HUMAN TUMOR ANTIGENS AND SPECIFIC TUMOR THERAPY

Organizers: Richard Metzgar and Malcolm Mitchell

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Molecular Characterization of TAA (Carbohydrate Epitopes)

T 001 STRUCTURAL AND FUNCTIONAL PROPERTIES OF A CELL ADHESION RECEPTOR COMPLEX EXPRESSED BY HUMAN MELANOMA CELLS, David A. Cheresh, Department of Immunology, Scripps Clinic and Research Foundation, 10666 No. Torrey Pines Road, La Jolla, CA 92037.

The disialogangliosides GD2 and GD3 play a major role in the ability of human melanoma cells to attach to Arg-Gly-Asp-containing substrates such as fibronectin and vitronectin, since pretreatment of these cells with monoclonal antibodies to the oligosaccharide of GD2 and GD3 can inhibit their attachment and spreading on such adhesive proteins. This report demonstrates that human melanoma cells (M21) synthesize and express a glycoprotein receptor that shores antigenic epitopes with the vitronectin receptor on human fibroblasts and is capable of specifically recognizing the Gly-Arg-Gly-Asp-Ser-Pro sequence. In the presence of calcium, GD2, the major ganglioside of M21 cells, colocalized with this receptor on the surface of human melanoma cells and their focal adhesion plaques.

To establish the structural and functional properties of this receptor on M21 human melanoma cells, stable variant cell lines were selected that express altered α chain levels. One of these variants, M21-L, fails to synthesize α chain protein or its mRNA, yet does produce normal levels of the β chain. In these cells the β chain does not reach the cell surface but rather accumulates within the cell. M21-L cells lacking the α chain are incapable of attaching to vitronectin, von Willebrand factor, fibrinogen, or an Arg-Gly-Asp-containing heptpeptide yet attach normally to fibronectin, whereas the unselected M21 cells attach to all of these adhesive proteins. In addition, a monoclonal antibody, LM609 generated to a functional site on the intact receptor, is capable of preventing M21 cell attachment to vitronectin, von Willebrand factor, fibrinogen, and the Arg-Gly-Asp peptide but not to fibronectin. Following a 2-min biosynthetic pulse-label, the newly synthesized α chain remains in free form for 5 min and then associates with previously synthesized β chain present in an intracellular pool. Once oligomerization takes place, the receptor gains the capacity to recognize Arg-Gly-Asp, and at this time the epitope recognized by monoclonal antibody LM609 is formed.

T 002 STRUCTURAL REQUIREMENTS FOR TUMOR-ASSOCIATED ANTIGENS AND IMMUNOGENS, Sen-itiroh Hakomori, The Biomembrane Institute and the University of Washington, 201 Elliott Avenue West, Seattle, WA 98119.

Essentially all human cancers are characterized by aberrant glycosylation expressed at the cell surface, many of which are defined by specific monoclonal antibodies. Four distinct classes of tumor antigens can be chemically defined. 1) The first type are structures that are extremely limited in normal cells or tissues but are expressed in large quantity in some tumors, such as incompatible histo-blood group antigens and Tn and sialyl Tn antigens. Their occurrence in common human cancer is limited. 2) A second type of tumor-associated antigen is modified type 1 or type 2 chain structures. Since expression of type 2 chain is highly limited in normal gastrointestinal tissues, modified type 2 chain structures, such as di- or trifucosyl Le^X, difucosyl Le^Y, and sialyl dimeric Le^X, are important tumor-associated antigens in common human adenocarcinomas. These antigens are expressed in other normal tissues. 3) A third group of antigens is comprised of polypeptide epitopes influenced greatly by O-glycosylation. A pentapeptide sequence, Val-Thr-His-Prol-Gly-Tyr, at the IIIcs region of fibronectin polypeptide becomes a tumor-associated antigen when Thr is O-glycosylated with α-GalNAc, i.e., a single O-glycosylation defines a unique antigenicity of a normally existing peptide sequence. This may be an important example of various other tumor-associated antigens associated with mucin-type glycoproteins. 4) A fourth group of tumor-associated antigen is formed by high density organization of a common structure, such as GM_3. In this case, only high density GM_3 is recognized by specific monoclonal antibody. Low density GM_3 can not be considered a tumor-associated antigen. A small proportion of GM_3 in melanoma has been found to be present as a lactone, which may be an important immunogen. This work has been supported by an Outstanding Investigator Grant from the National Cancer Institute, CA 42505, and by funds from The Biomembrane Institute.

T 003 GLYCOLIPIDS DETECTED BY HUMAN MONOCLONAL ANTIBODIES DERIVED FROM CANCER PATIENTS. Kenneth O. Lloyd, Memorial Sloan-Kettering Cancer Center, New York, NY 10021

Human monoclonal antibodies (hmAbs) can be produced by a number of techniques including the formation of human-human or human-mouse hybridomas and by Epstein-Barr virus (EBV) transformation of B cells (1). None of these methods is ideally suited to this purpose and further work is needed to improve procedures. A route that is cumbersome, but relatively successful, is first to transform lymphocytes with EBV and subsequently to produce human-mouse hybridomas from antibody-producing transformants.

Recently we have applied these approaches to the isolation of human monoclonal antibodies from lymphocytes of patients with melanoma (2). In our initial study, four of the six stable clones isolated were found to secrete antibodies detecting gangliosides. Two of the antibodies (FCM1 and HJM1) reacted selectively with melanoma cells whereas the two others (2-39 and 32-27) did not react with human cells but were reactive with heterophile antigens. The specificity of these four hmAbs was analyzed in detail by studying their reactivity with a large panel of gangliosides (3). Ab FCM1 reacted preferentially with GM3 but showed substantial reactivity with GD1a and sialylparagloboside also; GD3, GM2 and GD1b were weakly reactive. Only gangliosides with N-acetyl-type neuraminic acid were detected. The epitope detected by Ab FCM1 is therefore NeuAcα2→3Gal-. Ab HJM1 preferentially recognized $(NeuAc)_2$GD3 but also reacted quite strongly with $(NeuAc)_2$disialylparagloboside, (NeuAc-NeuGc)GD3, and (NeuAc-NeuGc)disialylparagloboside; di-NeuGc-containing gangliosides were unreactive. The basic epitope recognized by this antibody is therefore NeuAcα2→8NeuAcα2→3Gal-, although it will also tolerate -8NeuGcα- as an internal residue.

In contrast to these antibodies, Abs 2-39 and 32-27 reacted preferentially with N-glycolyl -containing gangliosides, thus explaining their anti-heterophile specificity. Ab 2-39 reacted optimally with (NeuGc)GM3, but also reacted well with (NeuGc)sialylparagloboside; all NeuAc-containing derivatives and disialylgangliosides were unreactive. The immunodominant structure is therefore NeuGcα2→3Gal-. Ab 32-27 has a more complicated pattern of reactivity. It reacted optimally with $(NeuGc)_2$GD3, but also showed a considerable degree of cross-reactivity with $(NeuGc)_2$disialylparagloboside, (NeuAc-NeuGc)GD3, and (NeuAc-NeuGc)disialylparagloboside. The epitope maximally reactive with Ab 32-27 is therefore NeuGcα2→8NeuGcα2→3Gal-, although it can also tolerate NeuAcα2→8- as a terminal residue.

(1) K. James and G.H. Bell. J. Immunol. Methods 100: 5 (1987); H. Yamaguchi, K. Furukawa, S.R. Fortunato, P.O. Livingston, K. O. Lloyd, H.F. Oettgen, and L.J. Old. P.N.A.S. 84: 2416 (1987);(3) K. Furukawa, H. Yamaguchi, H-F. Oettgen, L.J. Old, and K. O. Lloyd - in preparation

Growth Factors and Oncogenes as TAA

T 004 MONOCLONAL ANTIBODIES FOR THE DETECTION OF ACTIVATED AND NORMAL RAS p21s IN NEOPLASTIC AND PRENEOPLASTIC CELLS. W.P. Carney, P. Hamer, T. Pullano, H. Rabin, K. Trimpe, H. Wolfe, R. DeLellis and G. Cooper, Oncogene Research Group, Medical Products Department, E.I. Du Pont, 331 Treble Cove Road, N. Billerica, MA 01862, Departments of Pathology, Tufts New England Medical Center and Dana Farber Cancer Institute, Boston, MA 02115.

The ras gene family is composed of the Ha-, Ki- and N-ras genes which encode immunologically related proteins of approximately 21,000 daltons (p21). The ras p21s are associated with the inner face of the plasma membrane, bind GTP and possess intrinsic GTPase activity. The ras p21s have been highly conserved in eucaryotic organisms and have been implicated in cell proliferation and cell differentiation. The biochemical similarity of ras p21 to G proteins has led to the proposal that p21s may be involved in signal transduction. Transfection experiments using NIH3T3 cells as recipients of human tumor cell DNA have led to the discovery of activated ras genes that encode p21s with amino acid substitutions at positions 12, 13 and 61. Activated p21s have been detected in a wide variety of human and animal cancers including solid tumors and leukemias. Results indicate that approximately 40% of human colorectal cancers and preneoplastic colonic lesions have mutations at position 12 of the ras p21. Since activated p21s have not been detected in normal tissues, they may be considered to be specific cancer markers and their detection may be of diagnostic value. In this report, we describe a series of MAbs raised against mutations at position 12 which specifically react with ras p21s activated by position 12 mutations. Western blot analysis of a variety of cell lines demonstrated that MAb E184 specifically reacts with activated p21s containing glutamic acid at position 12, MAb R256 specifically reacts with activated p21s with arginine at position 12 and MAb DWP specifically reacts with activated p21s with valine at position 12. These reagents may be valuable in detecting the presence and frequency of activated p21s in cancer cells. Additional studies have suggested that overproduction of normal p21 may also be implicated in neoplastic progression; therefore the detection and quantitation of normal p21 may have clinical significance as well. To help understand the role of normal p21 in tumor development, we raised a series of MAbs broadly reactive with the three cellular forms of the ras p21. These latter MAbs are specifically reactive with ras p21 by flow cytometry and immunohistochemistry as well as by Western blotting and ELISA and thus may provide valuable tools for qualitative and quantitative analyses of ras p21 expression in normal, preneoplastic and neoplastic cells.

T 005 **B CELL GROWTH FACTOR RECEPTORS IN B-LINEAGE LYMPHOID MALIGNANCIES: THERAPEUTIC IMPLICATIONS**, Fatih M. Uckun, Dorothea E. Myers, Anthony S. Fauci, Chang W. Song, Jeffrey A. Ledbetter, Daniel A. Vallera, Lawrence L. Houston, Mridula Chandan, Patrick McFarland, Laura Chesky, Steven E. Swaim, Howard Mospowski, and Julian L. Ambrus, Departments of Therapeutic Radiology-Radiation Oncology, Pediatrics, and Bone Marrow Transplantation Program, University of Minnesota Health Sciences Center, Minneapolis MN 55455; Oncogen Corporation, Seattle, WA, Cetus Corporation, Emeryville CA, Laboratory of Immunoregulation, National Institute of Allergy and Infectious Diseases, Bethesda MD 20892. B cell growth factors (BCGF) are growth regulatory lymphokines that stimulate the proliferation of normal human B-lymphocytes. The stage in human B cell ontogeny during which B lineage cells express functional BCGF receptors and the role of BCGF in B lineage lymphoid malignancies are as yet unknown. The purposes of this study were 1) to analyze the expression of BCGF receptors and to elucidate the biological effects of biochemically purified BCGF in B-lineage lymphoid malignancies including B-lineage acute lymphoblastic leukemias (ALL), B-lineage chronic lymphocytic leukemias (CLL), hairy cell leukemias (HCL), prolymphocytic leukemias (PLL), and B-lineage non-Hodgkin lymphomas (NHL), and 2) to elucidate the interactions of B43 (anti-CD19), G28-5 (anti-Bp50, CD40), G28-7 (anti-CD22), and BA-5 (anti-p90, directed against a 90Kd BCGF receptor on B-lineage cells) MoAb and their toxin conjugates with BCGF. The estimated number of radioiodinated BCGF molecules bound per each B-lineage leukemia/lymphoma cell showed a pronounced interpatient variation indicating a marked heterogeneity in expression of BCGF receptors in B-lineage lymphoid malignancies. Biochemically purified BCGF induced PMA inhibitable $[Ca^{2+}]i$ fluxes as well as cAMP increases and stimulated the S-phase entry and proliferative activity in B-lineage leukemia/lymphoma cells as determined by DNA flow cytometry, ^{3}H-TdR incorporation and clonogenic assays. Our findings provide direct evidence for the expression of functional BCGF receptors on B-lineage leukemia/lymphoma cells and prompts the hypothesis that BCGF may play an important growth regulatory role in B-lineage lymphoid malignancies. B43 and G28-5 MoAb augmented the stimulatory effects of BCGF whereas G28-7 and BA-5 MoAb elicited inhibitory effects. Immunotoxins prepared by linking B43, G28-5, G28-7, and BA-5 MoAb to the ribosome inhibitory phytotoxin pokeweed antiviral protein were highly potent inhibitors of proliferative BCGF responses of target B-lineage leukemia/lymphoma cells. The opportunity is thus provided for using immunotoxins for more effective treatment of high risk or therapy refractory BCGF receptor positive B-lineage lymphoid malignancies.

Molecular Characterization of TAA (Protein Epitopes)

T 006 BIOSYNTHESIS AND MOLECULAR ORGANIZATION OF EPITHELIAL SIALOMUCINS.
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Mucus glycoproteins (mucins) are abundantly present on many tumor types and are likely to have an important impact on the biology of the tumor. Moreover, these molecules are tools to study cell transformation, progression of cancer and they are tumor markers for differential diagnosis and cancer monitoring by assaying their presence in serum.
MAM-6 is an epithelial sialomucin which we have employed as a model to study the molecular organisation and biosynthesis of mucins. It is a polymorphic epithelial sialomucin with a molecular weight over 400 kDa. We have cloned part of the MAM-6 gene from a cDNA library of T47D breast cancer cells. The gene consists of unique sequences, and repeated sequences of 120 bp. The polymorphism is localized in a fragment of the gene that contains the repetitive sequences. By pulse-labeling and immunoprecipitation experiments we have shown that the biosynthesis of MAM-6 proceeds via two lower molecular weight precursors and one undersialylated high molecular weight precursor. The processing of the first lower molecular weight precursor involves a rare proteolytic cleavage step which occurs in the endoplasmic reticulum. The high molecular weight precursor is generated by extensive O-linked glycosylation. The final maturation step occurs by sialylation of the high molecular weight precursor. The early MAM-6 precursors contain N-linked glycans suggesting that N-linked glycans are also present on the mature mucin. Although the copresence of the N- and O-linked glycans has been found on many molecules, no N-linked glycans have been reported on mucins previously.
Most monoclonal antibodies (MAbs) raised to this mucin were directed to the protein backbone. Some of these MAbs, however, were not reactive with the mature form of the molecule from all cell lines but were always reactive with the early precursors of MAM-6. These experiments show that the mature molecule may be differentially glycosylated causing masking of the protein epitopes. Alternatively, the MAM-6 protein backbone may be processed differentially. Such modifications may be responsible for the tissue and tumor preference of some MAbs directed to the MAM-6 epithelial mucin.

T 007 **IDIOTYPES AS TUMOR-ASSOCIATED ANTIGENS**, Syamal Raychaudhuri, Yukihiko Saeki, Jian-Jun Chen and Heinz Kohler, IDEC Pharmaceuticals Corporation, La Jolla, CA 92037.
Tumors express tumor-associated antigens to which the tumor host can respond. However, in most tumor hosts the anti-tumor response is not effective and the tumor continues to grow. We have addressed this failure of anti-tumor response in an animal system, the L1210/GZL tumor in DBA/2 mice. Towards this aim, tumor-antigen specific monoclonal antibodies were generated and used to produce monoclonal anti-idiotypic antibodies. Preimmunization with one such anti-idiotype induces protection against tumor take while another anti-idiotype accelerates tumor growth. Analysis of CTLs specific for the tumor showed equal numbers of CTL precursors in protected and unprotected mice. However, adoptive transfer with T cells induced protection only when taken from protected donors. This indicated the existence of a regulatory mechanism suppressing effective anti-tumor immunity. A panel of monoclonal anti-idiotypes was used to dissect possible network interactions in protected and unprotected groups of mice. A correlation between the expression of certain idiotopes in the sera and tumor growth could be established. A similar correlation was seen at the level of idiotype recognizing T helper cells expressing different idiotypic specificities in protected and unprotected mice. Collectively, these data demonstrate that a growing tumor depends on the establishment of regulatory idiotypic network interactions which suppress anti-tumor immunities and permit the tumor to continue to grow.

Genes Coding for TAA

T 008 SELECTIVE COAMPLIFICATION AND CLONING OF TRANSFECTED HUMAN DNA FRAGMENTS RELATED TO GENES ENCODING TWO MELANOMA-ASSOCIATED ANTIGENS. L.H.Graf, Jr.[1] M.Fagan[1], S.Ferrone[2], K.Kozlowski[1], C.D.Rosenberg[3], and J.Schrementi[1]. University of Illinois at Chicago, Chicago, IL[1], New York Medical College, Valhalla, NY[2], and Cornell University Graduate School of Medical Sciences, New York, NY[3].
In order to derive recombinant probes to investigate the specificity and the regulation of expression of human tumor cell-associated antigens (TAA's) we initiated interspecific transfer of genes encoding TAA's identified by mouse monoclonal antibodies (M'ab's). Use of highly competent mouse B16 melanoma recipient cell clone B78H1, and of an indirect red cell rosetting assay resulted in total human melanoma DNA-mediated transfer of genes encoding 4 TAA's, including 3 melanoma-associated surface antigens (MAA). This report describes selective coamplification in the mouse host cell background, and recombinant cloning of human chromosomal DNA fragments related to genes encoding 2 transfected MAA: "96K MAA," identified by M'ab 203.4, and "100K MAA," identified by M'ab 376.96. These MAA are surface glycoproteins which exhibit distinctive patterns of expression including modulation by interferons and other lymphokines, as well as developmental restriction, and preferential expression by neoplastic/metastatic cells. B78H1 transfectant cells express M'ab 203.4 or 376.96-reactive surface molecules closely resembling authentic 96K MAA or 100K MAA by immunochemical criteria. Their genomes contain characteristic arrays of presumably MAA gene-associated human alu family repeat sequence (h-alu)-positive restriction fragments after multiple rounds of transfection. An indirect coamplification procedure applied to 96K MAA and to 100K MAA gene transfectant cells led to increases in the copy numbers of all assayable donor DNA sequences in the host cell DNA's. The procedure entailed stepwise methotrexate (MTX) selection for increased dosage of one donor DNA component, the cotransfected mouse wild type dihydrofolate reductase (dhfr) gene vector, pFD11. MTX-amplified transfectant cell pFD11 dosages and DHFR activities were 50-100x the B78H1 host cells' native levels after stringent (50μM) MTX selection, and similar MTX-driven increases were observed in the transfectant cells' content of antigen gene-associated h-alu's and in their expression of the human antigens. Libraries containing genomic DNA fragments from coamplified transfectant cells overexpressing each antigen were constructed in bacteriophage vectors. Both 96K MAA and 100K MAA transfectant cell libraries yielded recombinant plaques containing MAA gene-associated h-alu's at frequencies of ~1/1000. Current characterization of h-alu$^+$ recombinant bacteriophage clones relative to functional 96K MAA or 100K MAA genes is a step towards construction of gene probes and vectors for analysis of the biological regulation of these antigens, and for development of their clinical potentialities. Supported by CA31937 and CA44107.

