AChR raised by direct immunization with AChR. Preliminary results suggest that immobilized F8-D5 can be used to isolate receptor. In experiments with a reconstituted vesicle system, preliminary results indicate that F8-D5 inhibits influx of ^{134}Cs. This procedure has also been used successfully to prepare antibodies to the adenosine receptor.

Initial attempts to understand the idiotypic mechanism on which the above procedures are based have suggested that anti-BisQ secreting cells can be found among splenocytes from unimmunized mice with markedly different levels occurring in different strains. This raises the possibility that the auto-anti-idiotypic response to BisQ may be strain dependent.

0140 ANTI-IDIOTYPIC ANTIBODIES AS PROBES FOR THYROID STIMULATING HORMONE-RECEPTOR, Nadir R. Farid, Thyroid Research Laboratory, Memorial University of Newfoundland, St. John's, Newfoundland, A1B 3V6.

On the premise that an antibody combining site is a mirror image of its antigen epitope, it is expected that an anti-idiotypic i.e. an antibody specific for the combining site of the first antibody will be homologous to the epitope. Anti-idiotypic antibodies raised against hormones or drugs would therefore, be anticipated to interact with their respective receptors. Thyroid stimulating hormone (TSH) is a glycoprotein hormone made up of an α and a β subunit. Little information is available concerning the TSH receptor and the manner whereby the two TSH subunits interact with the receptor. We have raised anti-TSH anti-idiotypic antibodies and found these to interact with the high affinity binding site for TSH on thyroid plasma membranes, to induce adenylate cyclase activation and iodide transport into dispersed thyroid cells as well as to promote the organization of these cells into follicular structures. The anti-TSH anti-idiotypic antibody interacted with an Mr $\sim$ 200,000 holoreceptor band resolved on SDS-PAGE from thyroid membranes and transferred to nitrocellulose paper. In another set of experiments we raised anti-idiotypic antibodies against monoclonal antibodies specific respectively for the α and β subunits of TSH. Neither the α nor β monoclonal antibody specific anti-idiotypic antibodies interacted with the TSH holoreceptor. Their combination inhibit binding of radiolabelled TSH to thyroid membrane. The mixture of anti-idiotypes increased basal cyclase activity significantly compared to normal IgG, induced follicular organization of dispersed cells, their uptake of ^{131}I as well as their binding to an Mr $\sim$ 200,000 holoreceptor on protein blotted thyroid membranes. As a result of the second set of experiments, we propose that the interaction of TSH with its receptor involves two signals delivered by the two subunits rather than a single signal requiring their combination.

Anti-idiotypic antibodies raised against highly purified hormones can be obtained in large amounts. They facilitate simple isolation of hormone receptors and are useful as probes for hormone-receptor interactions.

0141 USE OF MONOCLONAL ANTI-IDIOTYPIC ANTIBODIES AS PROBES OF THE MAMMALIAN REOVIRUS TYPE 3 RECEPTOR: IDENTIFICATION OF HOMOLOGY WITH THE MAMMALIAN BETA-ADRENERGIC RECEPTOR AND USE AS A SPECIFIC ANTI-VIRAL VACCINE. Glen N. Gaulton, Man Sung Co and Mark I. Greene. Department of Pathology, Harvard Medical School. Boston, MA 02115.

Syngeneic monoclonal anti-idiotypic antibodies which recognize the mammalian reovirus serotype 3 receptor have been constructed and successfully used to isolate reovirus receptors from a panel of both normal and transformed mammalian cells. Anti-idiotypic anti-receptor antibodies were prepared following immunization of syngeneic mice with a monoclonal antibody which specifically recognizes the binding domain on the type 3 viral hemagglutinin cell attachment protein. The specificity of anti-idiotypic antibodies was confirmed by their capacity to: 1) mirror the tropism of virus binding; 2) inhibit the binding of type 3 virus to cellular targets ; and 3) mimic many of the physiological effects of virus which are related to attachment. Biochemical analysis of anti-idiotype immunoprecipitates indicated that the mammalian reovirus receptor shares extensive amino acid sequence and functional homology with the mammalian beta-adrenergic receptor system. Reovirus type 3 receptors appear identical on all cells analyzed thus far, including lymphocytes and neuroblastoma cells, with a molecular weight Mr = 67,000Da and a heterogeneous PI of 5.7-6.0. Mammalian $beta_2$-adrenergic receptor purified from canine lung by ligand affinity chromatography was immunoprecipitated by anti-idiotype (90% recovery) and displayed an identical 2-D gel pattern to that seen in anti-idiotype immunoprecipitates of lymphoid and neuronal cells. Tryptic digests of both reovirus and beta-adrenergic receptors showed indistinguishable patterns, with major cleavage products of 57, 50 and 45KDa. Functional homology of these two receptors was confirmed by the observation of specific ^{125}I-hydoxyiodo-pindolol (HYP) binding to anti-idiotype purified receptor and by the immunoprecipitation of cell bound ^{125}I-HYP by anti-idiotype. Anti-idiotypic antibodies have also been utilized as an immunogen to elicit reovirus type 3 specific CTL, DTH and antibody responses. Priming of reovirus specific CTL responses was dependent upon immunization with anti-idiotype bearing hybridoma cells in contrast to DTH and antibody responses which were induced following the subcutaneous injection of purified syngeneic monoclonal antibody. The use of adjuvant was not required to elicit immune responsiveness although adjuvant didaugment anti-viral activity. Anti-idiotypic antibodies have recently been shown to mimic the physiological effects of virus binding in the induction of non-specific Ts cell activity and in receptor mediated inhibition of host cell DNA synthesis. These results indicate that modulation of the beta-adrenergic receptor by reovirus or anti-idiotype binding can have profound effects on host cell function which are not directly coupled to viral infectivity.

0142 PRODUCTION OF PROBES FOR THE STUDY OF CELL SURFACE RECEPTORS USING IDIOTYPE-ANTI-IDIOTYPE MOLECULAR MIMICRY. D. Scott Linthicum and Michael B. Bolger. Department of Pathology, University of Texas Health Science Center, Houston, Tx. 77025 and School of Pharmacy, University of Southern California, Los Angeles, CA 90033.

Identification and purification of some cell surface receptors is often hindered by several factors, foremost being low stability of detergent solubilized receptors, low density of receptors in specific tissues and lack of suitable high affinity probes for purification techniques. In the past few years an "idiotype-anti-idiotype molecular mimicry" strategy has been used to produce antibody probes for specific receptors with much success.

In 1978 Sege and Peterson (1) reported that rat antibodies directed against retinol binding protein (RBP) could be used as an immunogen in rabbits to produce a second set of antibodies (anti-idiotype) which mimicked the original ligand (RBP) in binding studies. Some of the anti-RBP antibodies apparently acted as a "negative mold" i.e., the internal image of the antigen-binding region (idiotype) acted as a molecular negative image of RBP, thereby acting as a template for some of the anti-idiotype antibodies; some of these anti-idiotype antibodies had the same molecular shape as the original ligand. Later studies by these same workers on insulin, anti-insulin and anti-idiotype antibodies demonstrated the same phenomenon. Since these initial studies, this concept has been put to work in the past several years for thyroid stimulating hormone receptor, beta-adrenergic receptors, acetylcholine receptors, chemotactic receptors, lymphocyte immunoglobulin receptors and viral receptors.

Over fourteen years ago, Lennon and Carnegie (2) proposed that some diseases may be caused by antibodies directed against hormonal or neurotransmitter cell surface receptors, thereby producing an "immunopharmacological blockade." This powerful hypothesis has been supported by a number of findings in myasthenia gravis, Graves disease and insulin resistant diabetes. To what extent immunopathological anti-receptor antibodies are generated during infectious or neoplastic disease as a result of the idiotype-anti-idiotype network is not known. Furthermore, the usefulness of this idiotype-anti-idiotype molecular mimicry approach in the identification, purification and modulation of cell surface receptors in neoplastic growth is intriguing and invites further investigation.

(1) Sege, K. and Peterson, P.A. (1978) Nature 271:167

(2) Lennon, K.A. and Carnegie, P.R. (1971) Lancet 1:630.

Monoclonal Antibodies to Glycolipids and Their Applications

0143 DISIALOGANGLIOSIDES GD_2 AND GD_3 ARE INVOLVED IN THE BINDING OF HUMAN MELANOMA CELLS TO THE CELL ATTACHMENT SITE OF FIBRONECTIN. D.A.Cheresh, M.D.Pierschbacher, M.A. Herzig and R.A. Reisfeld, Scripps Clinic and Research Foundation, La Jolla, CA 92037.

The attachment of human melanoma cells to fibronectin and to a hepta-peptide glycyl-L-arginyl-glycyl-L-aspartyl-L-seryl-L-prolyl-L-cysteine (GRGDSPC), which constitutes its principal cell attachment site, is inhibited by monoclonal antibodies (Mabs) MB3.6 and 126 that are directed against the disialogangliosides GD_3 and GD_2, respectively. This inhibition appears to be specific since it is not mediated by monoclonal antibodies of various isotypes directed against either protein or carbohydrate epitopes of a number of other major melanoma cell surface components, i.e., HLA antigens, a chondroitin sulfate proteoglycan, a sialylated glycoprotein and a neutral glycolipid. In contrast, melanoma cell adhesion to tissue culture treated plastic is not affected by pre-treatment with Mabs MB3.6 or 126, indicating that cell surface bound antibody does not compromise cellular functions unrelated to the adhesion to fibronectin. A study of the kinetics involved in this inhibition of melanoma cell attachment suggests an important role for GD_2 and GD_3 in the early phases of melanoma cell adhesion to immobilized fibronectin. Moreover, melanoma cells preattached and spread on a fibronectin substrate can be specifically detached by the exogenous addition of purified MB3.6. We provide evidence that these gangliosides may act synergistically with a putative protein receptor on the cell surface which is trypsin-sensitive in the presence of EDTA. In this regard, we demonstrate that melanoma cells pretreated with trypsin-EDTA show a reduced binding capacity to fibronectin and that Mabs MB3.6 and 126 are unable to further reduce this binding.

0144 EXPRESSION OF FUCOSE-CONTAINING GLYCOLIPIDS IN EMBRYONIC BRAIN
Gerald A. Schwarting and Miyuki Yamamoto, E.K. Shriver Center, Waltham, MA 02254

A monoclonal antibody (7A) raised against a homogenate of embryonic rat forebrain, marks the distribution of region- and developmental stage-specific cell surface antigens in the mammalian central nervous system. In the mouse, immunocytochemical staining revealed that 7A antigen is expressed almost exclusively in germinal layers of the cerebral cortex beginning as early as the 11th day of gestation and becomes undetectable by birth. Typical staining is seen at embryonic days 13 to 15, in which only the ventricular and the sub-ventricular zones of the cerebral cortex are intensely labelled, whereas most other areas of the brain are unlabelled. Tissue culture studies indicate that the 7A antigen is localized at the plasma membrane. Molecular species possessing the antigen are identified as neutral glycolipids which contain the X-determinant; Gal (β1-4) [Fuc (α1-3)]GlcNAc. . . Several glycolipids which possess this carbohydrate structure are expressed maximally in the rat cortex at E 15-17 but disappear by P14. These glycolipids are expressed in embryonic brains of many mammalian species (mouse, rat, rabbit, calf and human) and may be a cell surface component important in normal development of the central nervous system. We have also shown that Lewis a glycolipids and SSEA-3, globoside-derived glycolipids are also embryonic brain antigens in many mammalian species. Further studies of the localization and expression of these antigens in CNS should shed light on their potential role in differentiation and development.

Monoclonal Antibodies to Cell Surface Receptors, Glycoplipids, Lymphokines, Matrix and Cytoskeletal Components

0145 MONOCLONE DEFINITION OF A BIOLOGICALLY SIGNIFICANT PEPTIDE REGION OF HUMAN LEUKOCYTE INTERFERON, B. Altrock, D. Chang, H. Hockman, K. Duker and P. Lai, Amgen, Thousand Oaks, CA 91320

A neutralizing monoclonal antibody defined, biologically significant region of human leukocyte interferon has been mapped to an approximately 4 Kd region of the protein. The monoclone used (4F2) was generated to a high specific activity recombinant DNA derived analog of IFN-α, designated IFN-αCon_1, which differs from the naturally occurring IFN-αF subtype at ten amino acid positions. 4F2 was found to neutralize IFN-α biological activity. Monoclonal specificity was verified by competitive immunoassay using reference IFN-α and its antisera. The 4F2 antibody was found to selectively bind specific IFN-α subtypes found in buffy coat and lymphoblastoid IFN-α preparations which were identified by affinity purification and subsequent amino acid sequencing. The active region identified by 4F2 was mapped by Western analysis and amino acid sequencing of the products of site specific IFN-α protein cleavage. Though the amino acid sequence of the 4F2 epitope bearing peptide fragment was generally conserved throughout the IFN-α's, differences in monoclone reactivity with individual subtypes suggests that the configuration of this region may influence 4F2 binding and biological activity.

0146 TRANSFORMANTS EXPRESSING HUMAN CHROMOSOME #11 ENCODED CELL SURFACE ANTIGENS, Jerome Bill, Richard Davis, John Lehman and Carol Jones, Department of Pathology and Eleanor Roosevelt Institute for Cancer Research, University of Colorado Health Sciences Center, Denver CO 80262

J1 is a Chinese hamster ovary X human amniotic fluid fibroblast hybrid which has a single copy of chromosome #11 as the only human contribution to its genome. J1 expresses at least 5 different chromosome #11 encoded cell surface antigens which are recognized by monoclonal antibodies. Cotransfection of Chinese hamster ovary cells with pSV2-neo and genomic J1 DNA resulted in transformants resistant to G418 at a frequency of 5 x 10^{-4}-10^{-3}. Indirect immunofluorescent staining of G418 resistant transformants with 3 of the monoclonal antibodies identified cells expressing each of a_1, a_4, and a_7 antigens at frequencies of 10^{-4}-5x10^{-3}. Isolation of antigen positive transformants was accomplished by successive enrichment of populations initially positive at 5x10^{-5}-10^{-3}. Positive populations were diluted such that one of 20-50 hanging drops contained a positive cell which was identified by immunofluorescent staining. Positive drops were allowed to grow and the process was repeated. 6-50 fold enrichment was achieved in one cycle. Eventual cloning yielded a population of cells 99% positive for the a_7 antigen. This transformant is killed by 20 fold less antibody than J1 in a complement mediated cytotoxicity assay and displays 6 fold greater mean fluorescence by flow cytometric analysis. Efforts are in progress to clone the cells expressing the other antigens and to clone the genes responsible for expression of these human chromosome #11 encoded antigens.

0147 ANTI-Bp35 ANTIBODY INDUCES HUMAN B CELL PROLIFERATION: IMPLICATIONS FOR IN VIVO IMMUNOTHERAPY, Edward A. Clark and Jeffrey A. Ledbetter, University of Washington and Genetic Systems Corporation, Seattle, WA 98195

The BP35 (CD20) pan B cell antigen is an excellent candidate structure for monoclonal antibody (MAb) therapy since it is expressed at high density on B cell malignancies, is not readily modulated from the cell surface, and is not on stem cells. We have developed two mouse MAb to Bp35. When administered at 7 mg/kg in one dose to macaques, both MAb depleted circulating B cell levels, but the IgG2a (1F5) MAb was more effective than the IgG2b (2H7) MAb. Based on these studies, the 1F5 MAb has been prepared for therapeutic trials at two cancer centers. However, until recently nothing has been known about the possible function of the Bp35 molecule. Here we report that the 1F5 anti-Bp35 MAb stimulates resting B cells to proliferate. This proliferation does not require T cells, but is augmented by T cell derived growth factors, and cross-linking is required for activation. These features are similar to B cell proliferation induced by anti-Ig. However, Bp35 and sIg do not cocap. Unlike anti-Ig, anti-Bp35 does not require attachment to beads to function and, significantly, anti-Ig and anti-Bp35 induced proliferation are additive. Fab fragments of anti-Bp35, while not stimulatory themselves, do augment anti-Ig driven proliferation. Bp35 resembles the T cell antigen CD3 in several ways: both are pan B and pan T cell antigens distinct from antigen receptors; IgG2a MAb to Bp35 or CD3 induce proliferation, but IgG2b MAb do not; for both Bp35 and CD3 MAb, Fc domains, and presumably accessory cells, are necessary for proliferation. Both anti-Bp35 and anti-CD3 MAb induce proliferation in vitro, yet in vivo deplete B or T cells. The significance of these findings for immunotherapy of B cell malignancies will be discussed.

0148 MONOCLONAL ANTIBODIES AGAINST CHICK gp 115, A MATRIX GLYCOPROTEIN WITH BROAD DISTRIBUTION, Alfonso Colombatti*, Giorgio M. Bressan, Dino Volpin, and Ines Castellani, Institute of Histology, University of Padova, Padova, Italy and Oncology Reference Center*, Aviano (PN) Italy.

Hybridoma cell lines were generated producing monoclonal antibodies to chick gp 115, a 115,000-dalton glycoprotein widely distributed in the connective tissue. The specificity of the antibodies was determined by indirect radioimmunobinding: the extent of binding was a function of i) antigen and ii) antibody concentration; iii) binding of radiolabelled antibody was inhibited by cold antibody and iv) among many known extracellular collagenous or noncollagenous glycoproteins tested only gp 115 gave a strong positive binding reaction. The antibodies were used for indirect immunofluorescence and a strong staining reaction was detected in all blood vessels, around smooth muscle cells in several organs, and in the connective matrix of other tissues such as the liver, and the lung. Based on the competition of binding of ^{125}I-labeled purified antibody by cold antibodies, two separate determinants were identified on gp 115. Further analysis of the localization of the epitope was obtained by CNBr cleavage and partial digestion of gp 115 with Staphylococcus aureus V8 protease and α-chymotrypsin. Following CNBr cleavage a major fragment of Mr = 35,000 was recognized by 4 monoclonal antibodies, and fragments of comparable Mr were detected following V8 protease and α-chymotrypsin digestion.