T 009 FUNCTIONAL DIFFERENCES AMONG GENES ENCODING TUMOR-SPECIFIC ANTIGENS, Hans Schreiber[+], Barbara Starr[+], Mary Ann Fink[+], Hartmut Koeppen[+], Susan R. Ross* and Hans J. Stauss[+], [+]The University of Chicago, Chicago, IL 60637 and *The University of Illinois, Chicago, IL 60607.

Changes in class I antigens of the major histocompatibility complex (MHC) on tumor cells can influence the host response to tumors and alter the biologic behavior of cancer cells. It is unclear, however, why changes in MHC class I antigens encoded by different regions of the MHC complex appear to have different effects on T cell-mediated tumor rejection. By molecular cloning and gene transfer, we have recently isolated three different novel MHC class I genes (designated 216, 166 and 149) that encode unique tumor-specific antigens of the murine 1591 tumor (1,2). To explore the respective roles of each of these genes, we transfected each of these genes into a tumor variant which had lost all three genes and grew in normal mice. Transfection of any one of these three genes into tumor cells allows immunologic rejection in the majority of mice but some tumors grow progressively. Interestingly, the mechanisms of tumor escape are different for tumors transfected with the different class I genes. The 216 gene must be lost before tumor escape can occur while the expression of the 149 and 166 genes is not lost in reisolated escape tumors. The critical portions of these genes responsible for these differences in tumor rejection or tumor escape are being determined using hybrid genes. The general relevance of our findings is being tested using tumors transfected with other MHC class I genes.

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T 010 EXPRESSION OF A POLYMORPHIC GENE CODING FOR AN EPITHELIAL MUCIN IN BREAST AND OTHER CARCINOMAS, Joyce Taylor-Papadimitriou, Sandra J. Gendler and Joy Burchell, Imperial Cancer Research Fund, P O Box 123, Lincoln's Inn Fields, London WC2A 3PX, U.K.

The mammary gland is a complex tissue containing several lineages, including the lumenal or secretory epithelial cells and the basal or myoepithelial cells which line the ducts and alveoli. These two cell types can be distinguished in tissue sections by immunohistochemical staining with monoclonal antibodies to structural components and to functional products characteristic of each cell type. Such studies have focused attention on the lumenal cell, since the dominant cell in breast cancers expresses both the simple epithelial keratins and the mucin molecules expressed by the lumenal epithelial cells in the normal gland. In these particulars the breast cancer cell resembles other adenocarcinomas, such as those from the colon, lung and ovary.

A large number of antibodies have been developed which react with the mammary mucin and several are being used successfully in the diagnosis and treatment of some carcinomas. That antibodies directed to a normal cell product can show some specificity for cancer cells is due to the fact that the mucin can be processed differently in these cells, and thus display a different profile of epitopes from the normally processed mucin. We have studied the expression of the mucin using antibodies and partial cDNA clones (see Abstract, Gendler et al.) and find that in addition to variations due to processing, individual variations in molecular weight are seen due to a genetic polymorphism. Sequence data show that the polymorphism lies in the core protein which may contain varying numbers of a tandem repeat element. The epitopes recognised by core protein reactive antibodies lie within the repeat and that recognised by antibody SM-3, which shows considerable tumour specificity, is exposed in the mucin molecules found in breast cancers and masked in the normally processed mucin. By defining the structure of the mucin gene and identifying epitopes which are exposed in breast cancers, it should be possible to take a more directed approach to the production of tumour-specific antibodies.

Clinical Management and Diagnosis Using TAA

T 011 APPLICATIONS OF MONOCLONAL ANTIBODIES TO THE TAG-72 ANTIGEN, Jeffrey Schlom, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892

The TAG-72 (Tumor Associated Glycoprotein) antigen is recognized by MAb B72.3 (a murine IgG). TAG-72 has been purified, and can be briefly characterized as a mucin of >300,000d which is distinct from other carcinoma markers. It is expressed on a range of human carcinomas (colorectal, gastric, pancreatic, ovarian, endometrial, adenocarcinoma of the lung, and mammary) and is not found on normal adult tissues at appreciable levels, with the exception of secretory phase endometrium. MAb B72.3 has been shown to be: (1) a useful adjunct in the diagnosis of malignancy in human effusions and fine needle aspiration biopsies, and (2) a useful and novel reagent in serum assays to monitor for carcinoma recurrence.

Recent tumor targeting studies in over 300 patients have demonstrated that i.v. administered ^{131}I-B72.3 IgG can successfully localize approximately 70-80% of human gastrointestinal and ovarian carcinomas. Moreover, intraperitoneally (i.p.) administered ^{131}I-B72.3 can successfully localize peritoneal implants not detectable by conventional means. Both the i.v. and i.p. MAb B72.3 administration studies involved the direct analysis of biopsy materials to quantitate the specificity of MAb localization (i.e., analysis of cpm/g of tumor vs. cpm/g normal tissues).

Therapeutic clinical trials with MAb B72.3 are now in progress, as well as studies on the use of an intraoperative hand held gamma probe (subsequent to ^{125}I-B72.3 administration) to identify occult tumor at surgery.

Innovations now being pursued include: (A) The use of recombinant/chimeric B72.3. (B) The use of second generation MAbs to TAG-72 with higher K_a and enhanced tumor targeting potential. (C) The use of recombinant interferon to enhance cell-surface antigen expression and thus enhance MAb targeting.

TAA Defined by Cytotoxic T Cells

T 012 SPECIFIC RECOGNITION OF HUMAN TUMOR ASSOCIATED ANTIGENS BY NON-MHC-RESTRICTED CTL, Olivera J. Finn, Department of Microbiology and Immunology, Duke University Medical Center, Durham NC 27710.

Development of methods and reagents for maintenance of human lymphoid cells in vitro has raised again the possibility of identifying in tumor bearing hosts cells with anti-tumor reactivity and expanding their numbers for study or therapeutic use. We were interested in detecting an immune response to pancreatic adenocarcinoma, by analysing the draining lymph nodes from patients with this malignancy for the presence of tumor reactive lymphocytes. A number of pancreatic adenocarcinoma associated antigens have been identified by our collaborators and found to be expressed on exocrine pancreatic tumor sections as well as on in vitro established pancreatic tumor cell lines. If some of these or similar tumor associated antigens were stimulating a population of T cells residing in the draining lymph nodes, we reasoned that such a population could be expanded in vitro using antigenically cross-reactive allogeneic pancreatic adenocarcinoma cell lines as a continuous antigenic stimulus. Lymph nodes draining the pancreatic carcinoma bed were obtained from patients at the time of exploratory laparotomy. Lymphocytes were cultured with IL-2 alone or IL-2 and an irradiated monolayer of one of three allogeneic pancreatic adenocarcinoma cell lines, HPAF, T3M4, or CAPAN-2. Cells plated in IL-2 alone died over a period of seven to fourteen days, while cells maintained on any one of the three allogeneic tumor lines and IL-2 continued to proliferate. A number of T cell lines have been established which are highly specific in their recognition of pancreatic cell lines. We chose cell line W.D. for further studies. Regardless of the pancreatic tumor line used for its expansion, this T cell line shows high levels of proliferation in response to 9 out of 10 other pancreatic lines. The same tumor lines are efficiently killed while numerous other tumor targets remain uneffected. There is also no auto-immune reactivity against EBV transformed syngeneic B cells. W.D. T cells are CD3+ and CD8+ and antibodies against these molecules block their reactivity. Anti-class I and class II antibodies have no effect, confirming the MHC non-restricted nature of these cells. One pancreatic cell line which could not induce proliferation nor be killed by W.D. cells was found to lack DU-PAN 2 antigen present on all the other cell lines. DU-PAN 2 is present on the surface of the pancreatic tumors and also shed by them. Highly purified soluble preparations of this antigen induce specific T cell proliferation and block the killing of the tumor targets. The ability of this molecule alone to activate the CTL explains their apparent non-MHC-restricted phenotype, and its ability to block killing might explain why are these CTL apparently ineffective in vivo.

T 013 HUMAN TUMOR ANTIGENS DEFINED BY CYTOTOXIC AND PROLIFERATIVE T CELLS, Michael T. Lotze, Shohken Tomita and Steven A. Rosenberg, Surgery Branch, National Cancer Institute, Bethesda, MD 20892

We have developed autologous T cell lines and clones with unique reactivity to human tumor antigens. The tumor which we have studied most intensively is human melanoma for which specific proliferative and cytotoxic clones and lines of both CD4 and CD8 phenotype have been generated. In current experiments 3/6 parental cytolytic lines cultured directly from tumor in the presence of IL-2 were specific for the autologous fresh tumor and not reactive with allogeneic tumor or autologous nontumor targets (fibroblasts, B cell lines). Autologous tumor specific cloids could be generated by limiting dilution techniques with clonal frequency varying from 1/156 to 1/30,529 depending on the time that cloning was performed relative to the initial culture. Cultured tumor was susceptible to lysis by cloned T cell lines and increased from 40.3 to 54.1 lytic units/10^6 cells with one clone/tumor combination and 0.1 to 8.7 in another when targets were stimulated with 1000 U/ml of gamma interferon for 3 days. No lysis of the other cultured target was observed in the criss-cross experiment. Lysis of tumor 560 by cloid 12 ($CD8^+$) was completely blocked by an anticlass I MHC antibody, W6/32. No apparent bystander toxicity was observed of allogeneic tumor targets cocultured with autologous tumor cells lysed in these assays. Interleukin-4 (BSF-1) is a T cell derived glycoprotein (M.W. 20 kd, pI 6.2) which has multiple biologic functions including serving as a T cell growth factor. Preliminary experiments in our laboratory indicate that IL-4 is also capable of stimulating the growth of both antitumor clones and lines alone and in conjunction with IL-2 (stimulation index of 32.6 and 314, respectively). These combinations may be helpful in growing specific cytolytic cells. At low doses of IL-2, IL-4 inhibits the expansion of $NKH1^+$ cells. In 5/5 experiments IL-4 decreased the IL-2 induced generation of cells with lymphokine activated killing (LK) from normal peripheral blood. Patients pretreated with IL-2 however on immunotherapy protocols could be demonstrated to produce cells with LAK activity with IL-4 alone in 8/8 individuals. In one experiment relative LAK activity from cells in media < 0.01 lytic units (LU), IL-4 2.39, IL-2 12.11, and IL-4 and IL-2 14.18 was obtained from an IL-2 treated patient from unseparated cells and < .01, 2.7, 51.66 and 4.34 LU from separated null cells, respectively. Finally, we are evaluating the adoptive transfer of expanded TIL clones and lines in murine and human studies. Objective responses in patients treated with combinations of IL-2, cyclophosphamide and tumor infiltrating lymphocytes have been observed in several patients.

T 014 UNIQUE TUMOR-SPECIFIC ANTIGENS DEFINED BY CYTOTOXIC T CELLS, Patricia L. Ward, Teresa Hurteau, Hartmut Koeppen and Hans Schreiber, The University of Chicago, Chicago, IL 60637.

Most carcinogens are mutagens that could cause genetic changes resulting in the expression of tumor-specific antigens. A mutational etiology would be consistent with the finding that tumors induced by chemical or physical carcinogens can have antigens that are unique i.e. individually specific for a particular tumor even when tumors are of the same histologic type and induced in the same strain of mice by the same carcinogen. Unfortunately, there is no clear cut genetic evidence that these antigens result from mutations caused by the carcinogens or by other genetic changes during tumor evolution. Instead, it is possible that residual heterozygosity within inbred strains or new germline mutations might cause the appearance of such antigens; only isolated transplantation experiments have used autochthonous tissues (i.e. tissue from the mouse of tumor origin) which would provide evidence for the possible true tumor-specificity of these unique antigens. We have isolated a series of UV-induced tumors along with autochthonous normal cells and, when available, autochthonous second malignancies. With cytolytic T cell clones, we have found that these tumors do express antigens that are absent from autochthonous normal or malignant control cells and that these antigens are uniquely expressed on the particular tumor used as the antigen. These CTL probes will be used to determine the genetic basis for the seemingly endless diversity of unique tumor-specific antigens and to distinguish between somatic cancer-related mutation or random activation of preexistant silent genes as the cause for the appearance of these antigens.

TAA Identified by Human Monoclonal Antibodies

T 015 HUMAN MONOCLONAL ANTIBODIES TO TUMOR ASSOCIATED GANGLIOSIDES, Reiko F. Irie, Division of Surgical Oncology, UCLA School of Medicine, Los Angeles CA 90024.

Gangliosides are glycosphingolipids that contain sialic acids, are synthesized only by Deuterostomia and are most commonly expressed on cell surface membranes. Although more than 60 different ganglioside molecules have been identified approximately 25% have been reported to be found in human tissues. The expression of gangliosides at the cell surface can alter during the cell cycle, differentiation and neoplastic transformation. The sugar portions of gangliosides are immunogenic and many ganglioside antigens associated with human malignant cells have been identified by the use of monoclonal antibodies. Murine monoclonal antibodies to ganglioside GD3 and GD2 have been used by other investigators to treat melanoma patients in phase I clinical trials. Human monoclonal antibodies to ganglioside antigens can be developed if the ganglioside is immunogenic in man. Thus far five gangliosides that induce immune responses in man have been identified on human cancer tissues. Our laboratory has developed human monoclonal antibodies to three different epitopes of these immunogenic gangliosides. I will discuss the biological and therapeutic significance of these ganglioside antigens.

T 016 BIOCHEMICAL CHARACTERIZATION OF A HUMAN COLON TUMOR ASSOCIATED ANTIGEN IDENTIFIED BY A TUMOR DISCRIMINATING HUMAN MONOCLONAL ANTIBODY, N. Pomato, J. H. Murray, E. Bos, M. V. Haspel, R. P. McCabe, M. L. Berman, and M. G. Hanna, Jr., Bionetics Research, Inc., Rockville, MD and Organon International b.v., Oss, Holland, The Netherlands.

A tumor discriminating human monoclonal antibody (MCA 16-88) was used to identify a colon tumor associated antigen (CTAA 16-88). Under denaturing conditions, the antigen exists as a complex of closely migrating polypeptides. Based on immunochemical analysis with a defined panel of murine monoclonal antibodies, CTAA 16-88 has epitopes shared with cytokeratins 18 and 19. This was confirmed by development of cDNAs from genes whose products are recognized by MCA 16-88. Analysis of the DNA sequence, mRNA, genomic DNA and immunological reactivity of the recombinant proteins indicate the relationship with cytokeratins 18 and 19. Both the solubility properties and molecular size characteristics of the antigen indicate that the complex may represent altered forms of the cytokeratins. The ability of MCA 16-88 to differentiate between normal and colon tumor by histochemical analysis as well as by selective localization in clinical colon tumor metastases supports the quantitative and presumed qualitative differential expression of this antigen in transformed epithelial tissue. Whether this unique expression is a result of a transcriptional event or a post-translational event associated with increased proteolytic activity accompanying neoplastic transformation resulting in degradation products of the cytokeratin is yet to be determined. Nevertheless, this is the first step toward characterizing the repertoire of unique tumor associated antigenic determinants using reagents generated by autologous human immunity to tumor. Initial results on the characterization of antigen(s) recognized by a second tumor selective human monoclonal antibody (MCA 28A32) indicates that this protein is not related to cytokeratins and deffers from CTAA 16-88 by both size and biochemical characteristics.

Preclinical Studies and Animal Models of Specific Immunotherapy

T 017 EXPERIMENTAL IMMUNOTHERAPY WITH VIABLE REGRESSOR TUMOR CELLS, H. Kobayashi, Lab of Pathology, Cancer Institute, Hokkaido University School of Medicine, Sapporo, Hokkaido, 060 Japan

One of the most important aims for improving specific immunotherapy in the treatment of cancer focuses on how to produce anti-tumor immunity in the host strong enough to prevent the growth of challenged tumor cells. The use of LTD-50 is an appropriate way to measure the strength of such antitumor immunity in the host.

Our examinations using various lines of rat and mouse transplantable tumors have shown an increase in LTD-50 of only 1-10x in the immune response of crude membrane, oncolysate, formalin & mitomycin C treated tumor cells, as compared to the effect of the LTD-50 in the nonimmune control host.

Irradiated tumor cell-immunity showed a stronger response and its increase in LTD-50 was approximately 200x.

Histocompatibility gene-transfected viable tumor cells regressing autonomously in the host can also produce antitumor immunity, but the increase in LTD-50 was no more than 10-20x. Qercetin-treated viable (regressor) tumor cells also regresses autonomously in the host and can also produce immunity, but the increase in LTD-50 was no more than 20-40x.

As far as we have tested, the strongest antitumor immunity was observed when viable tumor cells infected with nonlytic foreign antigenic viruses (ex rat viable tumor cells infected with Friend virus) were used; its increase in LTD-50 was more than 2.000x in the subcutaneous immunization and more than 10.000x in the intradermal immunization. Such immunity was further enhanced by cyclophosphamide(CY) and bleomycin(BLM) when they were administered before and after immunization, except when CY by itself was given after immunization.

The immunity induced by virus-infected viable tumor cells is still stronger than the immunity induced by viable tumor cells mixed with BCG, a treatment that was already been used in human cancer immunotherapy. The mechanism for producing a much stronger anti-tumor immunity through the "viable vaccine" of tumor cells infected with foreign antigenic virus and its future possible application to human cancer immunotherapy is discussed.

T 018 IMMUNIZATION WITH PURIFIED GANGLIOSIDES, Philip O. Livingston, Memorial Sloan-Kettering Cancer Center, New York, N.Y. 10021.