0149 MONOCLONAL ANTI-IDIOTYPIC ANTIBODIES IN THE ACETYLCHOLINE RECEPTOR ANTIGEN SYSTEM: POSSIBLE MEANS OF THERAPY, Donard S. Dwyer and Simone Schönbeck, University of Alabama in Birmingham, Birmingham, AL 35294.

Autoantibodies against the acetylcholine receptor (AChR) cause the neuromuscular impairment observed in myasthenia gravis (MG). One monoclonal antibody (Mab) against the AChR, denoted ACR 24, can produce similar features of muscle weakness in some mice. In addition, about 30% of myasthenic patients have anti-idiotypic antibodies which are specific for ACR 24. These anti-idiotypes are found most frequently in recently diagnosed patients with mild disease. Thus, the ACR 24 idiotype has been shared from mouse to man.

Recently, monoclonal anti-idiotypic antibodies have been raised against ACR 24 and two other anti-AChR Mabs. The anti-idiotype against ACR 24 (FZ1.4) effectively competes with anti-idiotypes from MG patients for binding to ACR 24. Mice were then either pretreated with a combination of these anti-idiotypes prior to immunization with muscle AChR or were left untreated. The anti-idiotypes had a suppressive effect (as much as a 50% reduction in specific antibody) on the anti-AChR response in comparison to the untreated mice. The suppression was most apparent in mice with low to moderate anti-AChR titers. The anti-idiotypes had no effect on mice which were boosted with receptor and had high levels of anti-AChR antibody. We would speculate that ACR 24 is an important idiotype which ises early during the immune response to AChR in both mouse and man.

0150 ENRICHMENT OF A CHONDROITIN SULFATE PROTEOGLYCAN ON A METASTATIC MELANOMA CELL LINE, Daniel K. Hsu, David A. Cheresh, Ralph A. Reisfeld, Scripps Clinic & Research Foundation, La Jolla, CA 92037.

The identification of cell surface changes associated with the metastatic phenotype of tumor cells may aid in understanding their invasive properties. In this regard, we have examined the cell surface proteoglycan expression of a human melanoma tumor cell line A375-P and its metastatic variant A375-M (1). We have observed a two-fold enrichment of a cell-surface proteoglycan in the metastatic line A375-M over A375-P using the chondroitin sulfate proteoglycan-defining monoclonal antibodies (MAbs) 9.2.27 and 155.8 with fluorescence activated cell sorter (FACS) analysis, but no difference was observed in the expression of HLA-A,B,C between the two cell lines indicating that the elevated proteoglycan level is not simply due to increased total protein synthesis. Competetive antigen assays of cell lysates and culture media using the MAb 9.2.27-horseradish peroxidase conjugate showed similar increases of the total cell-associated 9.2.27-antigen and antigen shed into the culture media, respectively, in the metastatic variant over the parent line. Both parent and metastatic cell lines exhibited identical forward-angle light scatter by FACS suggesting that the change in proteoglycan expression is not caused by differences in cell size or shape. In addition, the two cell lines had similar growth rates and the observed differential antigen expression was consistent throughout the cell cycle.

(1) Kozlowski, J. M., et al (1984) Cancer Res. 44, 3522-3529.

0151 MONOCLONAL ANTIBODIES DETECT P-GLYCOPROTEIN, Norbert Kartner, Deanna Evernden-Porelle, Grace Bradley and Victor Ling, Ontario Cancer Inst., Toronto M4X 1K9

Numerous tissue culture models of multidrug resistance have been developed in the hope of elucidating the mechanisms of clinical resistance to combination chemotherapy. These cell lines have a number of features in common which define what is now termed the multidrug resistance phenotype. These features include a pleiotropic cross-resistance to unrelated drugs, reduced drug uptake, and a 170,000 dalton cell surface component (the P-glycoprotein), the expression of which correlates well with the expression of the multidrug resistance phenotype (Kartner, N. et al. Science 221: 1285, 1983). Little is known, however, concerning the role that P-glycoprotein plays in multidrug resistant cells, its normal cellular function, or its relevance to clinical multidrug resistance. To provide a means of furthering our understanding in these areas, we have prepared hybridomas from spleen cells of mice immunized with plasma membranes of drug resistant human and Chinese hamster ovary cells. Eight monoclonal antibodies specific for drug resistant membranes have been isolated and characterized. These antibodies detect P-glycoprotein in Western blots and in indirect immunofluorescence of fixed cells. In hamster P-glycoprotein, three spatially distinct epitopes have been defined, all of which appear to be exposed on the cytoplasmic surface of the plasma membrane. At least one of these epitopes appears to be highly conserved. Thus, monoclonal antibody reagents are now available which will be useful in the sensitive and specific detection of P-glycoprotein in experimental systems and in neoplastic human tissue. Supported by the National Cancer Institute and the Medical Research Council of Canada.

0152 FINE SPECIFICITIES OF MONOCLONAL ANTIBODIES TO THE ACTIVE SITE OF GASTRIN, Elizabeth W. Katt and John H. Walsh, CURE, Wadsworth Medical Center, UCLA School of Medicine, Los Angeles, Ca. 90073

Gastrin is both a classic gut hormone which regulates gastric acid secretion and a mitogen which is implicated in the tissue specific regulation of gastrointestinal cell growth. The gastrin receptor is found on parietal cells as well as approximately one-third of all gastrointestinal tumors. We report here the production of murine monoclonal antibodies against the active site of gastrin. In homology with the gastrin receptor these monoclonal antibodies crossreact with cholecystokinin, suggesting that anti-idiotypic antibodies to these monoclonals may cross react with the gastrin receptor. Thus far we have not been able to isolate autologous anti-idiotypic antibodies from a fusion following an immunization with gastrin or cholecystokinin. However, we have shown that the dominant specificities of antibodies to these ligands is limited and shared by rabbit and mouse.

0153 IDENTIFICATION OF BCDFγ_2 AND ITS RECEPTORS BY USING mIgG$^+$ BALB/c B LYMPHOID TUMOR CELLS, A20 - JIN KIM, BIOTECHNOLOGY RESEARCH CENTER/REVLON, DEPARTMENT OF IMMUNOLOGY. My main interest in the research has been to determine the antigen nonspecific signal(s) given by the T cell derived lymphokines (BCDFγ_2) and their receptors necessary for the differentiation of B cells into IgG secreting B cells. We are identifying BCDFγ_2 by using mIgG$^+$ BALB/c B lymphoid tumor cells as a model. In our recent studies, we found that supernatants prepared from a T cell hybrid (#91-4) and an allogeneic T cell clone (#19), after the stimulation of Con A, induced the IgG$_2$ secretion from mIgG A20 tumor cells. The number of IgG$_2$ secreting cells was determined by using reverse plaque forming cell assays after the tumor cells were incubated with various concentrations of T soluble factors for 3 days. We are in the process of comparing the activity of BCDFγ_2 to various other factors. The receptors against BCDFγ_2 will be analyzed by raising the monoclonal antibodies (mAb) against BCDFγ_2 receptors. These monoclonal antibodies will be generated by fusing NS$_1$ myeloma cells with spleen cells of rats which are immunized with A20 tumor cells. Hybrids will be selected on the basis of (1) the ability of the monoclonal antibodies to bind to A20 tumor cells, and (2) the ability to inhibit the BCDFγ_2 activity on A20 tumor cells.

0154 EFFECTS OF ANTI-EGF RECEPTOR MONOCLONAL ANTIBODIES UPON HUMAN TUMOR CELLS, John Mendelsohn, Hideo Masui, Anh Le and Carol MacLeod, University of California, San Diego, La Jolla, CA 92093
Four monoclonal antibodies (MoAbs) were produced against the receptor for epidermal growth factor (EGF) on human epidermoid carcinoma cell line, A431. Three MoAbs, which react with the polypeptide chain of the receptor, compete with EGF and each other for binding to the receptor. These 3 MoAbs inhibit EGF-stimulated functions in cultured cell lines, including cell proliferation and tyrosine protein kinase activity. One MoAb, which reacts with an oligosaccharide similar to blood group A, has a lower affinity for the receptor, does not block EGF binding, and has no effect on EGF activity upon cultured cells. All 4 MoAbs immunoprecipitate EGF receptors. Four cell lines bearing EGF receptors (3 epidermoid and 1 heptoma) are unusual, in that both EGF and the 3 blocking MoAbs inhibit their proliferation in culture. Among 10 cell lines tested, the 4 which are growth-inhibited in culture are also inhibited from proliferating as xenografts in athymic mice treated with anti-EGF receptor MoAbs. Inhibition *in vivo* is caused by both IgG1 and IgG2A isotypes. The selective inhibition of proliferation of some tumor cells by the MoAbs is not related to receptor number on the cells in culture, since 2 of the susceptible cell lines have elevated numbers of EGF receptors ($0.5 - 2 \times 10^6$/cell) while the other 2 have normal numbers ($3 - 5 \times 10^4$/cell). We are now exploring the possibility that these tumors have increased receptor numbers *in vivo*.

0155 A monoclonal antibody that inhibits BGDF (BCGF-II) function.
A. Muraguchi, K. Shimizu, Y. Kishi, and T. Kishimoto. Cellular Immunology, Inst. for Molecular and Cellular Biology, Osaka Univ., Osaka, 565, Japan.
The presence of a B cell growth factor (BCGF) with high molecular weight (BCGF-II) has been reported in human as well as in murine system. We have partially purified BCGF-II from a HTLV-tranformed T cell line. BCGF-II obtained from this cell line had a MW of 60K and a PI value of 5 to 6 and showed growth and differentiation activities, i.e. induction of not only in vitro proliferation but also in vitro differentiation and IgM secretion in BCL1 cells as well as in DXS-stimulated murine B cells, thus we refered this lymphokine as B cell growth and differentiation factor (BGDF). We have fused a non-secreting myeloma cell line and murine splenic cells previously immunized with a human-mouse B cell hybridoma, for the establishment of a hybridoma producing a monoclonal antibody (Mab) that recognizes a BGDF receptor. One of the Mabs we have established, called B1H5, has been found to inhibit BGDF-induced but not LPS-induced in vitro proliferation and differentiation of BCL1 cells. An isotype-matched another monoclonal antibody (IgM) did not show this inhibition. Distribution study revealed that the antigen recognized by B1H5 was expressed on various B cell lines, as well as normal activated B cells. In contrast, the antigen was not expressed on normal activated T cells or IL-2 dependent T cell lines. B1H5$^+$ cell lines produced high activity of BGGF in the culture supernatant. The result suggests that B1H5 antigen may be a candidate for BGDF receptor and the fact that B1H5$^+$ cell lines produce BGDF may support a notion of autostimulatory growth regulation in B cell tumors.

0156 MONOCLONAL ANTIBODY WHICH DETECTS A CLONOTYPE OF T CELL RECEPTOR ON EL4 AS A TOOL OF THERAPY FOR EL4 INJECTED MICE. Ryuji Nagasawa, Osami Kanagawa. Dale R. Wegmann and Jacques M. Chiller, Lilly Research Laboratories, La Jolla, CA 92037.
EL4, a C57BL/6 (H-2^b) derived thymoma, is known to express a T cell receptor of unknown specificity on its surface. In an attempt to determine whether monolconal anti-T cell receptor antibody might prove useful for therapy of thymomas, we derived a hybridoma antibody which reacts with a surface molecule on EL4 by fusion of EL4 immunized BALB/C spleen cells and Ag 8.653. This monoclonal antibody (A1) has been defined as an anticlonotype to the T cell receptor of EL4 on the following basis: First, A1 shows cytolytic activity in the presence of complement for EL4 cells only, and A1 shows no cytolytic activity for spleen cells of C57BL/6, C57BL/10 (H-2^b), (C57BL/6 x DBA/2)F_1 (H-2^b/H-2^d) mice, C57BL/6 derived cloned helper T cells, C57BL/6 derived cloned cytotoxic T cells or AKR (H-2^k) derived thymoma cells (BW 5147). Second, immunoprecipitation of cell lysate from ^{125}I-labelled EL4 cells with A1 reveals that the molecular weight of the cell surface antigen detected by A1 is about 90,000 in non-reduced and 45,000 in reduced sample, which corresponds to the molecular weight of the murine T cell receptor reported by others. When this cytolytic antibody was administered by daily peritoneal injections into EL4 injected C57BL/6 mice, prolongation of life was observed, as compared to EL4 injected C57BL/6 mice which did not receive the cytolytic antibody. These findings suggest that cytolytic monoclonal antibody to a clonotype of the T cell receptor may be helpful as therapy for tumors of T cell origin.

0157 REGULATION OF THE EXPRESSION OF INTERLEUKIN-2 RECEPTORS ON THYMOCYTES AND T-CELLS. Gabrielle H. Reem and Ning-Hsing Yeh, N.Y.U. Medical Center, New York, NY 10016
Studies of the expression of the cell surface receptors to interleukin-2 (IL-2) by human thymocytes and peripheral blood T lymphocytes indicate that freshly obtained T-cells and thymocytes did not express receptors to IL-2. Unlike T-cells, which are resting cells and require activation by lectins or antigens to proliferate, thymocytes proliferated spontaneously in vitro for the first 48 hours of culture without expressing receptors to IL-2. Activation of thymocytes with Con A resulted in the expression of receptors to IL-2 which were detected by the binding of the monoclonal antibody anti-Tac. Purified recombinant IL-2 (Hoffmann-La Roche) or highly purified natural IL-2 increased the expression of receptors to IL-2 by Con A activated thymocytes within 24 hours. After 48 hours thymocytes required IL-2 in order to continue to express IL-2 receptors and to proliferate. Anti-Tac, which binds to the IL-2 receptors, downregulated the expression of IL-2 receptors and inhibited IL-2 dependent proliferation. This inhibition could be reversed in a dose dependent manner by IL-2. Drugs which inhibit the synthesis of IL-2 and decrease the expression of receptors to IL-2, inhibit the proliferation of thymocytes after 48 hours of culture. The regulation of the expression of receptors of T-cells to IL-2 is similar to that described for thymocytes which have become dependent on IL-2 in order to proliferate. The observation that IL 2 is required for the expression of its own receptors on precursors of T-cells and on mature T-cells may be important in devising strategies aimed at introducing cytotoxic agents into leukemic cells with the help of antibodies which bind to the cell surface receptors to IL-2.

0158 MONOCLONAL ANTIBODIES TO VASCULAR ENDOTHELIAL CELL SURFACE COMPONENTS WHICH INTERFER WITH HEMATOGENOUS METASTASIS, Jean R. Starkey and Rokia M. Abdallah, Montana State University, Bozeman, MT 59717, and Washington State University, Pullman, WA, 99164.
Strategies to reduce tumor cell extravasation effectively reduce hematogenous metastasis. Since tumor cell attachment to vascular endothelial cells preceeds and is less avid than subsequent attachment to the subendothelial basement membrane matrix, blocking tumor: endothelial attachment should be a particularly effective metastatic approach. We prepared a series of monoclonal antibodies to vascular endothelial cell surface components using a) conventional immunization with whole cells in vivo and b) in vitro immunization using concentrated butanol extracts from endothelial cells grown in culture. Membrane components of interest were predicted to be present in the butanol extract since butanol extracted and subsequently washed endothelial cells (still 100% viable) bound fewer tumor cells than unextracted controls. No difference was evident between immunization protocols in the ratio of antibodies obtained to a) endothelial cell restricted antigens and b) less histiotypically restricted membrane components. Of 20 strongly positive clones isolated, 4 secreted antibody to sites restricted to endothelial cells, 2 secreted antibody to sites restricted to endothelial cells and leukocytes, and the rest, antibodies which also reacted with unrelated tissues. Seven of the clones secreted antibody which blocked tumor cell attachment to vascular endothelium in vitro and, in addition, 3 of these exhibited the ability to significantly alter tumor lung colonizing ability. The endothelial membrane components with which those antibodies with "blocking" activity react were found not to be restricted to endothelial or endothelial and leukocytic cells. NIH CA 32071

0159 A GLYCOLIPID ANTIGEN ASSOCIATED WITH BURKITT'S LYMPHOMA, Thomas Tursz, Joëlle Wiels, Eric Holmes*, Nancy Cochran* and Sen-itiroh Hakomori*, Institut Gustave Roussy, 94805 Villejuif - France and *Fred Hutchinson Cancer Research Center, Seattle, USA.

We have previously described a monoclonal antibody, referred to as 38.13, reacting with most Burkitt's lymphoma derived lines, either containing the Epstein-Barr virus (EBV) or not, but not reacting with EBV-positive lymphoblastoid cell lines. The target antigen, BLA, was shown to be a neutral glycolipid, identified as globotriaosylceramide (Gal α 1->4 Gal β 1->4 Glcβ 1->1 ceramide) (Science 220 : 509, 1983). This substance is known as the blood group antigen p^k, a normal intermediate in the P subtance synthesis. The enzyme activities involved in the synthesis and degradation of globotriaosylceramide, and the degree of exposure of this glycolipid have been studied with various Burkitt and non-Burkitt lymphomas and lymphoblastoid cell lines. The activity of UDP-Gal : LacCerα -galactosyltransferase of Burkitt lymphoma cells was consistently higher than that of non-Burkitt lymphoblastoid cell lines, and the enzyme activity can be correlated with the antigen expression at the cell surface. In contrast, the activity of α-galactosidase does not correlate with the expression of globotriaosylceramide. This mechanism is different from the two other situations in which globotriaosylceramide accumulation has been reported, namely p^k phenotype (blockage in β- NAcetyl-galactominyltransferase activity) and Fabry's disease (blockage in α -galactosidase activity).