The gangliosides GM2, GD2, and GD3 are differentiation antigens largely restricted to cells of neuroectodermal origin. They are expressed on most melanomas, astrocytomas, and neuroblastomas and have been shown to function as effective targets for monoclonal antibody therapy. The immunogenicity of these gangliosides has been explored in the mouse and in man by analyzing the humoral immune response after vaccination. In the mouse, vaccination with GM2 combined with Salmonella minnesota mutant R595 or BCG, but not with GM2 alone or whole cells expressing GM2, results in frequent production of anti-GM2 IgM, and similar vaccines containing GD3 (in place of GM2) result in higher antibody titers than seen with GM2. Pretreatment of the mice with low dose cyclophosphamide (to decrease suppressor cell activity) significantly increases the frequency and titers of anti-ganglioside antibodies. Based on these findings, my collaborators Drs. L.J. Old and H.F. Oettgen, and I, have treated disease-free patients with metastatic melanoma with a series of vaccines and used ELISAs on purified gangliosides to detect the serologic response. The specificity of observed reactions was further defined by immune staining of thin layer chromatography plates. Vaccines containing GM2 alone resulted in no antibody responses, vaccines containing R595/GM2 resulted in occasional antibody responses and those containing BCG/GM2 resulted in high titer antibodies in most patients. Pretreatment with low dose cyclophosphamide (200 mg/M^2) significantly increased the immunogenicity of R595 and BCG/GM2 vaccines. Cimetidine (1 gram/day PO) did not further augment the immunogenicity of GM2 vaccines in Cy pretreated patients. Nineteen of 24 patients immunized with Cy-BCG/GM2 produced high titer IgM antibodies (>1/80) and 8 produced high titer IgG antibodies. Disease progression was found to be significantly delayed in patients with high titer anti-GM2 antibody response after vaccination. Cy-BCG/GD2 or GD3 vaccines, however, resulted in no high titer antibody responses in the 12 patients immunized with each ganglioside. While GM2 is significantly more immunogenic than GD3 in man, the reverse is true in the mouse. This reflects the expression of these gangliosides on normal tissues, i.e. GD3 >GM2 in man and GM2 > GD3 in the mouse.

Recent Advances in Targeting Antibodies to TAA

T 019 **Successful Imaging of Metastatic Tumors Using ^{99m}Tc-Labeled Monoclonal Antibodies: A Prelude to Radiotherapy.**

Paul G. Abrams[1,2], Robert W. Schroff[1,2], Mehmet F. Fer[1], Darrell Salk[1], Janet F. Eary[2], and Wil B. Nelp[2]. NeoRx Corporation and the Division of Nuclear Medicine, University of Washington, Seattle, WA

Technetium-99m (^{99m}Tc) is the most commonly used radionuclide for nuclear medicine scanning because it provides superior image resolution and it is safe, inexpensive, and readily available. Using the diamino, dimercaptide ligand system developed by Fritzberg et al. (PNAS, in press), we investigated whether the 6-hour half-life of ^{99m}Tc was compatible with tumor imaging using monoclonal antibodies. To date, nearly 200 patients with melanoma, lung cancer, and colon cancer have been successfully imaged using Fab fragments of NR-M1-05, NR-Lu-10, and NR-Ce-01 antibodies. Toxicity, allergic in nature, was rare (1.5%), readily reversible, and never prevented completion of the study. Images were obtained in 6-20 hours. Previously known and occult metastatic disease was detected in liver, bone, skin, brain, lymph node, adrenal, ovary, spleen and lung, the latter having a lower detection rate for reasons as yet unexplained. Accumulation of the labeled antibody was observed in thyroid (cross-reactivity) and occasionally in areas of osteoarthritis positive on bone scan, and infrequently in inflammatory lesions. Excretion of the label via the renal and hepatobiliary routes complicated image interpretation. We conclude that ^{99m}Tc may be stably chelated to antibody fragments and that the resulting immunoconjugates provide useful staging information in a single diagnostic scan. Results of the imaging procedures were useful in patient management decisions. Elimination of hepatobiliary excretion will improve image interpretation. Radiotherapy applications using the same ligand with ^{186}Re are now being tested in clinical trials.

T 020 ANTI-RECEPTOR ANTIBODIES CAN FOCUS T-CELL ACTIVITY, Uwe D. Staerz, Basel Institute for Immunology, 4058 Basel, Switzerland.

The T-cell receptor dictates that T cells recognize foreign antigen in the context of self major histocompatibility complex (MHC) encoded molecules. Cells which are recognized as foreign by the T-cell system are efficiently rejected by direct cytolysis or in a lymphokine-mediated way. Antibodies, in contrast, recognize antigens without the constraints of MHC restriction. It might be advantageous to combine the superior effector function of T lymphocytes with the specificity of readily available antibodies. A system of hybrid monoclonal antibodies (HAb) was developed whose one binding site interacts with components of the T cell receptor complex and whose other binding sites see surface structures on predefined target cells. We will show that these HAb can efficiently target tumors and infected cells. The HAb, H1.10.16, linking F23.1$^+$ T lymphocytes to Thy1.1$^+$ SL2 targets was used to define the antibody mediated cytotoxicity in a tumor situation both in vitro and in vivo. As a model for an infection we chose the influenza virus.

Human Tumor Antigens and Specific Tumor Therapy

Active Antigen Immunotherapy

T 021 IMMUNOLOGICAL CHARACTERIZATION OF COLON TUMOR ASSOCIATED ANTIGENS, M. G. Hanna, Jr., J. H. Murray, N. Pomato, E. Bos, J. M. Jessup, R. P. McCabe, M. V. Haspel, and H. C. Hoover, Jr., Bionetics Research, Inc., Rockville, MD, Organon International b.v., Oss, Holland, The Netherlands, M.D. Anderson Hospital, Houston, TX, Massachusetts General Hospital, Boston, MA.

Active immunization with autochthonous tumor cells admixed with BCG has been used effectively as adjuvant therapy in a prospectively randomized Phase II clinical trial of patients with Duke's stage B_2 and C colorectal cancer. Significant improvement in five year survival and recurrence rates of tumors were seen in the immunized patients as compared to controls. A large number of human monoclonal antibodies (MCA) were obtained from the peripheral blood lymphocytes of patients undergoing active specific immunotherapy. Many of the human MCAs showed preferential binding to tumor cells. This suggests that the human MCAs are reacting with determinants that are immunogenic in man, the natural host. Two of these human MCAs, 28A32 and 16-88, were used in a combined radio-imaging and escalating dose phase I antibody therapy trial in colon cancer patients. Using I-131 labeled 28A32 and 16-88 immunoscintography has detected tumors as small as 2 cm in 12/13 and 8/9 patients respectively with minimal toxicity. Using these MCAs we have identified and isolated the cognate colon tumor associated antigens. Studies are underway to determine if these antigens will elicit an in vitro and in vivo cellular immune response. In vitro measurements of T cell activation by isolated tumor antigens include: (1) antigen induced cell proliferation; (2) increase in T cell activation antigens, such as TA-1, TLISA-1, IL-2R; and (3) antigen-induced cellular cytotoxicity. In addition, the antigens are being tested for their ability to induce a DCH in immunized patients. The ultimate goal of our research is to substitute a generic antigen vaccine for the current autologous tumor cell vaccines.

Combination Therapy (joint)

T 022 COMBINATIONS OF CHEMOTHERAPY AND BIOMODULATION IN THE TREATMENT OF CANCER, Malcolm S. Mitchell, Departments of Medicine and Microbiology, U.S.C. Cancer Center, Los Angeles, CA 90033.

Biological response modifiers ("biomodulators") have emerged as an important new class of agents for treating cancers. While they have usually been tested alone, several are now suitable for use in combination with older modalities such as chemotherapy. Chemotherapy is usually but not always immunosuppressive. Several drugs cause far less suppression than others, and many can be immunostimulatory. Such drugs as doxorubicin, bleomycin, DTIC and cis-platin are in this category, but even "classical" immunosuppressive compounds such as cyclophosphamide (CY) can cause immunostimulation at low doses by inhibiting cellular and humoral suppressor influences. Chemotherapy should generally precede immunotherapy rather than being given concomitantly, to permit recovery of the immune response or to eradicate suppressor influences. The cytoreductive effects of chemotherapy also allow the immune response to act more effectively on the remaining tumor cells. Under special circumstances, biomodulation has been useful before chemotherapy, perhaps altering vascular permeability of the tumor, but this sequence may make the immune response more vulnerable to suppression. Several clinical trials with therapy to restore T cells have potentiated chemotherapy, such as in small cell lung cancer and melanoma. Low-dose CY has potentiated delayed hypersensitivity to autologous melanoma cells, although clinical responses have been somewhat uncommon. Most recently the combination of low-dose CY preceding IL-2 has caused complete or partial remissions in nearly 30% of patients with melanoma. DTIC in therapeutic doses before IL-2 has caused responses of the same order of magnitude early in the study. Doxorubicin and interferon also appear to be synergistic, and are being exploited for that effect in current clinical trials. The use of biomodulators that increase the uptake of chemotherapy into cancer cells, particularly immunoconjugates, that decrease its toxicity (by stimulating bone marrow stem cells or antagonizing immunosuppression), cause tumor maturation or inhibit metastases are all on the horizon, and promise to potentiate the efficacy of chemotherapy considerably. Thus, the interaction of biomodulators and chemotherapy is mutual, with the potential of improving the activity of each.

T 023 PERSPECTIVES FOR COMBINED BIOTHERAPY OF CANCER: BUILDING UPON REGIMENS USING TOLERABLE DOSES OF INTERLEUKIN-2, Paul M. Sondel, Jacquelyn A. Hank, Jeff A. Sosman, Peter C. Kohler, Depts. of Pediatrics, Human Oncology and Genetics, University of Wisconsin, Madison, WI 53792.

Although interleukin-2 can activate immune cells with anti-tumor activity when given in very high doses, it can also turn on immune reactions which cause significant life-threatening toxicity. We have evaluated sustained treatments with lower doses of interleukin-2. This regimen does have side effects but these are not severe and are well tolerated by cancer patients admitted to a general hospital ward without requiring intensive care unit monitoring or support. This regimen, utilizing interleukin-2 alone, can activate within the patient a 100-fold increase in the level of circulating lymphokine activated killer (LAK) activity in their white blood cells. At least some patients receiving this treatment have shown greater than 50% shrinkage of all measurable renal cell carcinoma. Thus, interleukin-2 can be given safely, with acceptable toxicity, utilizing a regimen that induces dramatic LAK activity, as well as measurable anti-tumor effects.

IL-2 remains a relatively new treatment with much room and need for further development. Yet, in less than three years of clinical testing, it is clear that this strictly immunological approach can be utilized in a number of regimens to activate a patient's own immune system, and that this activated immune response can cause a shrinkage of sizeable cancers in some patients. IL-2 is not a "magic bullet", nor a panacea, nor is the LAK activity that is induced by IL-2. In fact, the majority of patients receiving IL-2 show some side effects without any measurable tumor shrinkage. Nevertheless, the induction of reproducible clinical anti-tumor effects by a well characterized molecule which acts only through the immune system, provides some hope that further development of this approach may someday enable better clinical results. This will most certainly require combination of this approach with other modalities. Numerous clinical and laboratory teams are working rapidly toward this goal.

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Late Addition

T 024 HUMAN MONOCLONAL ANTIBODIES, June Kan-Mitchell, Department of Microbiology, University of Southern California School of Medicine, Norris Cancer Hospital and Research Institute, Los Angeles, CA 90033.

To study tumor-associated antigens (TAAs) that are immunogenic to man, we have generated human monoclonal antibodies (HMAb) by fusing regional lymph node cells of cancer patients with a mouse myeloma cell line. Two IgG MAbs were thus isolated from a patient with melanoma and one with colon carcinoma. Both antibodies were produced in mg quantities and purified to 85% homogeneity for characterization by immuno-cytochemistry and -histochemistry.

The HMAb 2-139-1 from the melanoma patient was reactive against a cytoplasmic antigen in all melanoma cells tested. However, it did not identify a neuroectodermal differentiation antigen. That is, reactivity was not detected in skin melanocytes or banal nevus cells or other tumors of neural crest origin such as glioma and astrocytoma. Of interest was the absence of reactivity against normal tissues as well as fetal melanocytes and other fetal tissues. However, the antibody was reactive to different degrees to various carcinomas and was particularly crossreactive to colon carcinoma.

To determine whether HMAbs thus generated all express this unusual spectrum of reactivity, perhaps recognizing only a limited number of target antigens, a series of antibodies was developed from a patient with colon carcinoma. Although many of these "colon" HMAbs reacted to colon carcinoma and melanoma, others did not crossreact to melanoma. One of these, 14-31-10, selected for further characterization. The HMAb 14-31-10 also identified a cytoplasmic antigen in colon carcinoma but not in normal colonic epithelium. No binding was detected against a variety of normal tissues. In parallel studies of 2-139-1 in melanoma, 14-31-10 was found to be nonreactive to a variety of benign lesions of the colon such as diverticulosis and regional enteritis. However, some of the preneoplastic lesions such as villous adenomas were found to be reactive, although to a lesser degree.

Our results illustrate that HMAbs can add a new dimension to identifying novel TAAs. These HMAbs defined a family of cytoplasmic TAAs. They appear to discriminate between tumor and normal tissues. Because of this unusual spectrum of reactivity, these antibodies may have applications in pathological and serological diagnosis.

TAA Molecules and their Genes

T 100 ISOLATION AND SEQUENCING OF A cDNA CODING FOR THE HUMAN DF3 BREAST CARCINOMA-ASSOCIATED ANTIGEN, M.Abe, J.Siddiqui, D.Hayes and D.Kufe, DANA-FARBER Cancer Institute, Boston, MA 02115

The murine monoclonal antibody (MAb) DF3 reacts with high molecular weight glycoproteins detectable in human breast carcinomas. The correlation between DF3 antigen expression and human breast tumor differentiation, as well as the detection of a cross-reactive species in human milk, has suggested that this antigen might be useful as a marker of differentiated mammary epithelium. We have isolated a cDNA clone from a λgt11 library prepared from MCF-7 human breast carcinoma cells by screening with MAb DF3. The results demonstrate that this 309 bp cDNA, designated pDF9.3, codes for the DF3 antigen epitope. Southern blot analysis of EcoRI or PstI digested DNAs from 6 human tumor cell lines with ^{32}P-labeled pDF9.3 have revealed a restriction fragment length polymorphism (RFLP). Furthermore, hybridization of ^{32}P-labeled pDF9.3 with total cellular RNA from each of these cell lines demonstrated either one or two transcripts which varied from 4.1 to 7.1 Kb in each size. The presence of different sized transcripts detected by pDF9.3 was also found to correspond with the polymorphic expression of DF3 glycoproteins. Nucleotide sequence analysis of pDF9.3 has revealed a highly conserved GC rich 60 bp tandem repeat. These findings suggest that the variation in size of alleles coding for the polymorphic DF3 glycoprotein may represent different numbers of repeats. Finally, the pDF9.3 probe has been used to monitor RFLPs and patterns of DF3 gene expression in primary and metastatic human breast tumors.

T 101 CYTOLYTIC T LYMPHOCYTES WITH AND WITHOUT LYTIC GRANULES SUGGEST ALTERNATIVE PATHWAYS IN LYMPHOCYTE-INDUCED CYTOLYSIS, Gideon Berke, Cell Biology, The Weizmann Institute Of Science, Rehovot, 76100, Israel.

Demonstration of complement (C)-like 'rings' and of lytic granules and lytic proteins thereof, in certain cytocidal lymphocytes, for the most part growing in vitro in IL-2 or large granular lymphocytes (LGL), suggested a mechanism of lytic granule-exocytosis in lymphocyte-mediated cytolysis and a common terminal lytic step in lymphocyte and C-induced lysis. On the other hand, neither formation of C-like 'rings' nor lytic granules or lytic proteins thereof have been detected in preliminary studies of highly potent, peritoneal exudate cytotoxic T lymphocytes (CTL) (PEL) derived directly from the site of allograft rejection, or in cytocidal hybridomas thereof (PEL-hybridomas). We now report that when stimulated in vitro in the presence of interleukin-2 (IL-2), the small in vivo primed cytolytic PEL-CTL transform into large, dividing cytolytic T cells (PEL-blasts) which express the authentic PEL specificity in a short term lytic assay. PEL-blasts in contrast to PEL, possess massive quantities of lytic granules and serine (BLT)-esterase activity as well as non-specific cytolytic activity in a long-term assay. Hence the proposed lytic mechanism involving exocytosis of lytic granules and BLT-esterase(s) may apply to killing induced by granule-containing effectors such as LGL, activated lymphocytes, and to IL-2-dependent CTL lines such as PEL-blasts but not to killing by in vivo primed CTL like PEL or their hybridomas. These findings suggest two distinct pathways in lymphocytotoxicity (one one of which potentialy involves cytocidal lytic granules), and the modulation of lytic granule and BLT-esterase expression in cytolytic lymphocytes in IL-2.

T 102 EXPRESSION OF THE THOMSEN-FRIEDENREICH (T) ANTIGEN IN A MAMMARY ADENOCARCINOMA. K.L. Carraway and S.R. Hull, Univ. of Miami Med. Sch., Miami, FL 33101

The Thomsen-Friedenreich (T) antigen and its disaccharide component Galβ1,3GalNAc, which is recognized by the plant lectin peanut agglutinin (PNA), have been proposed as useful tumor markers because of their apparently specific occurrence in certain types of carcinomas. We have investigated the mechanism for the appearance of the disaccharide at the cell surface of ascites 13762 rat mammary adenocarcinoma cells using pulse-chase glucosamine labeling, proteolysis and PNA precipitation of the cell surface sialomucin ASGP-1. Glucosamine-labeled disaccharide appears at the cell surface in less than 10 min. Although the appearance of larger oligosaccharides continues to increase, the appearance of labeled disaccharide levels off within an hour. Analysis of intracellular vs. cell surface labeled oligosaccharides showed that all disaccharide synthesized more than an hour before reaching the cell surface is converted to larger oligosaccharides. Thus, the presence of the disaccharide at the cell surface results from its synthesis late in the transit pathway of the sialomucin to the cell surface.

T 103 EXPRESSION OF MELANOMA-ASSOCIATED ANTIGENS BY LM MOUSE FIBROBLASTS TRANSFECTED WITH DNA FROM MOUSE MELANOMA CELLS, Edward P. Cohen, Young S. Kim, Karen Hagen, and Ryszard Slomski, Dept. of Microbiology and Immunology, Univ. of Illinois at Chicago, IL 60612.