0160 REGULATION OF EXPRESSION OF CSF RECEPTORS AND OTHER FUNCTIONAL ANTIGENS ON HUMAN NEUTROPHILS, Mathew A. Vadas, Nicos A. Nicola and Angel F. Lopez. The Walter and Eliza Hall Institute of Medical Research, P.O. Royal Melbourne Hospital, Victoria 3050, Australia.

G-CSF (a pure murine colony stimulating factor that preferentially stimulates the maturation of neutrophils from mouse and human progenitors) and monoclonal antibodies WEM-G1 and 11 (recognizing granulocyte specific antigens of Mr 110000 and 95000 respectively) stimulate human neutrophil function. G-CSF enhances antibody dependent cytotoxicity (ADCC) of tumor targets by neutrophils but not eosinophils, WEM-G1 enhances ADCC by neutrophils and eosinophils and WEM-G11 (F(ab')$_2$ stimulates both ADCC and phagocytosis of Ig coated targets. Radio-iodinated G-CSF shows specific binding to human neutrophils that is inhibited by cold G-CSF and human CSF-β but not by GM-CSF or CSF-α. Incubation of human neutrophils with f-met-leu-phe (FMLP) at 37°C but not at 4°C resulted in rapid time and dose dependent loss of G-CSF binding (by 80% at 20') and simultaneous enhancement (by 330%) of the binding of WEM-G1 and 11. The capacity of the MAb's to stimulate neutrophil function was also increased by FMLP. In contrast to WEM-G's the binding of a MAb against β_2-microglobulin was unaltered. The changes observed were due to modulation of membrane structures rather than changes in affinity. Cytochalasin B caused a further dose and FMLP dependent enhancement of binding of WEM-G11 but not of WEM-G1 and a prolongation of the effect of FMLP or G-CSF receptors. These studies show an independent modulation of human neutrophil surface structures involved in function.

Monoclonal Antibodies to Lymphokines

0161 MONOCLONAL ANTIBODIES AGAINST GROWTH FACTORS AND RECEPTORS, Anthony C. Allison and Alain B. Schreiber, Institute of Biological Sciences, Syntex Research, Palo Alto, California 94304.

Monoclonal antibodies have been raised against several growth factors, including endothelial cell growth factor (ECGF) and fibroblast growth factor (FGF). These stimulate proliferation of endothelial cells and probably act locally to promote neoangiogenesis: ECGF in tissues of neural origin, including the pituitary and retina, FGF in the corpus luteum and placenta. A possible role of ECGF and FGF in tumor neoangiogenesis remains to be defined.

Monoclonal antibodies against the receptor for epidermal growth factor (EGF) can be agonistic or antagonistic, showning that signal transduction capability resides in the receptor moiety (1). They were also used to isolate the EGF receptor, which allowed the demonstration of sequence homology with the predicted V-erb-B oncogene product (2). Monoclonal antibodies against other receptors, e.g., on endothelial cells and T-lymphocytes, are mitogenic, and may define receptors for growth factors that are not yet identified.

A growth factor that promises to be of general interest is interleukin-1. It stimulates the proliferation not only of B- and T-lymphocytes but also fibroblasts and endothelial cells. It induces the production of proteinases by chondrocytes and demineralization of bone, and may contribute to neovascularization, tumor-related sclerosis and osteolysis.

1. Strosberg, A.D. and Schreiber, A.B. In Receptors and Recognition, Series B. (ed. Greaves, M.F.) London: Chapman and Hall 17:15, 1984.

2. Ullrich, A. et al. Nature, Lond. 309:418, 1984.

Monoclonal Antibody—Radionuclide Conjugates

0162 RADIOACTIVE ANTIBODIES IN CANCER DETECTION AND THERAPY: EXPERIMENTAL AND CLINICAL FINDINGS. David M. Goldenberg, Center for Molecular Medicine and Immunology, University of Medicine and Dentistry of New Jersey, Newark, NJ 07103.

A number of different anticancer antibodies, both conventional and monoclonal, have been shown to selectively localize in animal xenografts and human clinical specimens, and those labeled with a gamma-emitting radionuclide have been used to image cancer by the method of cancer "radioimmunodetection". Although the standard radionuclide used to date has been I-131, efforts to use In-111 and Tc-99m are in progress, but as yet have undesirable RES accretion. Antibody fragments appear to have certain advantages over whole antibody preparations because of more rapid clearance from nontarget tissues. Nevertheless, under current dose schedules, it has been necessary for us to continue to utilize computer-assisted dual isotope subtraction, especially when evaluating chest and liver involvement.

Studies at our Center with conventional goat and murine monoclonal antibodies against carcinoembryonic antigen (CEA), alpha-fetoprotein (AFP), human chorionic gonadotropin (HCG), prostatic acid phosphatase (PAP), colon-specific antigen-P (CSAp), and Reisfeld's high molecular weight melanoma antigen (9.2.27) have indicated good tumor localization with I-131 labeled preparations, approaching a sensitivity of about 90% in CEA radioimmunodetection. In a consecutive series of 51 colorectal cancer patients, 11 cases of occult cancer later confirmed by other methods were found; a lead time of up to 40 weeks was achieved. Using CEA, AFP, and CSAp antibodies in a small series of patients with liver neoplasms (primary and metastatic), radioimmunodetection revealed all of the tumors while CAT scans and NMR imaging disclosed 37.5% and 50%, respectively. Image resolution of radioimmunodetection ranges between 1.5 and 2.0 cm tumors. In the case of pancreatic carcinomas, use of single-photon emission tomography with a higher dose of radioactive antibodies to CEA appears to improve imaging results.

Based on animal results with I-131 anti-CEA IgG used to treat GW-39 human colonic carcinoma xenografts, showing a retardation of tumor growth and an increase in survival time, a Phase I/II clinical trial of radioimmunotherapy has begun. A dose of 25-75 mCi I-131 will be applied weekly for a 4-week course in patients with refractive and measurable CEA or AFP tumors. Current results indicate a blood T 1/2 of 24-36 hr. Evidence of immune complexes circulating in the blood after injection of radiolabeled antibody has been obtained.
Supported in part by NIH grants CA-37407, CA 37408, CA 37409, and CA 37412.

0163 **BIOCHEMICAL EFFECTS AND CANCER THERAPEUTIC POTENTIAL OF TUNICAMYCIN,** **Kenneth Olden[1,2] and Sandra L. White[1], [1]Howard University Medical School, Washington, D.C. 20060 and [2]National Institutes of Health, Bethesda, MD. 20205**

Tumor metastases apparently involves a number of distinct steps, many of which are thought to be mediated by surface glycoconjugants, and some of the changes in cell surface glycoconjugants that occur in association with oncogenic transformation may be related to the ability of tumor cells to metastasize. Therefore, by modifying the structure or preventing the biosynthesis of specific glycan components, it should be possible to interfere with growth and metastases of tumor cells. We have examined the effect of tunicamycin (TM), an inhibitor of the synthesis of the glycan moiety of asparagine-linked glycoproteins, on the viability (i) of transformed fibroblasts in culture, and of (ii) histocompatable mice receiving inoculations of L1210 cells. We find that TM (5×10^{-8} M) is cytotoxic toward a variety of transformed cell lines, including virally or chemically transformed fibroblasts from chicken embryo, rat kidney, human lung and mouse. The corresponding non-transformed cell lines were resistant to the same and even a 10 fold higher concentration of TM. The relationship between transformation and TM cytotoxicity was strengthened by the finding that chicken embryo fibroblasts, infected with temperature-sensitive viral mutants,are killed by the drug only at the temperature permissive for transformation. There is a direct correlation between the susceptibility of transformed cells to TM cytotoxicity and sensitivity to inhibition of protein glycosylation, sugar and amino acid transport, and glucose metabolism. For example, L-929 mouse fibroblasts are not killed by TM (1.5-2.0 ug/ml), and synthesis of glycoprotein is not inhibited.Membrane fractions prepared from normal and TM-sensitive transformed cells showed similar activities with respect to formation of lipid-linked N-acetylglucosamine per mg of protein, indicating that this is not the molecular basis for the hypersensitivity of transformed cells. We suggest that the selective cytotoxicity of TM for transformed cells is related to decrease glucose metabolism due to impairment in uptake of nutrients. TM treatment altered the cell surface morphology with increased numbers of microvilli and blebs, and reduced adhesion to collagen and fibronectin matrices. Also, the transformed fibroblasts, pretreated with TM <u>in vitro</u>,were less tumorigenic when implanted subcutaneously. Similarly, mice receiving inoculations of L1210 leukemic cells pretreated with 20uM TM <u>in vitro</u> survived almost twice (18-20 days) as long as those receiving implants of untreated cells. Additionally, mice receiving an initial inoculum of TM pretreated cells survived subsequent challenges with untreated L1210 ascites cells. Finally, preliminary studies suggest that TM-pretreatment of highly metastatic tumor cell lines <u>in vitro</u> markedly decrease their metastatic potential. Our results suggest that TM may be therapeutically useful as an anti-tumor agent to (i) selectively kill certain types of malignant cells, or (ii) to reduce tumorigenicity possibly by enhancing the immunogenicity of the tumor cells <u>in vivo</u>. **NIH grant GM-29804**

Cancer Vaccines

0164 A STRATEGY FOR THE DEVELOPMENT OF HUMAN MONOCLONAL ANTIBODIES TO SURFACE ANTIGENS OF CELLS OF SOLID TUMORS, M. G. Hanna, Jr., M. V. Haspel, R. P. McCabe, N. Pomato, and H. C. Hoover, Jr., Litton Institute of Applied Biotechnology, Rockville, MD 20850

Evaluation of the in vivo usefulness of human monoclonal antibodies in human cancer has been prevented by a paucity of monoclonals to relevant cell surface antigens. We have recently reported that vaccination of colorectal cancer patients with autologous tumor cells admixed with BCG significantly increased their delayed cutaneous hypersensitivity response to autologous tumor cells. We therefore reasoned that these tumor-immunized patients represented an ideal source of B cells sensitized to colorectal tumor-associated antigens. We now report the isolation of more than 30 monoclonal antibodies reactive with sections of colorectal carcinoma. Approximately one-half of these human monoclonal antibodies are directed against cell surface antigens. We also report the unexpected finding that the majority of the human monoclonal antibody-producing cell lines do not appear to be human-mouse heterohybridomas, but rather diploid human B cells. Direct antibody binding assays have been performed with the monoclonals to further investigate their tissue specificity. We have found that these antibodies recognize distinct antigenic determinants that are preferentially expressed on tumor cells compared to normal colon cells. Experiments with paired specimens of air-dried colon tumor cells and normal colonic mucosal cells showed cell surface binding with the tumor cells and significantly less reactivity with the normal colonic mucosa cells. Tests with frozen tissue panels of paired colon tumor and normal colonic mucosa showed cell surface antibody binding to the tumor cells and not with the normal colonic mucosa. None of the monoclonals reacted with frozen sections of normal breast, stomach, kidney, liver, skeletal muscle and skin. Furthermore, these human monoclonal antibodies did not react with CEA, human erythrocyte antigens and human lymphocyte antigens by various techniques. Our data further demonstrate that these transformed B cells and hybridomas are stable producers of human monoclonal antibodies. The antibody-producing transformed B cells have been shown to grow in continuous culture with increasing levels of monoclonal antibody production for a period of approximately 9 months. In addition, in vivo tumor localization experiments demonstrated preferential localization of the monoclonal antibodies within tumor tissue, indicating that the affinity of these human monoclonal antibodies may be sufficient for in vivo application.

0165 MONOCLONAL ANTIBODIES IN THE DEVELOPMENT OF ACTIVE SPECIFIC IMMUNOTHERAPY IN MELANOMA, June Kan-Mitchell, Department of Microbiology, University of Southern California School of Medicine, Los Angeles, CA 90033.

We have attempted to design a trial of active specific immunotherapy in which a more precise dose of tumor-associated antigens could be administered. Soluble extracts have been prepared from human melanoma cells from freshly obtained surgical specimens. The extraction was performed with 2.5% 1-butanol, a method originally devised to selectively remove tumor-associated antigens but not histocompatibility antigens from methylcholanthrene-induced mouse sarcomas (1). These butanol extracts have a high degree of immunogenicity for syngeneic mice, specifically protecting the mice against the induction of the same tumor, and prolonging the survival of mice after resection of large primary tumors. An estimate of the antigenic contents of our human melanoma extracts was made by a binding inhibition assay involving enzyme-linked immunosorbance, with the mouse monoclonal antibody 9.2.27 (2), which detects a chondroitin sulfate proteoglycan present in most human melanoma cells. To identify melanoma-associated antigens immunogenic to man, and therefore important to be included in tumor vaccines, human hybridomas were produced in our laboratory by the fusion of regional lymph node cells from melanoma patients with the mouse myeloma cell line M5(3). Six human monoclonal antibodies, 4 IgG and 2 IgM, had reactivities against melanoma cells in tissue culture and fixed tissue sections. Two of the IgG antibodies studied in greater detail bound to 23 of 23 melanomas and 2 ocular melanomas. There was no reactivity to normal melanocytes in 17 nevi: 2 blue, 3 compound, 2 congenital, 1 junctional, 7 epithelioid (Spitz) and 2 halo nevi. The only reactivity noted was against dysplastic nevi and lentigo maligna, which are, respectively premalignant and low malignancy forms of melanoma (4). Of most interest was that all of the human monoclonal antibodies identified cytoplasmic rather than surface antigens. These cytoplasmic antigens were distinct from carcinoembryonic antigen. A pool of butanol extracts from allogeneic melanoma was given subcutaneously without toxicity to 6 patients with Stage IV melanoma, either alone or preceded by low dose cyclophosphamide. Neither toxicity nor evidence of significant immunological effectiveness has thus far been observed in this Phase I trial. This effort is intended to be a prototype for the rational modification of dose, schedule and physical state of the immunogenic antigens, as well as the addition of adjuvants or forms of pretreatment in active immunotherapy. Monoclonal antibodies will play a significant role in the clinical trial, including future attempts to monitor the effectiveness of the therapy.

1. LeGrue, S.J., Kahan, B.D. and Pellis, N.R. J. Natl. Cancer Inst. 65:191, 1980.
2. Reisfeld, R.A., Morgan, A.C., Jr. and Bumol, T.F. In: Hybridomas in Cancer Diagnosis and Treatment, eds. M.S. Mitchell and H.F. Oettgen, N.Y., Raven, 1982.
3. Schlom J., Wunderlich, D. and Teramoto, V.A. Proc. Natl. Acad. Sci. USA 77:6841, 1980.
4. Imam, A., Kan-Mitchell, J., Kempf, R.A., Taylor, C.R. and Mitchell, M.S., In: Immunity to Cancer, eds. A.E. Reif and M.S. Mitchell, N.Y., Academic 1985, in press.

0166 APPROACHES TO AUGMENTING THE IMMUNOGENICITY OF TUMOR ANTIGENS, Philip O.
Livingston, Sloan-Kettering Inst., 1275 York Ave., New York, NY 10021

The treatment or prevention of cancer with specific vaccines has been envisaged ever since the first vaccines against infectious diseases were developed. Although several thousand patients have been injected with tumor cell preparations in this country and elsewhere over the past 50-60 years (1), the complexity of the studies has made an assessment of the value of this approach to cancer therapy impossible. Even under the most favorable clinical circumstances, and with a most carefully designed trial, the testing of human cancer cell vaccines is fraught with difficulties arising from the uncertainty of whether the vaccines contained in fact tumor-specific antigens, and whether the patients responded immunologically to these antigens. What is required for the development of an immunogenic cancer vaccine are methods to assess effectiveness that are both rapid and objective and that can be used to guide the process of vaccine testing step by step. With regard to vaccines against infectious diseases, serological responses to bacterial or viral antigens have served this purpose. With the development of serological typing systems for defining cell surface antigens of melanoma and other cancers, we now have serological tests of requisite sensitivity and specificity for this purpose. This is not to say that we think cell-mediated immunity is less important in the host's defense against cancer, on the contrary, just that at present serological techniques are more precise and better suited to screening approaches to vaccine construction. An additional benefit to the serological approach to monitoring vaccine trials is that it is likely to indicate vaccines yielding the most activated B cells for hybridoma and monoclonal antibody production.

We (2) and others (3) have vaccinated small groups of melanoma patients with a series of melanoma cell vaccines with the intent of constructing a vaccine that could consistently raise antibodies against these antigens, a vaccine that could then be used in larger clinical trials. However, none of the vaccines tested have consistently raised antibodies against melanoma antigens. For this reason we have conducted a series of trials in the mouse with whole cell and purified antigen vaccines (4). Approaches greatly augmenting the immunogenicity of these antigens (in terms of production of both antibody and activated B cells) have been identified. The serological responses and monoclonal antibodies produced and the clinical trials planned as a consequence of these studies will be discussed.

1. Livingston PO, Oettgen HF, Old LJ. IN: Immunological aspects of cancer therapeutics, E Mihich (ed), John Wiley & Sons, Inc., 363-404, 1982.
2. Livingston PO, Takeyama H et al. Int J Cancer 31:567-575, 1983.
3. Irie RF, Giuliano AE, Morton DL. JNCI 63:367, 1979.
4. Livingston PO, DeLeo AB et al. J Immunol 131:2601, 1983.