Most neoplastic cells that form surface antigens that might be "targets" of immune-mediated attack do not provoke anti tumor immune responses even if the tumor-bearing host is fully immunocompetent; tumor-associated antigens (TAA) are poorly antigenic in the authocthanous host. In an attempt to augment their immunogenic properties, we used DNA-mediated transfection to introduce genes from B16 melanoma ($H\text{-}2^b$) into mouse LM fibroblasts ($H\text{-}2^k$). LM cells (a thymidine-kinase-deficient variant was used to facilitate selection) were co-transfected with DNA from B16 cells and pTK, a plasmid carrying the mouse thymidine-kinase gene. Initial selection was performed in HAT medium. Viable colonies of transfectants expressing melanoma-associated antigens were identified *in situ* by erythrocyte resetting, (frequency = $4x10^{-3}$). Melanoma-antigen-expression was confirmed by flow cytometry. The proportion of cells staining above background with anti TAA serum ranged from 5 to 40 percent; in some the instances, the staining intensity exceeded that of B16 cells. Certain colonies formed unique melanoma-associated antigens, consistent with the expression of one of several TAA. As determined by MH reactions *in vivo* and CML reactions *in vitro*, the immunogenic properties of some transfected cell colonies exceeded those of B16 cells.

T 104 BIOCHEMICAL AND MOLECULAR STUDIES OF NGA, A NEUROECTODERMAL TUMOR-ASSOCIATED ANTIGEN, W.T. Dixon, K. Ohyama, R.C. McGarry, D.J. Demetrick, L.K. Sikora and L.M. Jerry, Oncology Research Group, University of Calgary, Calgary, Alberta, Canada T2N 4N1

Neuroglandular antigen (NGA) was first identified as a human melanoma-associated antigen recognized by a panel of murine monoclonal antibodies developed by this laboratory (Int. J. Cancer 39:138). Subsequently it has been shown to be present on human neuroblastoma cells and other human tumors with a neuroectodermal origin. Its expression can be modulated by treatment of target cells with various chemical agents known to cause *in vitro* cell differentiation. This tumor antigen consists of a core protein with an M_r of 22 kDa on SDS-PAGE which is modified co-translationally by the addition of at least 3 N-linked oligosaccharide moieties. These undergo processing and O-glycosylation in the Golgi to yield a heterogeneous family of glycoproteins (36-60kDa) with varying amounts of covalently attached carbohydrate. We have initiated screening of λgt11 human melanoma cDNA libraries for expression of NGA using a polyclonal rabbit antiserum directed against the deglycosylated form of NGA. Positive plaques have been obtained and are being confirmed by re-screening with an oligonucleotide probe derived from a partial N-terminal sequence of the NGA molecule. The function of NGA is unknown, however its over-expression in transformed melanocytes, especially metastatic melanoma, and other neuroectodermal tumors have prompted our efforts to isolate and clone the gene encoding NGA.
(Supported by MRC, NCIC and AHFMR)

T 105 A HIGHLY IMMUNOGENIC REGION OF THE POLYMORPHIC GENE CODING FOR THE HUMAN MAMMARY MUCIN IS MADE UP OF 60 BASE PAIR TANDEM REPEATS, Sandra J. Gendler, Joyce Taylor-Papadimitriou, Trevor Duhig and Joy Burchell, Imperial Cancer Research Fund, P O Box 123, Lincoln's Inn Fields, London WC2A 3PX, U.K.

Mucins, which are found on the surfaces of simple human epithelial cells and in their secretions, are highly immunogenic in rodents with the result that many monoclonal antibodies selected for epithelial or tumor specificity, are reactive with epitopes on these molecules. Since little is known about the detailed structure of mucins which are large complex molecules containing a high level of O-linked carbohydrates, we chose to approach the problem by analyzing the gene, and we recently reported the cloning of cDNA fragments of the human milk mucin that contain epitopes of the core protein (PNAS 84:6060, 1987). The immunogenic region consists of a 60 base pair tandem repeat unit that is repeated multiple times, resulting in a polymorphism that is detectable both in the expressed protein and in the DNA cut by various restriction enzymes (Nature 327:82, 1987). This region of the gene has the characteristics of a CpG island which is located within the translation unit near the 3' end of the gene. The tandem repeat codes for a 20 amino acid repeat unit which contains the epitopes defined by the antibodies HMFG-1, HMFG-2 and SM-3. Reactivity of SM-3 on sections of tumour and normal mammary tissue suggests that the mucin is abnormally glycosylated in carcinomas.

T 106 ANTIGENS ON MELANOMA CELLS RECOGNIZED BY CYTOTOXIC T LYMPHOCYTES (CTL) FROM HUMAN PATIENTS, P. Hersey, M. MacDonald and H. Werkman, Immunology & Oncology Unit, Royal Newcastle Hospital, Newcastle, N.S.W. 2300, Australia.

Antigens recognized by cloned cytotoxic T lymphocytes (CTL) from patients with melanoma were examined using methods based on the ability of antigen immobilized on nitrocellulose paper to stimulate proliferation of the CTL. The peak proliferative response was shown to occur at 3 days and was dependent on the presence of histocompatible antigen presenting cells (APC) in the cultures either in the form of autologous LCL (EBV transformed B cells) or histocompatible PBL. Presentation appeared to be via MHC class II antigens in that monoclonal antibodies (MAbs) against the latter but not class I antigens on the APC inhibited the proliferative responses. The response of the CTL appeared to be mediated by interaction with the CD3 T cell receptor complex in that pretreatment of the CTL clones with MAbs against CD3 inhibited their response to the antigen extracts irrespective of their phenotype. Extracts from several non-melanoma cells did not stimulate CTL clones specific for melanoma. At least two different specificities were detected in extracts from autologous and allogeneic tumor cells. The specificity of proliferative responses by CD3+ CD4+ and CD3+ CD8+ CTL appeared to be similar to their cytotoxic activity but CTL with the CD3+ CD16+ CD8+- phenotype (Type 2 CTL) had wider cytotoxic activity against TCs not stimulating proliferative responses. The antigen(s) responsible for the stimulation were shown in all instances to have mol. wts. of approximately 48kD. Preliminary analysis suggested that the antigen(s) have both protein and glycolipid (ganglioside) components but further analysis of the antigen is needed.

T 107 CHARACTERIZATION OF A NEWLY ESTABLISHED MONOCLONAL ANTIBODY TO HUMAN GLIOMA RELATED ANTIGEN, H. Hashimoto, Y. Hiasa, N. Konishi, Y. Murata, H. Matsuki, K. Kyoi*, S. Utsumi*, T. Sakaki* and T. Tada*, 1st Dept. of Pathology, and *Dept. of Neurosurgery, Nara Medical University, Nara, Japan.

IgG_1 monoclonal antibody to human glioma related antigen, non-glycoprotein, was established from tumor extract of anaplastic astrocytoma with sequential chromatographies of DEAE-Sephadex A-50, Sephadex G-100 and Con A-Sepharose affinity columns. Immunohistochemical examination of the paraffin-embedded and frozen sections of various human tissues and the cultured cells with the ABC technique revealed that the monoclonal antibody was strongly reactive with glioblastoma, malignant astrocytomas, gemistocytic astrocytomas, and protoplasmic astrocytomas; partially reactive with craniopharyngiomas and schwannomas. The reaction of the monoclonal antibody was expressed apparently on cytoplasms but was not within glial filaments which strongly reacted with anti GFAP and anti S-100 protein stains. It was not reactive with both normal human tissues but pancreas and carcinomas of other organs.

Histology	Positive/ Total
Paraffin sections	
Astrocytoma I, II	9/13
Astrocytoma III, IV	7/ 8
Glioblastoma	4/ 5
Oligodendroglioma	2/ 2
Medulloblastoma	0/ 7
Ependymoma	0/ 5
Schwannoma	2/ 6
Craniopharyngioma	2/ 3
Chordoma	0/ 1
Pituitary adenoma	0/ 4
Frozen sections	
Astrocytoma	2/ 2
Glioblastoma	2/ 2
Craniopharyngioma	1/ 1
Glioma cell lines	
SK-MG-1	++
SK-MG-4	++

T 108 CHARACTERIZATION OF A MELANOMA-ASSOCIATED CELLULAR ADHESION MOLECULE (MACAM-1). F. Hogervorst, C. Tyhof, C. Figdor. The Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands.

We have selected two hybridomas, NKI-M7 and PAF-1, after immunization with short-term cultured melanoma cells. Both inhibit the adhesion of melanoma cells to artificial substrates like PVC and to endothelial cells. Furthermore we observed that haptotactic migration of melanoma cells, induced by plasma fibronectin, can be diminished by these monoclonal antibodies (Mabs). They recognize overlapping epitopes. Immunoprecipitation studies revealed four polypeptides when analyzed under reducing conditions (120, 105, 29 and 27 kD) and two polypeptides under non-reducing conditions (145, 90 kD). These two Mabs specifically bind to melanoma cells and to endothelial cells of small vessels if tested on frozen tissue sections. However, if cultured cells of different types of tumors are used, they seem less specific. These results indicate that the antigen can be induced upon culturing. The staining pattern of NKI-M7 and PAF-1 with cultured cells is characteristic. We observe local concentrations at the border of the adhesion surface of cells. This same pattern is seen after staining with anti-vinculin Mab. Both vinculin and MACAM-1 can be found at the end of actin stress fibers. The antigen is also present in migration tracks and adhesion plaques.

These biological and biochemical data suggest that MACAM-1 is part of the integrin super family which is involved in adhesion, migration and differentiation processes. Cleveland digestion pattern of gp90 shows similarities with the pattern of gpIIIA, but not with gpIIA and the digestion pattern of gp145 is not homologous to gpIIB and gpIC.

T 109 HER2/neu: ROLE IN TRANSFORMATION AND POTENTIAL SIGNIFICANCE IN CANCER THERAPY, Robert Hudziak, Gail Lewis, Marcy Winget, Brian Fendley, H. Michael Shepard and Axel Ullrich, Genentech, Inc., South San Francisco, CA 94080.

The HER2 gene encodes a transmembrane glycoprotein with extensive homology to the human epidermal growth factor receptor. It is the human homologue of the rat neu gene and has many structural and biochemical features of a growth factor receptor, although no ligand has been identified. Recently, the HER2 gene was found to be amplified in about 30% of primary human breast tumors. In experiments designed to assess the role of the HER2 gene in oncogenesis, we found that overexpression of unaltered HER2 coding sequences in NIH 3T3 cells resulted in cellular transformation and tumorigenesis. We have also shown that amplification of the HER2 gene will protect the cells from the cytotoxic effects of macrophages and tumor necrosis factor-alpha. Monoclonal antibodies specific for the extracellular domain of HER2 will partially reverse both the transformed phenotype and TNF resistance, and in one case an antitumor effect has been demonstrated in a mouse model.

T 110 OLIGOSACCHARIDES OF THE DF3 ANTIGEN OF THE BT-20 HUMAN BREAST CARCINOMA CELL LINE. S.R. Hull, A. Bright, K.L. Carraway, M. Abe and D. Kufe, Univ. of Miami, Miami, FL 33101 and Dana-Farber Cancer Inst., Boston, MA 02115

DF3, a murine monoclonal antibody, reacts with mucin-like glycoproteins in human breast carcinoma cells and cell lines. Glucosamine-labeled DF3 antigen was purified from the media of the BT-20 human breast carcinoma cell line by sequential chromatography on wheat germ-Agarose, Sephadex G-200 and anti-DF3-Sepharose. Oligosaccharitols were obtained from the antigen by β-elimination with alkaline borohydride and co-chromatographed by ion suppression HPLC with previously characterized oligosaccharitols from the major sialomucin of the 13762 rat mammary adenocarcinoma. Of the three major oligosaccharitols of the DF3 antigen only one corresponded to a component of the 13762 cells, the peanut lectin-binding disaccharide Galβ1,3GalNAc. Anion exchange and desialylation studies indicated that the other two DF3 oligosaccharides were the mono- and di-sialylated derivatives of the disaccharide. Thus the Thomsen-Friedenreich antigen and its sialylated derivatives are major carbohydrate components of this tumor cell surface antigen.

T 111 CDNA CLONING OF P3.58, A CELL SURFACE ANTIGEN CORRELATED WITH TUMOR PROGRESSION IN MELANOMA. Barbara Stade, Uwe Hupke, Bernhard Holzmann, Gert Riethmüller, Judith P. Johnson, Institute for Immunology, Univ Munich, Munich 2 West Germany.

Expression of the P3.58 antigen, gp89, by malignant melanoma in situ is correlated with tumor progression. While benign nevocellular nevi and thin melanomas which have a good prognosis are rarely positive, the frequency of P3.58 expressing tumors increases in parallel with the probability of development of metastatic disease. In contrast to the majority of tumor associated antigens, the expression of the P3.58 antigen on melanoma cells is modulated by exposure to interferon- and tumor necrosis factor α. A survey of normal tissues indicates that the expression of P3.58 is limited to some endothelia and to activated B lymphocytes and monocytes. In vitro studies suggest that the antigen plays a role in macrophage-lymphoid cell contact and its biochemical characteristics are similar to those of two putative LFA-1 ligands, the ICAM adhesion molecule and the lymphocyte homing receptor. Using a series of monoclonal antibodies prepared against isolated denatured gp89, a cDNA clone encoding the P3.58 antigen has been isolated from a melanoma lambda expression library. Southern analyses indicate a single gene in the melanoma and show that the same band is present in a P3.58+ L cell transfectant. Sequence and structural analysis of the P3.58 antigen are in progress and may provide information on the relationship of this molecule to other adhesion molecules and its function in malignant melanoma.

T 112 TUMORPROGRESSION IN HUMAN MALIGNANT MELANOMA: MOLECULAR CLONING OF THE MUC18 ANTIGEN *Jürgen M.Lehmann, Gert Riethmüller and Judith P. Johnson,* Institute for Immunology, Univ. of Munich,8000 Munich2,F.R.G.
The multistep process in the course of tumor progression of melanocytes leads to stepwise changes not only of histological and clinical features, but also to changes in the antigenic phenotype of melanocytes (1). The 113,000 dalton glycoprotein MUC18 is such an antigen (2), whose expression is linearly correlated with tumor thickness and thus with poor prognosis and the eventual development of metastatic disease. The MUC18 antigen was found neither on benign nevi nor on very thin malignant melanomas (≤0.75mm). Furthermore the antigen has not yet been found on other types of tumors and its expression in normal tissues appears limited to smooth muscle of blood vessels and hair follicles. For molecular cloning, a human melanoma lambda gt11 cDNA library was screened using four monoclonal antibodies directed against the denatured antigen. Four cross-hybridising positive clones (size 1.2 - 3.0kb) identifying a 3,0kb mRNA in melanoma were selected for further studies and sequencing. Northern blot analyses showed a tissue distibution similar to that of MUC18 antigen expression.
References: 1.Holzmann et al. Int.J.Cancer 39:466 (1987)
2.Lehmann et al. Cancer Res. 47:841 (1987)

T 113 APPLICATION OF MONOCLONAL ANTIBODIES TO MAMMARY MUCINS IN HUMAN CANCER, P. Jeremy McLaughlin, Xing Pei-xiang, Joe J. Tjandra and Ian F.C. McKenzie. Department of Pathology, University of Melbourne, Parkville, Vic. 3052, Australia.
Many monoclonal antibodies currently showing potential as clinically useful reagents are directed against epitopes on a high molecular weight mucin-type molecule. This molecule is a major glycoprotein of human milk fat globule membrane (HMFGM) but is also expressed, often in an altered form, on breast carcinoma cells. Using human milk fat globule membranes in native or chemically/enzymically altered forms, or breast cancer cell membranes as immunogens we have developed a series of murine monoclonal antibodies reactive with epitopes on HMFGM. Many of these antibodies are strongly reactive with a large proportion of malignant breast tumours, but also react weakly with certain normal tissues including breast. Serum assays have been developed using one or more of these monoclonal antibodies, which specifically and sensitively detect breast carcinoma, even in early stages. Some antibodies were used successfully in immunolymphoscintigraphy in early breast carcinoma for detection of lymph node metastases. Thus monoclonal antibodies to epitopes of human milk fat globule-type mucins may have clinical application in diagnosis and staging of breast carcinoma.

T 114 NEURONAL CELL DIFFERENTIATION AND ANTIGEN MODULATION OF HUMAN NEUROBLASTOMA AND GLIOBLASTOMA CELLS BY RETINOIC ACID PLUS HERBIMYCIN-A. Peter N. Preis[1,2], Guenther Hochhaus[1], Hideyuki Saya[1], Werner Scheithauer,[2] Victor Levin[1], and Wolfgang Sadee[1]; [1]University of California San Francisco, CA 94143; [2]Vienna University, 2nd Department of Internal Medicine, A-1090 Vienna, Austria.
We investigated the effect of herbimycin-A (herb-A) and retinoic acid (RA), given alone or in combination on cell growth, cell differentiation and colony formation of two human neuroblastoma cell lines, SK-N-SH and SH-SY5Y, and two glioma cell lines, U-87MG and U-251MG. Cell differentiation was evaluated by determining changes in cell morphology and expression of the μ opioid receptors in the neuroblastoma cell lines, and by investigating the stress fiber arrangement and expression of the glial fibrillary acidic protein (GFAP) in the glioma lines. In both SK-N-SH and SH-SY5Y, we observed cytostasis and the selection of a differentiated neuronal cell type upon incubation with a combination of low concentrations of herb-A (236 nM) and RA (10μM). Selection of the neuronal phenotype was rapid and near complete after a 3-5 day incubation period. Longer incubation led also to a complete suppression of colony formation. In parallel with neuronal maturation, the μ opioid receptors resent in both neuroblastoma cell lines were up-regulated three-fold by the combination of RA and herb-A. RA and herb-A induced less morphological differentiation and growth inhibition in the glioma cell lines. The striking effects of herb-A and RA on neuroblastoma cells warrant further study of their therapeutic potential against neuroblastoma.

T 115 NOVEL SQUAMOUS CELL CARCINOMA DIFFERENTIATION ANTIGENS RECOGNIZED BY MAB 174H.64
John Samuel and B.M. Longenecker, University of Alberta, Edmonton, AB T6G 2H7

We have developed a MAb (174H.64.R24) which selectively recognizes certain molecular markers associated with squamous cell carcinoma (SCC). Histopathological studies of the frozen tissue sections demonstrated selective binding of this antibody to SCCs of human, bovine and murine origin. Tumors of other histological types did not show antibody binding. These studies also demonstrated the preferential binding of the antibody to the perpheral layer of the cancer tissues consisting of the stem cell population of the tumor. Studies on normal tissues demonstrated selective binding of the antibody to the epidermal basal layer of human, bovine and murine skin. Adjacent layers of the epidermis did not show antibody binding suggesting that the antigens are lost during the differentiation of the epidermal cells. The SCC antigens detected by 174H.64.R24 were characterized as proteins with molecular weights 245, 135 and 125kD from a bovine SSC tissue using western blotting technique. A murine lung SCC model was developed using the murine SCC cell line KLN-205. These cells did not demonstrate antibody binding when grown in vitro, whereas the lung tumor obtained after an in vivo passage in DBA/2 mice showed binding with the MAb. KLN-205 cells derived from fresh lung tumor when grown in vitro continue to express the antigens for one week and thus offer an in vitro model for further studies. The antigens described by MAb 174H.64 would serve as useful markers for studying the stem cell populations of normal epidermis and squamous cell carcinoma.