0167 USEFULNESS OF MONOCLONAL ANTIBODIES IN ACTIVE SPECIFIC IMMUNOTHERAPY, Malcolm
S. Mitchell, University of Southern California Cancer Center, Los Angeles, CA 90033.
Monoclonal antibodies to tumor-associated antigens potentially have several important roles in the development of regimens for active specific immunotherapy. In the past, most clinical trials of tumor "vaccines" have not attempted to determine the content of immunogenic materials in the preparations, but this practice is becoming rarer. It is now possible with an increasing number of human tumors to determine which specific epitopes are present on the tumor cells, the lysates, or the extracts used for the immunization. Murine monoclonal antibodies to melanoma, colon carcinoma, pancreatic carcinoma, and ovarian carcinoma, to name just a few, are now available for this purpose. The inhibition of binding of monoclonal antibodies to tumor targets expressing a particular epitope, immunoprecipitation by monoclonal antibodies and subsequent radioautography, or identification of antigens bound to monoclonal antibodies on an immunosorbent column are various means by which semiquantitative estimates can be made of the content of specific antigens. Human monoclonal antibodies, now in their early stages of development, can potentially permit the identification of those antigens that are immunogenic in man, which therefore should be included in vaccines. It is interesting in this regard that many of the human monoclonal antibodies identify cytoplasmic antigens rather than surface antigens. Cytoplasmic antigens have not heretofore been considered appropriate immunogens in vaccine preparations, but their recognition by tumor-bearing patients suggests that they might be. The titration of the humoral immune response to the antigens contained in the vaccine may be facilitated by monoclonal antibodies. This last application may be limited by the low concentration of serum antibodies to a given epitope, but panels of antibodies to detect whole antigens may overcome this difficulty. Finally, human monoclonal antibodies may well be generated most efficiently by fusing lymphocytes derived from patients who have been treated with vaccines, which could greatly improve their development. These intersections make it highly appropriate to consider active specific immunotherapy in the context of this conference.

0168 EXPERIENCE WITH TUMOR CELL VACCINE IN HUMAN MELANOMA, Donald L. Morton, Division of Surgical Oncology, John Wayne Cancer Clinic, Jonsson Comprehensive Cancer Center, UCLA School of Medicine, Los Angeles, CA 90024.

We began a randomized prospective study of adjuvant immunotherapy with tumor cell vaccine (TCV) in 1973. Fourty-nine Stage II melanoma patients received intradermal immunizations of TCV prepared from cultured allogeneic melanoma cells every 1-2 weeks after lymphadenectomy. Their clinical course and humoral immune responses were monitored for a mean follow up of 5 years. The patients who received TCV had a longer disease-free interval (53%) compared to those who had no postoperaive treatment (41%), although the differences were not statistically significant. Serum levels of anti-tumor associated antibodies were evaluated for those patients who were free of disease for more than 120 days after lymphadenectomy. A significant correlation between IgM antibody responses and prolonged survival was observed. Of 35 patients, 6 achieved marked elevations in IgM antibody titers and 5/6 (83%) were free of disease at 5 years. In 1979, patients with disseminated melanoma were entered into a pilot study of intralymphatic infusion (ILI) of TCV in an effort to augment antibody responses. Of 44 evaluable patients, 22 developed elevated antibody titers and 12 (55%) had no disease progression for at least 3 months after the treatment. In contrast, 20/22 (91%) patients who failed to develop antibody titers had disease progression. Subsequently, in 1981 we began a randomized prospective trial of ILI-TCV for Stage II melanoma patients. Of 44 evaluable patients, 23 developed increased antibody titers; however, most titer elevations were transient and dropped markedly within a month. Patients with these transient titers had no clinical advantage. Based on our hypothesis that the sudden decrease in antibody titer might have been due to suppressor cells that inhibited antibody production and that the use of a highly immunogenic TCV might induce greater immune responses, we improved our current TCV immunotherapy protocol as follow: 1) low-dose cytoxan was given before TCV immunization to minimize the generation of suppressor cells, 2) highly antigenic TCV would be developed to maximize specific immune responses, and 3) vaccine would be administered intradermally rather than by ILI. Three melanoma cell lines containing the highest concentrations of three well-defined immunogenic antigens, gangliosides GD2, GM2 and a melanoma-associated lipoprotein (MW 180,000-190,000) were selected from 30 human melanoma cell lines. The effect of the new TCV immunotherapy is presently being evaluated.

Monoclonal Antibodies to Oncogene Products

0169 USE OF MONOCLONAL ANTIBODIES TO THE SRC GENE PRODUCT TO PROBE THE EXPRESSION OF THE CELLULAR SRC PROTEIN IN POLYOMA VIRUS TRANSFORMED CELLS. Joan S. Brugge, Leah A. Lipsich, J. Bolen*, M. Israel*, Wes Yonemoto. Dept of Microbiology, State University of New York, Stony Brook, NY 11794; *Pediatric Branch, National Cancer Institute, Bethesda, MD. 20205

We have prepared monoclonal antibodies to the Rous sarcoma virus transforming protein, $pp60^{v-src}$. These reagents offer many advantages over other immunological probes used for the detection of $pp60^{v-src}$: 1) Many of the antibodies recognize the cellular homolog of the src protein from all avian and mammalian species; 2) several of the antibodies recognize other tyrosine kinase transforming proteins including the fps, ros, and yes transforming proteins; and 3) all of these antibodies allow the expression of the tyrosine kinase activity of $pp60^{src}$ within the immune-complex. For these reasons, these antibodies are ideal reagents to probe the expression of the cellular src gene product and other related tyrosine kinases in naturally occuring tumors and in cells transformed by other tumor viruses.

We have used the anti-src protein monoclonal antibodies to examine the interaction between the middle tumor antigen (MTAg) transforming protein encoded by polyoma virus and the cellular src protein. The MTAg transforming protein has been shown to be physically associated with the cellular src protein in polyoma virus transformed cells. It has been speculated that polyoma virus transformation may be mediated by this interaction with the cellular src protein. We have found that the tyrosine kinase activity of the src protein molecules which are bound to MTAg is enhanced 10-50 fold in lysates from polyoma virus transformed or infected cells. The $pp60^{c-src}$-associated tyrosine kinase activity is not elevated in cells infected with transformation defective mutants of polyoma virus. These results suggest a novel mechanism for the activation of a cellular oncogene; namely, that the interaction between MTAg and $pp60^{c-src}$ activates the tyrosine kinase activity of the cellular src protein. We have recently found that the cellular src protein molecules which are bound to MTAg can be distinguished from src protein molecules which are not associated with MTAg by the presence of a unique site of tyrosine phosphorylation within the amino terminal domain of the molecule. We are presently investigating whether this modification is involved in the activation of tyrosine kinase activity of the src protein in polyoma virus transformed cells. We are also investigating whether the tyrosine kinase activity of $pp60^{c-src}$ or related tyrosine kinases are activated in any naturally occurring tumors.

0170 IN VIVO AND IN VITRO EFFECTS OF MONOCLONAL ANTIBODIES WHICH RECOGNIZE A CELL SURFACE ONCOGENE PRODUCT. Jeffrey A. Drebin, Victoria C. Link, and Mark I. Greene. Department of Pathology, Harvard Medical School, Boston, MA 02115.

Activated cellular oncogenes, capable of transforming NIH3T3 cells in DNA transfection assays, have been identified in a variety of human and animal cancer cells and cell lines. Some of these activated oncogenes are cellular homologs of activated oncogenes previously identified in acutely transforming retroviruses, while others appear to be only distantly related or unrelated to retroviral oncogenes. Members of one such family of activated cellular oncogenes have been identified in a number of independently derived, ethylnitrosourea induced rat neuroblastomas. These oncogenes, termed neu oncogenes, are related to, but distinct from, the retroviral erbB oncogene and its cellular homolog, the Epidermal Growth Factor receptor (EGFr) gene. Cells transformed by neu oncogenes express a 185 Kd cell surface phosphprotein (p185) which is antigenically related to, but distinct from, the EGFr. It is thought that p185 is the neu oncogene product. We have raised monoclonal antibodies that bind to cell surface determinants of the p185 molecule. Addition of purified anti-p185 monoclonal antibody 7.16.4 to cultures of neu transformed NIH3T3 cells results in the rapid and reversible down modulation of cell surface p185 expression. Although not directly cytotoxic, incubation of neu transformed cells with antibody 7.16.4 also results in the reversion of the transformed phenotype of the neu transformed cells; antibody 7.16.4 dramatically inhibits the ability of neu transformed NIH3T3 cells to form anchorage independent colonies in soft agar and to grow in low serum. It has no effect on the transformed phenotype of NIH3T3 cells transformed by src or ras oncogenes. Monovalent F(ab) fragments of antibody 7.16.4 have no effect on soft agar colony formation by neu transformed cells, suggesting that cross-linking of cell surface p185 by intact antibody is required to revert the transformed phenotype. These studies indicate that specific interference with an oncogene encoded protein can cause reversion of at least some of the neoplastic characteristics of tumor cells containing the relevant oncogene. Antibody 7.16.4, which is an IgG2a, also inhibits tumor formation in nude mice by neu oncogene transformed cells. This effect may be mediated by an antibody dependent cellular cytotoxicity mechanism, as described for other IgG2a antibodies reactive with cell surface determinants. However, the fact that neu transformed cells require continued expression of p185 in order to maintain the neoplastic phenotype *in vitro* suggest that tumor cells may not be able to escape from monoclonal antibody attack by selection for p185 negative variants *in vivo*. Thus monoclonal antibody therapy targeted at determinants whose expression is linked with the mechanisms responsible for transformation may prove extremely useful in the treatment of certain neoplasia.