T 116 PURIFICATION AND CHARACTERIZATION OF A HUMAN MELANOMA CELL SURFACE ANTIGEN DEFINED BY A HUMAN MONOCLONAL ANTIBODY
Dirk Schadendorf, Hiroshi Yamaguchi, Lloyd J Old & Pramod K Srivastava, Samuel Freeman Lab,Sloan-Kettering Institute, New York,NY 10021

Human monoclonal antibodies have been generated that identify glycolipid and glycoprotein antigens on the surface of melanoma cells (1-3). One such antigen, initially identified by a monoclonal antibody DSM1 derived from lymphocytes of a cancer patient immunized with autologous and allogeneic melanoma cells, is expressed predominantly on tumors of neuroectodermal origin (11/14 melanomas, 6/8 astrocytomas). This antigen has been purified by sequential application of lectin affinity, ion exchange and Mono P FPLC chromatography. It is present in plasma membrane as well as cytosol of tumor cells. Detergent solubilization experiments indicate the existence of an aqueous soluble form, a detergent soluble form and a detergent insoluble form of the antigen. Western blotting of antigen extracts shows size heterogeneity in the membrane (60-65 kd) and cytosolic forms (52-62 kd) of the antigen. This may be due to differences in carbohydrate moieties on the protein, but its precise structural basis is presently unclear. Aminoacid composition analysis of a homogenous preparation reveals relative abundance of aspartic acid/asparagine and serine and threonine residues and an absence of methionine residues. This is the first instance of purification and characterization of a glycoprotein antigen defined by a human monoclonal antibody.

(1) Houghton AN,Brooks H,Cote,R,Taormina MC,Oettgen HF & Old LJ (1983) J Exp Med 158,53. (2) Tai T,Paulson JC,Cahan LD & Irie RF (1983) PNAS USA 80,5392.(3) Yamaguchi H,Furukawa K, Fortunato S,Livingston PO,Lloyd KO,Oettgen HF & Old LJ (1987) PNAS USA 84, 2416.

T 117 PURIFICATION AND COMPOSITION OF HUMAN TUMOR-ASSOCIATED GLYCOPROTEIN (TAG-72) DEFINED BY MONOCLONAL ANTIBODIES CC49 AND B72.3,
D.G. Sheer, J. Schlom, and H.L. Cooper, National Cancer Institute, Bethesda, MD 20892.

Human mammary and other carcinoma cells secrete and express on their cell surfaces complex, mucin-like glycoproteins ($M_r > 10^6$) that are recognized as tumor-associated antigens by a variety of monoclonal antibodies. Monoclonal antibodies CC49 and B72.3, which recognize non-identical epitopes on the human carcinoma-associated antigen TAG-72, were used to develop a procedure to yield large amounts of purified antigen suitable for analyzing amino acid sequence. Xenografts of LS174T cells (a human colon carcinoma cell line) grown in nude mice were solubilized, extracted with several chaotropic agents and treated with perchloric acid. The acid soluble antigen was subjected to CC49 affinity chromatography, gel filtration and ion exchange chromatography using FPLC and HPLC methodologies. A B72.3/CC49 double determinant assay showed greater than a 1000 fold purification. Radiolabeled protein on SDS:PAGE demonstrated an apparently homogeneous high molecular weight mucin and an additional protein with an apparent M_r of 63000 under reducing conditions and 50000 under nonreducing conditions. Chemical deglycosylation using trifluoromethanesulfonic acid yielded low molecular weight proteins, which could be analyzed for amino acid sequence, and also yielded a number of discrete hydrophobic peptides after trypsinization. The purified mucin and the 63kDa protein revealed amino acid compositions similar to other purified mucins, and to reported linker proteins of mucins, respectively.

T 121 CYTOTOXICITY WITH BISPECIFIC MONOCLONAL ANTIBODIES REACTIVE WITH KIDNEY TUMOR AND CD3. J. van Dijk[1], R.J. van de Griend[1], R.L.H. Bolhuis[2], G.J. Fleuren[1], S.O. Warnaar[1], [1]Department of Pathology, State University Leiden, [2]Rotterdam Radio-Therapeutic Institute, The Netherlands.
Recently we described monoclonal antibodies (mAbs) that recognize renal cell carcinoma (ROC). One of these mAbs, G250, reacts with 95 % of primary and with 88 % of metastatic ROC in immunohistochemistry, but not with normal kidney tissue. To investigate the possible usefulness of G250 antibody in immunotherapy, bispecific antibodies were produced by fusing anti-CD3 (γ2a) producing hybridoma with variants of the hybridoma G250 producing immunoglobulins (Ig) with different isotypes specific for human kidney tumor. Ig producing hybridomas and those positive in immunohistochemistry on kidney tumor and spleen for production of bispecific mAb were selected. In cytotoxicity assays ROC were used as target cells and clones TCRαβ or TCRγδCD3$^+$ lymphocytes as lytic effector cells. The various bispecific antibodies produced, the γ1-γ2a, γ2a-γ2a, and γ2b-γ2a combination of G250 and anti-CD3, all induced target cell lysis by all types of T lymphocytes.
These bispecific mAbs may therefore proof effective in immunotherapy by retargeting selected effector cell populations.

T 122 ANTIBODY RECOGNITION OF THE TUMOR SPECIFIC BCR-ABL JOINING REGION IN CHRONIC MYELOID LEUKEMIA, Janneke van Denderen, André B.C. Hermans, Toon Meeuwsen, Netty Zegers, Wim J.A. Boersma, Gerard Grosveld and Willem van Ewijk. Dept. Cell Biology, Immunology and Genetics, Erasmus University, Rotterdam, The Netherlands.
In the leukemic cells of 96% of all CML patients the Ph´ chromosome is present. The Ph´ is formed by a reciprocal translocation between chr 9 and chr 22. The c-abl oncogene has moved from chr 9 into the breakpoint cluster region (bcr), within the bcr gene on chr 22, resulting in a chimeric bcr-abl gene. The fused gene encodes a 8.5 kb chimeric mRNA which is translated into a 210 kD protein. The breakpoint in the bcr gene occurs either between bcr exon 2 (b2) and 3 (b3), or alternatively between bcr exons 3 (b3) and 4 (b4). Therefore in the mature bcr-abl mRNA either b2 or b3 is spliced to abl exon a2, which results in two alternative $P210^{bcr-abl}$ proteins, comprising either the b2-a2 or the b3-a2 junction. As such, the two different amino acid sequences at the point of the junction represent tumorspecific determinants. Based on the nucleotide sequence we synthesized a peptide consisting of 10 amino acids spanning the b2-a2 junction. Rabbits were immunized with this peptide. From one rabbit we isolated an antiserum, (BP-1), which reacts in a very specific way with the b2-a2 junction in the b2-a2 $P210^{bcr-abl}$. B3-a2 $P210^{bcr-abl}$ is not recognized by BP-1. Antisera raised according this strategy, directed against recombination sequences, may further aid in the clinical diagnosis of CML and in the distinction of the various malignant disorders, where bcr-abl rearrangements are involved.

T 123 IMMUNE RESPONSES OF PANCREATIC CANCER PATIENTS RECEIVING ACTIVE IMMUNOTHERAPY, Zeinab Wahab, H.F. Seigler and R.S. Metzgar, Duke Univ. Med. Ctr., Durham,NC 27710.
A Phase I active immunotherapy trial for pancreatic adenocarcinoma patients has been initiated at the Duke University Medical Center. The protocol focuses on vaccination with tissue cultured, irradiated allogeneic pancreatic tumor cells (HPAF) grown on human serum. The line lacks Class II HLA antigens but expresses Class I antigens. The scientific objectives of the trial are to evaluate the immunogenecity of HPAF cells in man and to characterize and isolate the immunogenic molecules. Although 4 patients have thus far been vaccinated, only 2 have received at least 5 injections of the cells over a 3 month period. Cell mediated cytotoxic and proliferative immune responses could be demonstrated in these 2 patients, but we could not easily resolve whether these cellular responses were directed to Class I antigens. Antibody responses could be demonstrated by indirect immunofluorescence and by a Western blotting technique. Both patients receiving 5 or more injections reacted specifically with a 30 KD molecule by Western blot analysis with a lysate of HPAF and other xenografted pancreatic nude mouse tumors. This band was not noted with comparable melanoma xenografted tumor lysates. Pre-immune sera from these patients and normal sera failed to detect this 30 KD molecule. Human polyclonal antibodies reacting with this molecule were affinity purified from nitrocellulose blots and were shown to remain reactive with HPAF cells by immunofluorescence. The specificity and molecular properties of the 30 KD antigen are currently being defined. Our future clinical goals are to augment the immune response generated by the active immunotherapy with HPAF cells by pulsed administration of IL-2 and possibly other lymphokines.

T 118 CLONING AND EXPRESSION OF cDNA CLONES ENCODING A MAJOR HUMAN ADENOCARCINOMA/-EPITHELIAL CELL SURFACE ANTIGEN, J. Strnad, A. E. Hamilton, L. S. Beavers, G. Gamboa, R. J. Schmidt, L. D. Apelgren, J. R. Sportsman, T. F. Bumol, J. D. Sharp, and R. A. Gadski, Lilly Research Labs, Eli Lilly and Co., Indianapolis, IN 46285.
The use of monoclonal antibodies (MoAbs) for site directed drug therapy is now under intense worldwide clinical evaluation. We are investigating the use of the murine MoAb KS1/4 which defines a 40,000 dalton surface glycoprotein antigen (KSA) found on adenocarcinoma tumor cells and a subset of normal epithelial cells. Here we describe the cloning and sequencing of overlapping cDNA clones which encode the antigen as expressed in P3-UCLA, a human adenocarcinoma cell line. KSA is synthesized as a 314 residue (34,922 dalton) preproprotein that is then processed to a 233 residue (26,340 dalton) surface protein. KSA appears to have a single transmembrane domain of 23 residues that separates the highly polar 26 residue cytoplasmic domain from the extracellular domain. The N-terminal region of the propeptide is rich in cysteines and contains three potential N-glycosylation sites. Computer assisted analyses at both the DNA and protein levels have found no significant similarities of this antigen to known sequences, but a GC-rich 5' terminus is evident. Northern analysis shows that transcription of KSA can be detected in RNA isolated from normal colon but not in RNA isolated from normal lung, prostate, or liver. Truncated forms of KSA have been expressed to determine the biological significance of this surface glycoprotein.

T 119 MEASUREMENT OF *IN VITRO* GLYCOSPHINGOLIPID SYNTHESIS AND DEGRADATION WITH CARBOHYDRATE-SEQUENCE SPECIFIC MONOCLONAL ANTIBODIES, Cheryl L.M. Stults, Bruce J. Wilbur and Bruce A. Macher, Department of Chemistry/Biochemistry, San Francisco State University, San Francisco, CA 94132.
Several human tumor antigens recognized by monoclonal antibodies have been identified as glycosphingolipids. The level of expression of the antigens depends on the activities of specific glycosyl transferases and glycosyl hydrolases. To monitor the activities of these enzymes an enzyme-linked immunosorbent assay (ELISA) technique has been developed. Glycosphingolipids were immobilized in microtiter wells and treated with either α-galactosidase or β-galactosyltransferase. Product formation was determined with carbohydrate-sequence specific monoclonal antibodies and an avidin-biotin enzyme system (ABC reagents). This procedure has several advantages over conventional methods: product identification is much simpler and is done on each sample; detergents are not required; small amounts of reagents are consumed; results can be obtained within a few hours; and no radioisotopes are required. This assay provides a convenient way to monitor the progress of glycosphingolipid metabolizing enzyme reactions. These studies were supported by grants CA 32826 and GM 40205.

T 120 A 140kDa CELL SURFACE GLYCOPROTEIN FROM PANCREATIC CANCER CELLS WITH N- AND O-SULFATE ESTERS ON ASPARAGINE-LINKED GLYCANS
Göran Sundblad*, Shama Kajiji, Vito Quaranta, Hudson Freeze and Ajit Varki
UCSD Cancer Center and Scripps Clinic and Research Foundation, La Jolla, CA.
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We have described two new classes of sulfated N-linked oligosaccharides (GlcNAc-Asn; complex-type) from total cellular $^{35}SO_4$-labelled molecules of different mammalian cell lines. The first class carries sialic acids and 6-O-sulfate esters, while the second more highly charged class carries heparin/heparansulfate-like sequences. We have now characterized a sulfo-phosphoglycoprotein of 140kDa from human FG-met-2 pancreatic cancer cells whose glycans share properties of both these classes. The molecule was localized to the cell surface by electron microscopy using a mouse monoclonal antibody (S3-53) and by cell surface iodination. Digestion with Peptide: N-glycosidase F (PNGaseF) indicated a minimum of 4 N-linked glycosylation sites. PNGaseF released more than 90% of the (^{3}H)$GlcNH_2$-label and 40-70% of $^{35}SO_4$-label from the immunoprecipitated 140kDa molecule. The labelled chains were partially susceptible to both neuraminidase and to heparin/heparan degrading enzymes. Nitrous acid treatment showed that both N- and O-sulfate esters were present. Thus, this 140kDa glycoprotein contains at least 4 asparagine-linked chains substituted with a heterogeneous mixture of sulfated sequences, including some that share properties with heparin/heparan sulfate.

T 124 ESTABLISHMENT OF A MOUSE T LYMPHOCYTIC LEUKEMIC CELL CLONE (LAC-1) AND ITS BIOLOGICAL CHARACTERISTICS, Chen Wei, He Qiuzao, Xie Qi, Zhu Yidong, Dept. of Immunology, Shanghai Medical University, Shanghai, PRC.
A mouse T lymphocytic leukemic cell clone (LAC-1) was established from the L783 mouse leukemia by means of limiting dilution method. The LAC-1 clone has been continuously propagated in culture for 3 years, and gave the following biological characteristics: (1). Electrophoretic behavior studies indicated that LAC-1 cells migrated at a fast mobility rate of 1.50 ± 0.19 (μ/s/v/cm) and displayed only one peak. (2). Surface property and phenotype detection showed they were Thy1.1$^+$, Thy1.2$^-$, Lyt1$^-$, Lyt2$^-$, SmIg$^-$, and C_3R^- leukemic T cells, resembling early thymocyte precursors. (3). Proliferation response of LAC-1 cells in exponential growth rate could be found when cell culture were supplemented with IL-2, and a characteristic dose-dependance of IL-2 could also be demonstrated. (4). The cells multiplied 100 times within 6 days and the doubling time were 15.25 hours. The mitotic indexes attained its peak on day 2 (58‰). (5). Morphologically, typical lymphocytic leukemic cell characteristics were shown by light and electromicroscope. (6). The chromosome num. of LAC-1 cells were hyperdiploid with a modal number of 41. (7). MuLV particals were observed in the cytoplasm of LAC-1 cells by electromicroscope. X-C assay and reverse transcreptase activity ditection were both positive. (8). LAC-1 cells retained carcinogenicity in syngeneic SW-1 mice and nude mice, but transplantation of LAC-1 cells to other various H-2 type mouse strains resulted in complete failure.

T 125 GLYCOLIPID CHANGES ASSOCIATED WITH DIFFERENTIATION OF B CELL LINES. Joëlle Wiels, Cécile Tétaud and Thomas Tursz. Institut G. Roussy, 94805 Villejuif Cedex, France.
Burkitt's lymphoma (BL) is a B cell proliferative disorder, associated with Epstein-Barr Virus (EBV) and characterized by a chromosomal translocation. Tentative assignement of BL cells in the pathway of normal B cell differentiation has been widely discussed. We have previously shown that BL cells are characterized by accumulation of a specific glycosphyngolipid (GSL): globotriaosyl-ceramide (Gb3). This GSL is not expressed by lymphoblastoid cell lines (LCL) obtained by EBV infection of normal B lymphocytes, which are supposed to be more mature than BL cells. As it has been demonstrated that carbohydrate chains of GSL undergo rapid and dramatic changes during development and differentiation, we have studied GSL from different B cell lines along with B cell differentiation markers. All BL cells express predominantly Gb3. LCL derived from peripheral B lymphocytes have a very low level of Gb3 and large amount of globoside; they also express gangliosides, mainly GM3. In contrast LCL obtained from tonsil B cells do contain Gb3 as well as globoside and GM3. After treatment of BL cells with TPA which induces differentiation (attested by several B cell antigens), Gb3 decreases, synthesis of globoside is newly induced and synthesis of the ganglioseries GSL is significantly enhanced. These results suggest that glycosphyngolipids could represent new markers for B cell differentiation.

TAA as Diagnostic or Clinical Management Aids; Cytotoxic T Cells to Tumor Specific Antigens

T 200 AUGMENTATION OF CYTOLYTIC ACTIVITY OF PERIPHERAL BLOOD LYMPHOCYTES OF VACCINIA MELANOMA ONCOLYSATE (VMO) TREATED MELANOMA PATIENTS IN THE PRESENCE OF AUTOLOGOUS ANTIBODY AND INTERLEUKIN-2. Jerry A. Bash, Evelyn S. Darnell and Marc K. Wallack, Mount Sinai Medical Center, Miami Beach, Florida 33140.

Melanoma patients treated with VMO have been shown to have augmented antibody responses to melanoma-associated antigens at the same time that cell-mediated responses are often diminished. The importance of antibody in clinical responses is inferred by the association of improved clinical course in conjunction with increased IgG antibody as detected in an ELISA using melanoma target cells. Likewise, reactivity of patient serum with autologous melanoma cells has been demonstrated by immunoperoxidase staining. Lysis of melanoma cells by fresh antibody-containing sera has not been observed, whereas the addition of normal peripheral blood lymphocytes results in augmented lymphocyte-dependent antibody (LDA) mediated killing. Although direct cytolysis of melanoma targets by patient lymphocytes (CTL) could be achieved after restimulation with melanoma cells in vitro, this required addition of exogenous interleukin-2 (rIL-2, Hoffmann-LaRoche). The most potent cytolytic effect was achieved in the presence of antibody and rIL-2 activated effector (LAK) cells. LAK activity and LDA activity were additive at low E:T ratios and could be further differentiated on the basis of kinetics and rIL-2 concentration. These in vitro results suggest that melanoma patients with circulating anti-melanoma antibody and deficient cellular immunity might be benefited by low-dose interleukin-2 treatment to enhance LDA activity.