0171 PROTEIN PRODUCTS OF THE MYC ONCOGENE DEFINED BY ANTISERA PREPARED AGAINST SYNTHETIC PEPTIDES, Robert N. Eisenman, Stephen R. Hann, and Craig Thompson, Division of Basic Sciences, Fred Hutchinson Cancer Research Center, 1124 Columbia St., Seattle WA 98104

In order to identify and characterize the proteins encoded by the myc oncogene in normal and neoplastic cells we have prepared antisera against a series of synthetic peptides corresponding to various regions of the putative myc coding sequence previously derived from nucleotide sequencing data. Although all the peptides gave rise to antibodies reactive with myc proteins the most effective were those corresponding to the twelve C-terminal amino acids of myc. This region differs by only a single amino acid between c-myc from avian, murine and humans. However this single difference in the penultimate amino acid is sufficient to render antisera against the three peptides essentially non-cross reactive.

These anti-myc-peptide sera (anti-myc 12C) have been affinity purified and used to identify myc encoded proteins. Criteria for identification include ability to precipitate (i) proteins synthesized in vitro using mRNAs hybrid-selected with myc DNA; (ii) proteins synthesized in heterologous cells following transfection with myc DNA and ; (iii) previously characterized proteins encoded by avian acute leukemia viruses possessing the myc gene (1,2). The myc-encoded proteins were found to be phosphorylated nuclear proteins with half-lives on the order of 20-30 minutes. While myc proteins appeared to have affinity for DNA most of the myc protein in the nucleus appears to be associated with the nuclear matrix in a manner independent of bulk cellular nucleic acid. Mitotic cells show a distinctly cytoplasmic distribution of myc protein (3). Furthermore we have demonstrated that synthesis of myc proteins in proliferating normal and malignant human and avian cells occurs at an apparently constant rate throughout the cell cycle.

(1) Hann, S.R., Abrams, H.D., Rohrschneider, L.R. and Eisenman, R.N., Cell 35: 789-798. 1983.
(2) Hann, S.R. and Eisenman, R.N. Mol. Cell Biol. 4 in press.
(3) Eisenman, R.N., Tachibana, C.Y., Abrams, H.D., and Hann, S.R. Mol. Cell Biol. in press.

0172 ONCOGENESIS BY RECEPTOR GENE TRUNCATION, A. Ullrich, L. Coussens, J.S. Hayflick, T.J. Dull, A. Gray, A.W. Tam, J. Lee, H. Riedel, Y. Yarden*, T.A. Libermann*, J. Schlessinger*, J. Downward+, E.L.V. Mayes+, N. Whittle+, M.D. Waterfield+, and P.H. Seeburg, Department of Molecular Biology, Genentech, Inc., South San Francisco, California 94080, *Department of Chemical Immunology, The Weizmann Institute of Science, Rehovot, Israel, +Protein Chemistry Laboratory, Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2A 3PX, UK.

Isolation of monoclonal antibodies which recognize the human epidermal growth factor (EGF) receptor has made it possible to use immunoaffinity chromatography for receptor purification. Partial protein sequence analysis and the subsequent isolation and characterization of cDNA clones reveal close similarity between the entire predicted amino acid sequence of the avian erythroblastosis virus (AEV) transforming gene v-erb-B and the EGF receptor transmembrane and cytoplasmic domains. The single 23 amino acid transmembrane region separates a cysteine-rich 621 residue extracellular EGF binding domain from the 542 amino acid long, cytoplasmic, tyrosine-specific protein kinase domain. The receptor gene is amplified and rearranged in A431 epidermoid carcinoma cells and primary brain tumors (glioblastoma) resulting in receptor overproduction and generation of truncated gene products. Two approaches were taken to further investigate the involvement of EGF receptor gene products in oncogenesis. First, a variety of primary human tumors and leukemic cells were analyzed for possible aberrant expression of the receptor gene. Second, in vitro modification of the isolated receptor cDNA sequences and subsequent introduction and expression in tissue culture cells was carried out to identify molecular characteristics responsible for oncogene generation. Insights obtained from these experiments will be presented.

Special Lecture

0173 MONOCLONAL ANTIBODIES AND CANCER METASTASIS, , Isaiah J. Fidler, Department of Cell Biology, University of Texas System Cancer Center, M.D. Anderson Hospital and Tumor Institute, Houston, TX 77030

The major challenge facing the oncologist in treating cancer is how to eradicate metastases. Despite major advancements in surgical resection of neoplasms, and in the development of adjuvant therapies, most deaths from malignant neoplasms are caused by metastases which are resistant to conventional therapeutics. This resistance is due to a large extent to the constant evolution of cancer cells, resulting in the generation of neoplasms which are biologically heterogeneous. Heterogeneity is not restricted to primary neoplasms only. Multiple metastases often exhibit inter- and intralesional diversity which is expressed in characteristics such as metastatic potential, antigenicity, hormone receptors, and sensitivity to chemotherapeutic agents. The source of this heterogeneity can be due to both the nature of the process of cancer spread, and to the process of tumor progression. Studies with murine tumors of recent origin demonstrate that metastases are not produced by the random survival of malignant cells. Rather, metastases are produced by the survival and growth of minor but specialized subpopulations of cells. Moreover, many metastases can be clonal and originate from different progenitor cells. This may explain, in part, why antigenic heterogeneity can exist among different metastases.

We have recently demonstrated that NK cell-deficient nude mice can be used to ascertain the metastatic potential of human neoplasms. The successful metastasis of human tumors in young nude mice offers a unique opportunity to use these animals as a model for the selection and isolation of metastatic tumor cells from heterogeneous human neoplasms. Evidence supporting the validity of this in vivo model is provided by data of successful selection of metastatic cells from human melanomas, prostatic and colon carcinomas.

Testing of therapeutic agents against human tumor cells proliferating in a relevant organ site (lung, liver or lymph nodes) can be accomplished by using NK-cell deficient nude mice. For such studies, the mice are inoculated with preselected metastatic cells and therapeutic agents are administered after metastases are established. Five to six weeks after treatment, the mice are killed and the metastatic incidence (number of foci, size, location) is compared between treated and control groups.

In summary, malignant neoplasms of human origin can produce metastases in organs of NK-depleted nude mice. This model can therefore be useful for the isolation of metastatic cells from heterogeneous human tumors and affords the opportunity to investigate the response of human tumor cells growing as visceral metastases, rather than as subcutaneous solid tumors, to therapeutic agents.

Monoclonal Antibodies to Cell Surface Receptors

0174 IDIOTYPE-SPECIFIC REGULATION OF IMMUNOGLOBULIN EXPRESSION IN MURINE PLASMACYTOMA CELLS, Richard G. Lynch, Department of Pathology, University of Iowa College of Medicine, Iowa City, IA 52242.

Idiotype-specific immunoregulatory signals directed to the idiotopes of surface membrane immunoglobulin on murine plasmacytoma cells influence the proliferation, differentiation and immunoglobulin expression by the neoplastic cells. Monoclonal and polyclonal anti-idiotypic antibodies specific for combinatorial determinants (V_L + V_H) of M315 (IgAλ_2, anti-TNP) effect a modulation of cell surface M315 from MOPC-315 cells in vitro without influencing the viability or ultrastructure of these cells or their secretion of M315. Idiotype-specific suppressor T cells effect a rapid, highly selective inhibition of M315 synthesis and secretion. Inhibition is rapid in onset, reversible and effected by a diffusible product. The idiotype-specific suppressor T cells are Ly_t 1^-2^+ and express Id^{315}-specific recognition structures on their surface membranes. The inhibition of M315 synthesis results from a down-regulation of λ_2-mRNA expression. An aberrently rearranged, but transcribed λ_1 gene on the homologous chromosome is coordinately regulated with λ_2. These studies demonstrate that neoplastic B cells can be useful tools in the analysis of mechanisms that regulate immunoglobulin expression. (Supported by CA32275 and CA28848).