T 201 A HUMAN MONOCLONAL IgM ANTIBODY REACTIVE WITH ANTIGEN ASSOCIATED TO ADENOCARCINOMAS, Per Borup-Christensen, Karin Erb, Jens Chr. Jensenius and Bjarne Nielsen, Institute of Surgery/Biomedical Laboratory, Institute of Medical Microbiology and Institute of Pathology, Odense University, 5000 Odense C, Denmark.

A human IgM antibody (B9165) developed in our laboratory reacts with an antigen located on human tumor of preferentially endodermally derived epithelial tissues such as colo-rectal cancer, mammary cancer and ovarian cancer while binding to normal tissues were restricted to mammary and prostate epithelium. Electron miscrscopy of colon cancer tissue showed that B9165 reacts with molecules associated with intermediate filaments, while no binding to normal colon epithelium was observed. Incubation on live colon cancer cells showed that some of the antigens might be located on the cell surfae. The antigen has molecular weight of 43 K judged by Western-blotting of colon cancer extract and has a pI of 5.4-6.2 judged by iso-electric focussing. Based on these results B9165 is a candidate for the investigations concerning the ability to localize tumors in patients with adenocarcinomas.

T 202 POTENTIATION OF HUMAN MELANOMA VACCINE: RELATION BETWEEN METHOD OF IMMUNIZATION, IMMUNOGENICITY, AND TUMOR PROGRESSION: J.C. Bystryn, M. Dugan, R. Oratz, J. Speyer, D.F. Roses, M.N. Harris, N.Y.U. School of Medicine, New York, N.Y. 10016.

To examine whether the immunogenicity of a polyvalent, purified, allogeneic human melanoma vaccine can be potentiated; 53 pt with resected Stage II melanoma were entered sequentially into several immunization protocols (differring in schedule, adjuvant, or physical properties of vaccine). IgG antibodies and cellular response to melanoma were measured prior to and 6-10 weeks following onset of immunization. The immunization procedures which most frequently potentiated antibody responses induced cellular responses less often, and vice-versa. Recurrences were less common and disease free interval was prolonged in patients with a cellular as opposed to an antibody response to immunization .

IMMUNE RESPONSE	NO PTS	NO (%) WITH RECURRENCE	DISEASE-FREE INTERVAL
cellular (a)	20	8 (40%)	120+wks
antibody (a)	7	6 (86%)	39 wks
none	29	13 (45%)	65+wks

(a) includes 3 pts with both types of response

These results suggest that the type of immune response induced by melanoma vaccines is influenced by the method of immunization, that potentiation of cellular as opposed to IgG antibody responses may slow the progression of melanoma, and that consequently, the development of effective melanoma vaccines should be guided by their ability to preferentially augment cellular immunity to melanoma.

T 203 MECHANISMS OF ACTION OF SUPPRESSOR CELLS FROM METASTATIC LYMPH NODES
Federico Cozzolino, Maria Torcia, Donatella Aldinucci, Anna Rubartelli, IV Dept. Intern. Med., University of Firenze, Italy.
It has previously been shown that invaded lymph nodes from patients with larynx or bladder cancer contain T lymphocytes that can be induced to proliferate specifically in response to autologous malignant cells, following addition of exogenous IL-2. A positive response requires, however, that a subset of suppressor T cells with a CD3 CD8 CD11 Leu-7+ surface phenotype is removed from the suspension. Using these culture conditions, cytotoxic T cell lines against autologous malignant cells were obtained. In the present study, we investigated the mechanisms of action of suppressor cells. We found that these cells exert their action mainly through the release of soluble factors that inhibit both the proliferative response and the maturation of CTL induced by IL-2. The adsorption of suppressor cell supernatants with Sepharose coupled to anti-Tac MoAb removes most of the suppression. Gel filtration of supernatants shows a peak of suppression in the MW range between 55 and 75 kD. Furthermore, suppressor cells, that do not proliferate in response to IL-2, bind and internalize radiolabeled IL-2, although ten times less than PHA-activated peripheral blood T cells. From these results, we conclude that in invaded lymph nodes the specific immune response against malignant cells is possibly modulated by suppressor cells through the release of soluble IL-2 receptors and, to a much lesser extent, through the internalization of endogenous IL-2.

T 204 HUMAN MONOCLONAL ANTIBODIES REACTING WITH CANCER ASSOCIATED ANTIGENS,
Karin Erb, Per Borup-Christensen, Jens Chr. Jensenius and Bjarne Nielsen, Institute of Medical Microbiology, Institute of Surgery/Biomedical Laboratory and Institute of Pathology, Odense University, 5000 Odense C, Denmark.
Lymphocytes from lymph nodes draining the tumor region in patients with colo-rectal cancer were fused with two different human B-lymphoblastoid cell lines, LICR-LON-HMy-2 and WI-L2-729-HF2 to generate hybridomas synthesizing antibodies reacting with tumor-associated antigens. In this way 220 hybridomas were obtained which produced antibody reacting with colon cancer. All established clones produced IgM. Five of the human monoclonal antibodies have been further analyzed. Four of these (G4146, B9165, D4213 and F10279) showed binding to cancer-associated antigens by immunocytochemical analysis on different cancer cell lines and normal human leucocytes and by immunohistochemical analysis on sections of frozen malignant and normal tissues, while the fifth (F11348) showed reaction with all cells and tissues tested. Western blots of tumor extracts showed binding of G4146 to two components from colon cancer cells with M_r of 59K and 61K, while B9165 bound to a 43K component and F11348 to several components with M_r from 30 to 200K. D4213 and F10279 showed no binding in this analysis. The result obtained demonstrate the successful application of the hybridoma technology for producing human monoclonals with reactivity to tumor-associated antigens.

T 205 MONOCLONAL ANTIBODY DEFINED ANTIGENS CA-195 AND CA19.9 IN COLON CANCER: COMPARISON WITH CEA.
M.K. Gupta, R. Arciaga, R.M. Bukowski, and P.K. Gaur, The Cleveland Clinic Foundation, Cleveland, OH 44195 and Hybritech, Inc., San Diego, CA 92121
Monoclonal antibody CC3C195 has been shown to recognize sialyted human Lewis A blood group antigen just like the previously described antibody CA19.9. We have previously reported a good correlation of CA195 and CA19.9 in patients with pancreatic cancer. Here we compare CA195 with CA19.9 and CEA in colon cancer patients. These markers were measured by immunoradiometric assays in a total of 193 patients which included 69 normal controls, 44 patients with benign colon and GI diseases and 80 patients with colon cancer (Duke A = 3, B = 25, C = 27 and D = 25). A cut off of 15 u/ml for CA195, 37 u/ml for CA19.9 and 5 ng/ml for CEA was used. The diagnostic sensitivities for these assays were 36%, 35% and 43.7% respectively. Elevation of CA195 and 19.9 was more frequently seen in Duke C, where sensitivities of these markers were 37%, 33% and 33% respectively. Also at this stage, addition of either marker CA195 or CA19.9 to CEA significantly enhanced the sensitivity and detection of disease (from 33% with CEA alone to 59% when CEA combined with CA195 or CA19.9). Use of all three markers simultaneously gave the sensitivity of 66%. In contrast, CEA was more frequently elevated than CA195 or CA19.9. when metastatic disease was present, (Duke D, 76% for CEA vs. 56%, for CA195 and 52% for CA19.9). This data suggested the possibility of early elevation of CA195 than CEA in colon cancer. This was further confirmed in 7 patients with colon cancer who were monitored with serial measurement of CEA and CA195. In general, CA195 showed the same trend as CEA with disease progression and regression in these patients. However two patients with progressive disease, who had frequent (monthly) measurements of these markers, CA195 elevation was seen 2 and 3 months prior to elevation of CEA (CA19.9 was only elevated in 1 patient concurrently with CA195). In conclusion, our results suggest the measurement of CA195 may add important lead time in detection of recurrent and progressive disease.

T 206 CORRELATION BETWEEN SERUM TUMOR ASSOCIATED ANTIGEN AND THYMIDINE KINASE LEVELS AND TUMOR PROLIFERATIVE ACTIVITY IN LYMPHOMAS, LUNG CANCER AND BREAST CANCER

'Matti Lehtinen, [2]Tenho Hietanen, [3]Olli-Pekka Kallioniemi,'Ritva-Kaarina Aaran, [2]Tuija Wigren, '[2]Tuula Lehtinen, and [2]Antti Ojala,'Dept. of Biomedical Sciences, University of Tampere, Depts. of[2]Clinical Oncology and[3]Clinical Chemistry, University Central Hospital, Tampere SF-33101, Finland

Concomitantly taken pretreatment serum samples and tumor biopsies were analyzed by RIA and DNA-flow cytometry in 50 newly diagnosed cancer patients. Twenty-two patients with small cell lung cancer (SCLC, 10 limited and 12 extensive cases) had the highest mean S_2-phase cell fraction (SPF) 16.2% (range 6.3 to 23.4); the mean SPF for 12 patients with advanced breast cancer (BCA) was 11.5% (range 3.3 to 22.6), while the lowest mean SPF of 6.5% (range 1.4 to 17.2) was seen among 16 non-Hodgkin lymphoma (NHL) patients. Correlation between serum thymidine kinase (TK) levels and tumor SPF was good in the NHL patients only ($r=0.52$, $p\leq 0.1$). The tumor specified tumor associated antigens (TAA), neuron specific enolase (NSE) in SCLC, CA 15-3 in BCA and B_2-microglobulin in NHL showed moderate to low correlation with the tumor SPF ($r\approx 0.41$, $r=-0.18$, $r\approx 0.16$, respectively). However, in 12 extensive SCLC cases a statistically significant correlation between serum NSE and the primary tumor SPF was seen ($r=0.77$, $p\leq 0.05$). It appears that factors other than the proliferative activity of the tumor also determine the serum levels of TAA:s in patients with solid tumors. Tumor mass but also tumor progression e.g. bone marrow involvement are plausible determinants.

T 207 A NOVEL RNA-PROTEIN COMPLEX DEFINED BY MONOCLONAL ANTIBODIES TO NIH 3T3 CELLS TRANSFORMED BY TRANSFECTION WITH DNA FROM A HUMAN PANCREATIC ADENOCARCINOMA LINE, M.A. Hollingsworth, M.L. Everett and R.S. Metzgar, Duke Univ. Med. Ctr., Durham, NC 27710.

In an attempt to identify novel transformation related antigens of human pancreatic adenocarcinoma, we have raised monoclonal antibodies to NIH 3T3 cells that have been transformed by transfection with DNA from the human pancreatic adenocarcinoma cell line HPAF. Ten monoclonal antibodies have been obtained which recognize at least 4 antigens that are expressed on HPAF cells and HPAF-transfected NIH 3T3 but are not expressed on non-transfected or spontaneously transformed NIH 3T3 cells. One antigen (S1D11) recognized by three of the antibodies, is an RNA protein complex. Radioimmunoprecipitates from HPAF cells reveal two RNA species with approximate sizes of 1.5 and 3.0 kb and at least one protein greater than 200 K.D. The complex can be immunoprecipitated from supernatants of HPAF cells where it appears within 5 minutes of adding radiolabel to the cells during pulse-chase experiments. Treatment of cells with Monensin inhibited the appearance of the complex in supernatants. The antibodies will not immunoprecipitate purified HPAF RNA, nor do they bind to purified RNA immobilized on nitrocellulose. The large protein (>200 KD) can be specifically eluted from immunoaffinity columns suggesting that the epitope(s) recognized by these antibodies are on the protein moiety of this ribonucleoprotein complex. The antigen shows diffuse cytoplasmic and focal nuclear staining by immunofluorescence. Preliminary experiments suggest that the antibodies to this antigen can inhibit DNA replication in isolated HPAF nuclei and that this inhibition can be reversed when immunoaffinity purified antigen is added to the nuclei-antibody mixture.

T 208 KILLING SQUAMOUS CELL CARCINOMA IN VITRO VIA ANTIBODY-DEPENDENT CELL CYTOTOXICITY USING HUMAN CELLS AND ANTIBODY, Wayne M. Kocn and William J. Richtsmeier, Johns Hopkins School of Medicine, Baltimore, MD 21205.

We have developed a reference killer assay using standard ^{51}Cr release technique and employing human antibody and effector cells against cultured human squamous cell carcinoma (SCC) targets. Sera from several patients with pemphigus vulgaris served as the source of polyclonal human antibodies for antibody-dependent cell cytotoxicity (ADCC). One of two cell lines established from SCC of the upper aerodigestive tract was incubated with killer cells and the serum. Normal human serum was used as a negative control. Killer cells were obtained from peripheral blood leukocytes of normal human donors and activity was tested with and without prior stimulation by interleukin 2 (IL-2). Antibodies from three patients showed no activity, while those of three others enhanced cytolysis by 10 to 50% over the IL-2 stimulated cells alone. Human cell lines which do not express pemphigus antigen were not affected by the addition of serum to the killer assay. In an effort to explain this range of activity the sera were analyzed for antibody titer and IgG subclasses. High levels of ADCC correlated with strong binding of antibodies of the IgG2 isotype. ADCC may be one way in which the immune system responds to SCC. Our system demonstrates that effective tumor cell killing is possible using appropriate human leukocytes and serum. By testing material obtained from an individual patient, it has the capability to identify which component(s) is responsible for ineffective tumor surveillance.

T 209 A RADIOIMMUNOASSAY FOR CA 125 EMPLOYING TWO NEW MONOCLONAL ANTIBODIES, M. J. Krantz[1], G. MacLean[2], B. M. Longenecker[3] and M. R. Suresh[1], [1]Biomira Inc., [2]Cross Cancer Institute, and [3]Dept. of Immunology - Univ. of Alberta, Edmonton, Alberta T6N 1E5, CANADA.

Mice were immunized with a partially purified mucin fraction containing high levels of the CA 125 antigen, that was obtained from an ovarian cancer patient. Monoclonal antibodies were generated by standard protocols, and screened by ELISA for reactivity with the immunogen. A number of the selected antibodies were able to inhibit a commercially available assay for CA 125, and two of these, B27.1 and B43.13, were further characterized. Both antibodies were of the IgG1 subclass, and both could be purified in one step from ascites fluid by HPLC on Bakerbond ABx™. The previously described anti-CA 125 antibody, OC 125, recognizes a repeated epitope and is used in a homologous sandwich format. In contrast, the two new antibodies gave very weak radioimmunoassay signals in the homologous sandwich format, suggesting that the corresponding epitopes are non-repeated. Blocking experiments showed that B27.1 and B43.13 recognize different sites on the ovarian cancer associated mucin molecule. A heterologous sandwich radioimmunoassay was developed, using B27.1 as solid phase and B43.13 as tracer. A group of ovarian cancer sera were tested in this assay, and the results were compared to the levels of CA 125, determined in the commercial assay. A correlation coefficient of 0.96 was obtained. We conclude that B27.1 and B43.13 recognize unique non-repeated sites that are sterically near the repeated sites recognized by OC 125. The high degree of correlation in serum assays indicates that coexpression of the sites recognized by OC 125 and the two new antibodies is conserved on mucins secreted by different individual cancer patients. It further indicates that B27.1 and B43.13 will be useful for the determination of CA 125 antigen levels in the sera of ovarian cancer patients. In an initial study of serial serum samples obtained from ovarian cancer patients, CA 125 levels determined in the new assay corresponded well with subsequent progression or regression of disease.

T 210 ANALYSIS OF CLONES AND LINES GENERATED FROM TUMOR-DERIVED ACTIVATED LYMPHOCYTES (TDAL). James R. Maleckar, Colleen S. Desch, and John R. Yannelli. Biotherapeutics, Inc, Franklin, TN 37064.

TDAL have recently been used as a source of biotherapy for cancer patients. This study focused on growth, phenotype, lytic activity, and methods for expanding TDAL while maintaining their ability to kill autologous tumor cells. In 20/20 cases lymphocytes have been grown from tumor sources, including melanoma, renal cell carcinoma, and lymphoma. T lymphocytes comprised greater than 90% of each population and, in about half the cases, lytic activity against the autologous tumor cells was observed at one month. Lysis of non-autologous tumor cells was not seen. Cultures were initiated with finely minced tumor chunks in media containing 10% human serum and 1000 u/ml rIL2. In some experiments, cultures were also supplemented with conditioned media from lymphocyte activated killer (LAK) cell cultures. In several cases, killing and growth were maintained for several months with antigen restimulation. In addition, in 3/3 cases, lytic activity and growth could be recovered after cryopreservation if cultures were restimulated with the antigen. Using limiting dilution procedures, clones were derived from a lymphoma specimens one week after the culture had been initiated. These clones were grown in the presence of LAK cell conditioned media as a source of growth factors and additional rIL2 was not added. The clones were selected on their ability to lyse the autologous target. The successful development of techniques for growing TDAL while maintaining their specificity indicates that TDAL are a promising resource for the biotherapy of cancer.

T 211 PROGNOSTIC SIGNIFICANCE OF SPECIFIC ANTIBODIES DIRECTED TO GANGLIOSIDES CONTAINING N-GLYCOLYLNEURAMINIC ACID DURING IMMUNOTHERAPY OF HUMAN MELANOMA WITH VACCINIA-MELANOMA ONCOLYSATES (VMO), Jacques Portoukalian, Jean-François Doré and Eleuthère Leftheriotis, INSERM U.218 Centre Léon Bérard and Institut Mérieux, Lyon, France.

The presence of several gangliosides containing N-Glycolylneuraminic acid (NGNA) was revealed in human melanoma tumors and established cell lines by immunostaining on TLC plates with a specific chicken antisera. Since these compounds are known to be strongly immunogenic (H-D antigens) because of the N-Glycolyl replacing the usual N-Acetyl group on the sialic acid molecule, we tested the presence of related antibodies in the sera of melanoma patients, as compared to antibodies to other gangliosides of melanoma. Nearly all melanoma patients were found to have detectable antibodies to NGNA-containing gangliosides. These antibodies were mostly IgM, with low levels of IgG. Very few patients had antibodies to GD2 or GM2 , and none to GM3 and GD3 containing N-Acetyl neuraminic acid. Melanoma patients treated with VMO were monitored with an ELISA assay for the level of specific antiNGNA antibodies during the immunotherapy on a three-years period. It was shown that an early IgG response was correlated with a good prognosis, whereas an exclusive IgM response or a lack of response were found mostly in patients with further recurrences. Thus, the assay of antiNGNA antibodies during VMO treatment is a possible monitor of the stimulation of the immune system.

T 212 TARGETING MONOCLONAL ANTIBODIES TO MICROMETASTATIC CELLS IN COLON AND BREAST CANCER PATIENTS, Ilona Funke, Günter Schlimok*, Barbara Bock, Birgit Schweiberer and Gert Riethmüller, Dept. of Immunology, Universität München, Goethestr. 31, 8000 München 2, and *Zentralklinikum Augsburg.
Early dissemination of tumor cells from small primary carcinomas is the leading cause of the failure to cure many human cancers. In an attempt to better define the early stage of metastasis we have developed a double staining procedure which allows to unequivocally characterize single tumor cells in bone marrow. Together with an iodine labeled monoclonal antibody specific for cytokeratin restricted to simple epithelia, we defined additional cellular markers e.g. EGF receptor, myc protein, transferrin receptor and Ki 67 nuclear antigen with the help of alkaline phosphatase labeled antibodies. Tumorigenicity of cytokeratin+ tumor cells isolated from bone marrow was demonstrated by development of liver metastases from such cells after transplantation into nu/nu mice. In vivo labelling of carcinoma cells in bone marrow was shown in 5 of 10 patients after infusion of 500 mg of 17 1A antibody. Reproducible detection of cytokeratin positive cells was achieved during therapy with monoclonal antibody thus demonstrating the feasibility of monitoring immunotherapy directed to micrometastatic cancer cells.

T 213 INTRAVESICAL BCG: WHAT ARE THE KINETICS OF THE INFILTRATING LYMPHOCYTIC SUBSETS? Marvin Rubenstein, Michael Shaw, Veronica Ray, Paul Targonski, and Patrick D. Guinan, Chicago, Il.
Intravesical bacillus Calmette-Guerin (BCG) appears effective in managing superficial bladder cancer. The method of action of BCG and how it might differ from more conventional intravesical chemotherapy remains controversial. We have previously demonstrated that the predominant infiltrating cell in BCG treated bladders was the lymphocyte and that the predominant cell in Thiotepa treated bladders was the polymorphonucleated cell. In an effort to determine the specific lymphocytic response to BCG the bladders of rats treated intravesically with BCG were examined immunohistologically for markers identifying various lymphocyte subsets. Fifty-seven Copenhagen X Fischer rats treated with intravesical BCG were sacrificed at two week intervals and their bladders were examined employing monoclonal antibodies specific for Total T (W3/13), Helper T (W3/25) and Suppressor T cells (Ox8). The results indicated that there was a predominant T-helper lymphocyte response in BCG treated bladders and there also was an increase in the H/S ratio over time. This effect appears to peak at six weeks although the H/S ratio remained elevated. The results of this investigation could support an immunologically mediated mechanism for the favorable effect of BCG in superficial bladder cancer. They might also explain why, serendipitously, a six week induction is perhaps the most appropriate.

T 214 A FAST PROCEDURE FOR CONCOMITANT PURIFICATION OF PROSTATIC CARCINOMA TUMOR MARKERS (PAP AND PSA) FROM HUMAN SEMINAL FLUID, Dario Rusciano, Anna Berardi, Lorenzo Pacenti and Benedetto Terrana, Sclavo Research Center, Siena Italy.
Carcinoma of the prostate represents to date one of the most frequent causes of death from neoplasia in men over fifty years of age, and its importance is destined to rise, as the mean age of the population increases. The problem is aggravated by the fact that more than 50% of the patients are already affected by metastatic spread of the primary tumor at the time of diagnosis. The existence of tumor markers specific for this kind of neoplasia, i.e. Prostatic Acid Phosphatase (PAP) and Prostate Specific Antigen (PSA), represents a helpful clinical aid in the management of this disease. As the availability of highly purified protein antigens is essential for the development of diagnostic kits, many multistep protocols have been described so far to purify PAP and PSA from the prostate gland or from seminal fluid, all of which require several purification steps and result in a scanty recovery of the purified protein. We describe here a rapid and efficient purification procedure, which allows the concomitant purification of both PAP and PSA, and requires only two chromatographic steps.

T 215 HUMAN SQUAMOUS CELL CARCINOMA (SCC) TAA- AND ONCOGENE-EXPRESSION AND LOCALIZATION, Charles Shuler, Jens Hoellering and George E. Milo, The Ohio State University, Columbus, Ohio 43210.

Recently we have developed monoclonal antibodies to identify differentiative function and structure (Cancer Res. 45:239) of malignant (OSU 22-3) and normal (OSU EP-16) cells in SCC. Operationally several different tumor cell phenotypes have been identified by the patterns of immunohistochemical staining with monoclonal antibodies and in situ hybridization with oncogene cDNA probes. The distribution of cells positive for the different antibodies was nonuniform. Regional concentrations of positive cells were present that by microscopic examination were indistinguishable. The patterns of in situ hybridization also demonstrated intratumor variations. Intensive oncogene cDNA hybridization was detected in 11 of 15 tumors. High levels of both H-ras and c-myc were present in individual cells in tumor projections invading the underlying connective tissue. Keratin mRNA was identified in the tumor epithelial cells by in situ hybridization but all vector controls were nega- tive. Genomic DNA isolated from specific subpopulations of SCC was transfected into normal human keratinocytes. The transfected cells ultimately produced foci of morphologically altered cells that demonstrated an antigen pattern similar to the parental tumor cells. The distribution of cell populations in the tumor suggests possible relationships between oncogene expression, antigen presence and malignant potential. The spatial nonuniform distribution of cells in the tumors suggests that the different populations may exhibit differing biologic potentials. The present study permits subpopulations of the tumor to be identified and further characterized. Supported in part by NIH-NCI CA25907 and P30 CA16058.

T 216 EVALUATION OF LEWIS^X EPITOPE ASSOCIATED WITH A GLYCOPROTEIN IN THE SERA OF PATIENTS WITH ADENOCARCINOMA, Anil K. Singhal, Suzanne Nance and Sen-itiroh Hakomori, The Biomembrane Institute, and Departments of Pathobiology, Microbiology and Immunology, Univ. of Washington, Seattle, WA 98119.

Lewis^X(Lex) epitope with (galβ1 $\rightarrow$4 [Fucα1 $\rightarrow$3] glcNAc) determinant is expressed on glycolipids and high molecular weight glycoproteins in tumor tissues (1,2). The present study examined the presence of Lewis^X antigen in sera of normal and cancer patients. Sera were fractionated over a sephacryl 200 gel filtration column (2·5 x 90 cm) and the Le^X epitope was detected by monoclonal antibodies SH1 and SH2. Lewis^X antigen was present in the void volume fraction of the gel filtration column in 12/14 adenocarcinoma patients. Although the Le^X antigen was detected in the void volume fractions of 4/12 normal sera, its levels were significantly lower compared to cancer sera. Lewis^X activity appears to be present on a glycoprotein of approximately 200 KD as determined by immunoprecipitation with SH1, followed by gel electrophoresis. Lewis^X antigen quantitated by sandwich assay using MAbs SH1 and SH2 was present in 67% of adenocarcinoma patients whereas only 7% of normal sera were positive. This information will be helpful in developing diagnostic assays in cancer.

1 Hakomori et al. J. Biol. Chem. 259:4672 (1984)
2 Rettig et al. Cancer Res. 45:815 (1985)

T 217 CHARACTERIZATION OF THE SPONTANEOUS HUMAN ANTI-COLON CARCINOMA IMMUNE RESPONSE AT THE T CELL LEVEL, M.Slaoui[x], C.Bruyns[x], T.Schuurs[°] and J.Urbain[x],[x]Laboratoire de Physiologie Animale,Université Libre de Bruxelles,67,rue des Chevaux,B1640 Rhode-St-Genèse,Belgique and [°]Organon International B.V., Oss, the Netherlands.

We have designed experiments aimed at identifying the nature of the spontaneous anti-tumor response in patients bearing colon carcinomas. The T cell response was analyzed by harvesting proximal or distal lymph node T cells, or tumor infiltrating lymphocytes (TIL) from tumor patients and restimulating them in vitro with the autologous tumor cells, in the presence of 1 U/ml of RIL-2 and 10% supernatant from PHA stimulated PBL. After 2 rounds of stimulation, the T cells were cloned twice and characterized. Interestingly, all T cell clones derived from the TIL population had a cytolytic phenotype (OKT8$^+$ OKT4$^-$ OKT3$^+$) and were able to lyse a Fc receptor + target cell in the presence of OKT3 mAbs, demonstrating that all these clones belong to the cytotoxic compartment of T cells. In contrast, only 50% of T cell clones derived from the draining lymph nodes belonged to the cytotoxic compartment. This strongly suggests a preferential infiltration of the tumor burden by cytolytic T cells. The specificity of the CTL clones was analyzed in a direct ^{51}Cr release assay. 2 out of 9 TIL derived clones demonstrated a significant specific lysis of the autologous tumor cells without significant lysis of autologous normal lymphocytes. Experiments are now under way, in order to identify the nature of the TAA recognized by these CTL clones, as compared to the TAAs identified on colon carcinoma cells using monoclonal anti-tumor antibodies.

T 218 DIFFERENT FINE BINDING SPECIFICITIES OF MONOCLONAL ANTIBODIES TO GANGLIOSIDE GD2, Tadashi Tai,[1] Ikuo Kawashima,[1] Nobuhiko Tada,[2] and Takao Fujimori,[3] 1-Tokyo Metropolitan Institute of Medical Science, Tokyo, 2-Tokai University School of Medicine, Kanagawa, 3-Meiji Institute of Health Science, Kanagawa, Japan.

The fine structural specificities of six monoclonal antibodies(MAbs) to ganglioside GD2 were studied. These MAbs were produced by hybridomas obtained from A/J mice immunized with EL4(C57BL/6 derived-T lymphoma). The binding specificities of these MAbs found to differ from each other by virtue of their binding to structurally related authentic standard glycosphingolipids as revealed by three different assay systems(enzyme immunostaining on thin-layer chromatography, enzyme-linked immunosorbent assay and immune adherence inhibition assay). The MAbs examined could be divided into three binding types. Three MAbs A1-201, A1-410, and A1-425 bound specifically to ganglioside GD2 and none of the other gangliosides tested. Two other MAbs A1-245 and A1-267 reacted not only with GD2, but also several other gangliosides having the sequence NeuAcα2→8NeuAcα2→3Gal(GD3, GD1b, GT1a, GT1b, and GQ1b). The reactivities with these gangliosides varied to some degree. In addition, these MAbs were found to react with both GD3(NeuAc, NeuAc) and GD3(NeuGc, NeuAc), but not with GD3(NeuAc, NeuGc) or GD3(NeuGc, NeuGc). The last MAb A1-287 also reacted with several other gangliosides(GT1a, GQ1b) but with lower avidity than A1-245 and A1-267. These findings suggest that each MAbs to ganglioside GD2 may have an individual binding specificity and avidity. These MAbs should be powerful tools not only in analyzing the biological functions of ganglioside GD2 on cell surface membranes, but possibly also in the diagnosis and therapy of human tumors.

TAA as Targets for Imaging and Therapy

T 300 RICIN A CHAIN IMMUNOTOXINS FOR OVARIAN CANCER THERAPY, R.W. Baldwin[1], V.S. Byers[1,2], L.G. Durrant[1], R.A. Robins[1], M.J. Embleton[1], M.C. Powell[3], E.M. Symonds[3], and P.J. Scannon[2], 1. Cancer Research Campaign Laboratories, University of Nottingham, U.K., 2. XOMA Corporation, Berkeley, CA., 3. Department of Obstetrics and Gynaecology, University Hospital, Nottingham, U.K.
Monoclonal antibodies (MoAbs) reacting with antigens expressed upon ovarian cancer cells have been developed for targeting cytotoxic agents in the treatment of ovarian cancer. MoAb selected for this purpose were initially evaluated by their reactivity with ovarian cancer cells, and then by flow cytometry analysis of MoAb binding to tumor cells obtained by collagenase disaggregation of primary and metastatic ovarian tumors. MoAb XMMCO-791 binding to a gp72 glycoprotein reacted with more than two thirds of the tumors and other MoAbs showing ovarian tumor reactivity included B55 binding to tumor-associated mucin-like antigen and 228 reacting with CEA. These studies on the phenotypes of ovarian cancer cells have provided MoAbs reacting with essentially all malignant cells within ovarian tumors. MoAb XMMCO-791 localization in ovarian tumors has been demonstrated by immunoscintigraphy of 78 patients. For ^{111}In-labeled MoAb the sensitivity and specificity of tumor localization were 92% and 82% respectively. With ^{131}I-MoAb the sensitivity and specificity were 100% and 75%. Analysis of resected tumor specimens demonstrated uptake of radioactivity in tumor to be at least 4 times that in uninvolved ovary. An immunotoxin constructed by linking XMMCO-791 to ricin A chain is cytotoxic for ovarian cancer cells. In vitro cytotoxicity tests indicate that the immunotoxin is 2000-fold more cytotoxic than free ricin A chain and 30,000-fold more active than a control melanoma immunotoxin.

T 301 BIOLOGIC, THERAPEUTIC, AND IMAGING RESULTS WITH MONOCLONAL ANTIBODY KC4G3 IN PATIENTS WITH BREAST AND LUNG CANCERS, D. Dienhart, R. Schmelter, J. Lear, G. Miller, S. Sedlacek, S. Glenn, D. Bloedow, K. Kortright, P. Bunn, University of Colorado Cancer Center, Denver, CO, and Coulter Immunology, Hialeah, FL.
KC4G3 is an IgG3 murine monoclonal antibody which recognizes a high molecular weight (>400,000 dalton) antigen present in human milk fat globules (HMFG) and on the cell surface of most human epithelial cancers including breast (164/178) and non-small cell lung (149/154) cancers. Prestudy circulating KC4G3 antigen was detected in all advanced breast cancer patients (24-1571 ng/ml, mean 379) and 95% of lung cancer patients (0-1702 ng/ml, mean 295). KC4G3 was given intravenously in doses from 1-500 mg alone or mixed with 1 mg KC4G3-DTPA-111-Indium (5 mCi) in 20 patients with non-small cell lung cancer and 11 with metastatic breast cancer. Serum clearance analyzed by radioimmunoassay and gamma counting showed a greater serum antibody clearance in lung compared to breast cancer (0.035 ml/min/kg vs. 0.017 ml/min/kg; $p<0.05$). The antibody clearance was not dose related, fit 1 or 2 compartment models, and closely compared to 111-Indium distribution and clearance kinetics. The serum half-lives were 23 and 36 hours for lung and breast cancers, respectively. Less than 15% was excreted in urine over 96 hrs. Sustained KC4G3 serum levels >10 micrograms/ml were achieved with twice weekly administration of >250 mg. Gamma camera imaging was optimal at 72 hrs. and detected the majority of large (>3 cm) tumors in both lung and breast cancer patients (80% and 63%, respectively), but a minority of small tumors (23% and 26%, respectively). Nonspecific uptake in liver, spleen and marrow contributed to the poor sensitivity. Twice weekly intravenous administration of unlabeled KC4G3 produced only minor allergic reactions and could saturate tumor cells in tissues. However, there were no objective responses and 10/12 patients developed human anti-mouse IgG. We conclude that immunoconjugates are needed for anti-tumor responses and methods to decrease nonspecific binding will be needed for better imaging and therapy.

T 302 IDIOTYPE VACCINES AGAINST HUMAN T CELL LEUKEMIA AND LYMPHOMA. Malaya Chatterjee,* and Heinz Kohler.** Roswell Park Mem. Inst.*, Buffalo, NY 14263 and IDEC, Inc.**, LaJolla, CA 92037.
The concept of idiotype vaccine against tumor-associated antigens is being studied in human T cell leukemia and lymphomas. T-cell acute lymphoblastic leukemia (T-ALL), and T-cell lymphoma are generally not curable and would be excellent candidate diseases for anti-idiotype therapy. The starting point of our work to develop an idiotype vaccine was the availability of specific murine monoclonal antibody against a unique human T-cell leukemia/lymphoma associated cell surface glycoprotein, GP-37 (MW 37,000), not found on normal T cells, which represents an appropriate target for immunotherapy of T-cell leukemia/lymphoma patients. This hybridoma (Ab1) has been used to generate monoclonal syngeneic anti-idiotype antibodies (Ab2) expressing the image of the tumor-associated antigen. Our conclusion that some of the Ab2s may carry the internal image of the GP-37 antigen has been derived from the following observations - (1) Ab2 inhibited the binding of Ab1 to GP-37 antigen and target cells, (2) the binding of Ab1 to Ab2 was inhibited by isolated GP-37 antigen, (3) Ab2 reacted with rabbit antisera specific for GP-37, and (4) Ab2 induced the production of antigen specific Ab3 (Ab1') in mice and rabbits in absence of any exposure to GP-37 antigen. Following preclinical testing and confirmation of anti-tumor immunity in animal systems, a phase I clinical trial will be initiated in selected patients with T-cell leukemia/lymphoma with the use of these internal image anti-Id antibodies.

Collectively, we hope these studies will lead to a novel idiotype approach to the therapy of human T-cell leukemia/lymphoma and, at the same time, provide further insight into the biology of the immune network.

T 303 THE RADIOIMMUNODETECTION OF GASTRIC CANCER IN VIVO, Z.W.Dong, M.Y. Zhang,S.M.Wei,J.R.Zhu,Z.Y.Mu,Beijing Inst.for Cancer Res. China

Monclonal antibodies(MoAbs) 3F4, 3G9 and 3H11 against gastric cancer were raised by using immunizing Balb/c mice with five human gastric cancer cell lines in sequence. These MoAbs were reactive with all target cell lines and 85-94% of human gastric cancer tissues(n=46), and they did not react with normal cells and tissues(33 kinds). It is suggested that their corresponding antigens might be common ones associated with gastric cancer.

MoAbs 3G9 and 3H11 were labeled with ^{131}I and injected into tumor-bearing nude mice. Clear image of tumor was obtained from 48 to 96 hrs. with r camera. The localisation capacity of labeled MoAbs were evaluated with Tumor/Normal Tissues ratio and Localisation Index(L.I.). At 96 hrs, Tumor/Liver and L.I. for MoAb 3G9 could reach 4.72± 1.56 and 4.13±2.09(n=6), while 8.26±1.66 and 6.08±1.51(n=4) for MoAb 3H11 respectively. With kinetic analysis it was shown that the time of half life of the labeled MoAb 3H11 in blood was much shorter than 3G9(18hrs versus 42hrs). When the F(ab') 2 fragment of MoAb 3G9 was labeled and injected into animal model, the clearance of its radioactivity from circulation was much faster with the time of half life 14hrs. Clearer image was obtained at 24hrs, and Tumor/Liver was 28.92±9.48 and 53.80±5.04(n=3) at 48hrs and 96hrs respectively.

T 304 THERAPEUTIC VACCINE AGAINST MELANOMA ASSOCIATED ANTIGEN P97 IN MURINE TUMOR MODEL, Charles D. Estin, Ulrike S. Stevenson, Ingegerd Hellstrom, and Karl Erik Hellstrom, Oncogen, 3005 1st Ave., Seattle, WA 98121

To develop a therapeutic tumor vaccine against the human melanoma associated antigen, p97, we have cloned and expressed p97 in the murine metastatic melanoma K1735. Transfected sublines have been derived expressing a range of human p97 surface antigen densities. Sublines expressing antigen levels an order of magnitude higher than human melanoma cells are non-tumorigenic in immunocompetent mice. Conversely, lower expressing transfectants are highly tumorigenic in syngeneic hosts, providing a model for testing vaccines directed against p97. The most successful vaccine in this system is a recombinant vaccinia virus expressing p97 cDNA, v-p97NY. Immunization with v-p97NY induces tumor rejection against intravenously inoculated p97-expressing murine tumor lines when the vaccine is provided two days post-challenge. The growth of tumor lines expressing different p97 levels was quantitated in vitro and in vivo, with and without v-p97NY vacination. From these data it appears that total antigen load is a critical factor in determining the balance between tumor growth or immune-mediated rejection. Therapeutic vaccination with v-p97NY may alter this balance in favor of immuonological activation.

T 305 HUMAN MONOCLONAL ANTIBODIES REACTIVE WITH THOMSEN FRIEDENREICH ANTIGEN, Michelle S. Glasky and Christopher L. Reading, Departments of Tumor Biology and Hematology, The University of Texas System M.D. Anderson Hospital, Houston, TX 77030

We have generated human monoclonal antibodies reactive with the tumor-associated Thomsen Friedenreich (T) antigen by the EBV-hybridoma technique. T antigen is expressed on approximately 90% of adenocarcinomas from a variety of organs. Normal individuals have circulating antibodies to T antigen due to immunization by intestinal flora bacteria carring the immunodominant carbohydrate moiety, βGal(1→3)βGalNAc). B lymphocytes, isolated from the peripheral blood of normal laboratory volunteers, were selected for reactivity to T antigen by panning on asialoglycophorin-coated petri dishes and transformed with EBV. T antigen-reactive cells constituted 1.5 to 7 percent of the peripheral blood B lymphocytes. Cells secreting anti-T antibody, as determined by hemagglutination and ELISA, were either cloned or fused immediately with the murine myeloma cell line P3X63Ag8.653 (653). T-antigen specific transformed cell lines of the IgM and IgA classes have been produced which secrete up to 20ug/ml/10^6 cells/24hr of specific antibody. Cloned, antigen-reactive transformed cells were unstable and would not yield viable hybrids after fusion with 653 cells. Uncloned non-specific cell lines always gave hybrids. Therefore, uncloned antigen-reactive lymphoblastoid cells were fused with 653 cells shortly after transformation (within 4 weeks). The results of fusions with these antigen-reative cell lines will be presented.

T 306 TUMOR-UNIQUE ANTIGENS ON T CELL CHRONIC LYMPHATIC LEUKEMIA (T CLL) RECOGNIZED BY MONOCLONAL ANTIBODIES. TREATMENT OF T CLL WITH ANTI-IDIOTYPIC MONOCLONAL ANTIBODY, Carl Harald Janson, Mahmood J. Tehrani, Håkan Mellstedt and Hans Wigzell, Dept of Immunology, Karolinska Institute and Depts of Oncology and Imm. Res. Lab. Karolinska Hospital, Stockholm, Sweden.

When tumor therapy is approached with a monoclonal antibody (Mab) concept, a tumor-unique, stabile and nonsecreted tumor antigen is called for. The idiotypic molecules on B lymphocyte (Ig) and T lymphocyte (TCR) membranes are clonally distributed and unique antigens. As normal and malignant B cells use somatic hypermutation, the membrane bound Ig molecule is not constant over time, leading to tumor escape. Furthermore, secretion of Ig (blocking factor) is undesired. The TCR is a unique, constant and nonsecreted antigen.

We have produced five murine Mab against idiotypic determinants on the TCR of two patients (E.L. CD3,4; I.U. CD3,8) with chronic lymphatic leukemia (T CLL). The anti-idiotypic nature of the Mabs was concluded from specificity, molecular weight of antigen and CD3 comodulation and coprecipitation. The initial Mabs were of IgG1 and IgG2a subclasses. IgG2a spontaneous switch variants have been selected. The Mabs mediated no significant complement cytotoxicity and ADCC. However, in ADCC with IL-2 stimulated effector cells significant lysis was seen. The Mabs could induce DNA synthesis in normal allogenic lymphocytes (<4% reacted with Mab) but not in the tumor cells. Patient I.U. was treated intravenously with 1+1+10 mg of Mab (IgG2a) over a period of four weeks. An 80% reduction of total circulating tumor cells was reached using 2 x 1 mg of Mab. 10 mg but not 1 mg induced detectable complement activation. Side effects noted were fever, chills, nausea, vomiting, diarrhea, tachycardia, increase in systolic blood pressure and shortness of breath.

Furthermore, in the process of selecting for anti-idiotypic Mabs we have found two Mabs reacting with tumor-unique antigens, not being the TCR molecule, on the membrane of a T CLL tumor cell. These antigens have, to our knowledge, previously not been described. One antigen has the relative molecular weight of 74-80kD under nonreducing and 80kD under reducing condition. The other antigen is a dimer with a molecular weight of 74-80kD nonreduced and 38kD reduced.

T 307 PROTECTIVE TUMOR-ASSOCIATED ANTIGENS (TAAA) IN COLORECTAL CARCINOMA. J. M. Jessup, K-F Chi, and R. Hostetter. UT System Cancer Center, Houston, TX 77030.

Autoantibodies from 47 patients have been prospectively analyzed to identify protective TAAA. Western blots of extracts of primary and metastatic colorectal carcinomas were probed with autologous IgG collected at surgery. Seventeen patients had or developed clinical metastasis (poor prognosis) and 18 patients remained free of disease a median of 18 months (good prognosis). Both groups had a similar number of TAAA (4.0±.3 and 4.1±.4 TAAA per patients, respectively). There were 13 TAAA of 98 to 26 kDa - each recognized by autoantibodies in 15% or more of the patients. Two antigens (58 and 41 kDa) were organ-associated antigens (OAA) recognized by 65 to 83% of sera from both groups and present in extracts of normal colon. However, 2 TAAA were significantly associated with the good prognosis group: 38 kDa (56% vs 12% in the poor prognosis group, $p<0.01$) and 26 kDa (33% vs 6%, $p<0.05$). A group of 12 patients with Dukes B2 and C colon carcinoma entered a randomized Phase III autologous tumor cell-BCG adjuvant trial. Although a pilot study demonstrated that this vaccine improved survival by 15% and boosted both autoantibody and cell-mediated immunity to TAAA, surgery and vaccination induced antibody to a new TAAA in only 1 of 5 vaccinated patients and none of 7 control patients treated with surgery alone. Normal sera reacted with the OAA but not the 38 and 26 kDa TAAA. These results indicate 1) colorectal carcinoma patients develop immunity to their tumors, 2) the repertoire of serologically defined antigens is limited, 3) the immunodominant OAA are probably not associated with outcome, but 4) responses to 38 and 26 kDa TAAA may protect against recurrence.

T 308 MONOCLONAL ANTIBODY L/1C2 REACTIVE WITH A HUMAN CARCINOMA ASSOCIATED ANTIGEN. David A. Johnson[1,5], M.C. Gutowski[1], E.K. Berger[1], M.J. Borowitz[2], J.N. Eble[3], R.S. Maciak[1], J. Kan-Mitchell[4], M.S. Mitchell[4] and J.L. Zimmermann[1]. Lilly Research Laboratories, Eli Lilly and Co., Indianapolis, IN 46285 (DAJ, MCG, EKB, RSM, JLZ); Duke University Medical Center, Durham, NC 27710 (MJB); Veterans Administration, Richard C. Roudebush Medical Center, Indianapolis, IN 46223 (JNE) and; University of Southern CA Cancer Center, Los Angles, CA 90033 (MSM, JKM).

A murine IgG3 monoclonal antibody is described which defines a human carcinoma associated antigen. The antibody, designated L/1C2, reacted with the surface of 15 of 16 viable human carcinoma cell lines and was detected in frozen sections of 70 of 78 human carcinomas. Melanoma cell lines and frozen sections of melanomas and a lymphoma were unreactive. Normal tissue reactivity included vessel endothelium, plus some ducts, glandular structures, and epithelial surfaces. Similar normal tissue reactivity patterns were seen with Rhesus monkey tissue samples. Immunoprecipitation studies indicate that L/1C2 reacts with a glycoprotein and, fluorescence analysis suggests this antigen is internalized following reaction with the L/1C2 antibody. The antigen identified by the L/1C2 antibody doses not appear to be identical to other described carcinoma associated antigens. This antibody may prove valuable in exploring drug or toxin immunoconjugate cytotoxicity.

T 309 AN IN VITRO MODEL FOR TUMORIMMUNOTHERAPY WITH ANTIBODY HETERO-CONJUGATES (AHC)*, Gundram Jung# and Hans J. Müller Eberhard
Research Institute of Scripps Clinic, La Jolla, CA 92037

We describe here an in vitro model in which two different antibody hetero-conjugates, an anti target-anti CD3 and an anti target-anti CD28 conjugate, bound to tumor cells, induce activation of resting human T cells. Activation is monocyte independent and proceeds in the presence of human serum. Once activated T cells destroy the AHC coated tumor cells which provided for their activation. These results imply that certain AHC upon in vivo application may achieve both, induction as well as mediation of T cell cytotoxicity on the surface of tumor cells.

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* Antibody heteroconjugates (AHC) may be defined as conjugates of two different monoclonal antibodies. Here this term is specifically used for conjugates consisting of tumor specific antibodies and certain antibodies capable of activating T cells under appropriate conditions.

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T 310 TARGETING OF T LYMPHOCYTES FROM RENAL CANCER PATIENTS TO TUMORS USING CROSS-LINKED ANTIBODY AGGREGATION. L. A. Kerr, J. Donohue, H. Zincke, P. Leibson, and D. J. McKean, Mayo Clinic, Rochester, MN 55905

The requirement of relevant antigen for activation of cytolytic T lymphocytes can frequently be bypassed by perturbation of the Ti-CD3 complex on the membrane surface. By physically cross-linking the activated CTL to the target cell of interest cellular lysis occurs. Anti-CD3 antibody chemically cross-linked to antitumor antibody is a potent reagent in this regard. We have tested a combination of growth factors including IL-2, IL-4, IL-3 and anti-CD3 to rapidly expand cytolytic effector populations from renal tumor bearing patients' peripheral blood and lymph nodes. Anti-CD3 and IL-2 are the most effective combination with proliferation of effector cells 2 to 1000 times greater than with other lymphokines alone or combined. After two weeks in culture, LAK activity predominates and no increase in lysis due to heteroaggregates is noted. After 8 weeks in culture, however, no LAK activity is recorded and CTLs with variable antigen specificities dominate cultures. In the presence of the tumor specific monoclonal antibody heteroaggregates cytolysis of tumor is greatly increased (25:1 E:T rates 30-55% lysis versus 0-4% lysis of controls). Irrelevant anti-target cell heteroconjugates demonstrate little bystander effect (4% lysis above controls). These results including the use of combinations of heteroconjugates will be discussed in light of what we know of lymphokine activation.

T 311 SUPPRESSION OF TUMOR GROWTH BY CYTOTOXIC T LYMPHOCYTES(CTL):DEVELOPMENT OF HUMAN ANTITUMOR CTL THERAPY, Keizo Kitsukawa, Shigeyoshi Fujimoto, Shinji Hamasato, Masaru Takata and Nagamasa Maeda, Department of Immunology, Kochi Medical School, Kochi, Japan.

Establishment of a rational cancer immunotherapy requires the development of a way to augment anti-tumor effector cells which destroy tumors in tumor-bearing hosts. We have proven that one of the anti-tumor effector cells is the CTL against tumors having Thy-1 and Lyt-2 antigens on their cell-surface. The CTL specific for syngeneic sarcoma S1509a could be induced in splenic cells of syngeneic animals immunized with mitomycin C treated homologous tumor cells. I.v. CTL transfer clearly destroyed growing tumors in hosts pretreated with cyclophosphamide to inactivate the suppressor T cell. The destruction of growing tumors was abrogated by removal of Thy-1 or Lyt-2 positive T cells , suggesting that the CTL is essential in tumor destruction. We applied CTL therapy to a human case involving a 74 year old female patient with recurrent breast cancer, and liver and lung metastases with marked left pleural effusion, because of strong side effects due to chemotherapy. The patient's CTL against the autochthonous breast cancer cells obtained from the pleural effusion were induced in vitro. The CTL were shown to possess HLA-restricted killing activity and CD3 and CD8 antigen positive cell-surface phenotype. The CTL($5x10^7$ cells) were injected at two week intervals. Metastatic regions in the lungs and liver began to regress and by the sixth month had disappeared without any side effects. She was discharged seven months after treatment and is leading a normal life. We wish to emphasize that successful experimental results in mice are potentially applicable to human cancer therapy.

T 312 MONOCLONAL ANTIBODIES IN CANCER THERAPY: SELECTION OF THE MOST APPROPRIATE REAGENT FOR DIFFERENT APPROACHES. Delia Mezzanzanica, Silvana Canevari, Gabriella Della Torre, Maria I. Colnaghi, Istituto Nazionale Tumori, Milano, Italy.
Five monoclonal antibodies (MAbs) MBr1, MOv17, MOv18, MOv19 and MLuC2, raised against tumors of epithelial origin, were selected, due to their restricted pattern of reactivity, for therapeutic applications. To define the most appropriate application for each one, the following parameters were analyzed: immunoreactivity after radiolabelling, the affinity constant, the binding kinetics to the relevant antigen and the fate of the antigen-antibody complex after binding to the cell surface. The latter was evaluated as antigen stability and as mode and quantity of MAb-induced internalization. Immunoelectron microscopy and pH 2.8 desorption indicated that: MOv17 induced internalization through coated vesicles of about 60% of the bound antigen, MBr1 induced internalization through uncoated vesicles of about 1/3 of the bound antigen, whereas the other three MAbs were quite incapable of inducing internalization. Accordingly, MOv17 and MBr1 gave rise to active immunoconjugates when linked with a ribosome inactivating protein. On the other hand, the stability of the MOv18-recognized antigen enabled us to efficiently retarget cytotoxic T lymphocytes by bispecific anti-tumor/anti-CD3 MAbs. Finally, the maintainance of immunoreactivity after the labelling of MOv19 and MLuC2 (from 50 to 70%) could be exploited for ^{131}I radioimmunotherapy.

T 313 SYNERGISTIC ADCC OF BREAST CANCER CELLS BY ANTIBODY 113F1 and IL2, D.B.Ring, S.T. Hs ieh-Ma, J.A. Kassel, Dept. of Immunology, CETUS, Palo Alto, Ca 94303. 113F1 is a monoclonal mouse IgG3 that precipitates a 37/60/93/200 kd glycoprotein complex from surface labeled SK-Br-3 breast cancer cells. The antigen is found on a wide range of human tumors and cancer cell lines, as well as certain normal tissues; most staining in normal tissues is limited to the luminal surfaces and cytoplasm of epithelial cells. When 33 mouse monoclonals were tested for ADCC against human breast or ovarian cancer cell lines, 113F1 showed the highest activity with either mouse macrophages or human monocytes or nonadherent mononuclear cells as effectors. ADCC was enhanced when either monocytes or nonadherent cells were pre-incubated with recombinant IL2. When the concentrations of effector cells, 113F1, and IL2 were all limiting, cytotoxicity due to 113F1 plus IL2 was greater than the sum of killing observed with 113F1 alone, indicating synergy between antibody and lymphokine.

T 314 DEVELOPMENT AND CHARACTERISATION OF MONOCLONAL ANTIBODIES TO HUMAN BLADDER CANCER, P.J. Russell[1], K.Z. Walker[2], J. Wotherspoon[2], S. Stewart[3], from [1]Urological Cancer Research Unit, Royal Prince Alfred Hospital and Department of Surgery, Univesity of Sydney, [2]Cellular Immunology Research Centre, University of Sydney, and [3]Department of Nuclear Medicine, Royal Prince Alfred Hospital, Sydney, Australia.
A panel of 8 monoclonal antibodies (MAbs) was generated against a human bladder cancer cell line (UCRU-BL-17CL) derived from an invasive transitional cell carcinoma, exhibiting features of squamous and adenocarcinoma. Hybrid supernatants were assayed by surface immunofluorescence staining using small numbers of cells embedded in agarose. The MAbs have been tested against a series of human cancer cell lines derived from genitourinary, ovarian, colon, and stomach cancers, as well as leukaemic and melanoma cell lines. Most of the MAbs were specific for bladder cancer, but interestingly, one MAb (BLCA 38) raised against bladder cancer reacted even more strongly with ovarian, colon and stomach cancer cell lines. MAbs are also being extensively tested against normal cells. None of the panel reacted with normal bladder. On examination of reactivity with cells from normal peripheral blood, it was found that two of the antibodies reacted against group A1 red blood cells but not against other blood group antigens. The UCRU-BL-17CL is known to express blood group A antigen. The panel of MAb are being further characterized with respect to cellular reactivity and the nature of the antigens recognized. They may be potentially useful for examination of urine specimens for the diagnosis of bladder cancer. Antibodies labelled with ^{131}I are being examined for their ability to localize to xenografted tumours in nude mice.

T 315 EXPERIMENTAL SEROTHERAPY OF MOUSE T-CELL LEUKEMIA WITH ANTI-GANGLIOSIDE (GD2) MONOCLONAL ANTIBODIES, Nobuhiko Tada[1], Norikazu Tamaoki[1], Tadashi Tai[2], Ryuzo Ueda[3], Munehiro Oda[4] and Takao Fujimori[4], [1]Dept.Path., Tokai Univ.Sch.Med., Isehara, Kanagawa, [2]Tokyo Metrop.Inst.of Med.Sci., Tokyo, [3]Aichi Cancer Res.Inst., Nagoya, and [4]Meiji Inst.of Health Sci., Odawara, Japan.

Six monoclonal antibodies have been established by a cell hybridization between spleen cells of a A/J mouse which was hyperimmunized with EL4 cells, a C57BL/6-derived T-cell leukemia cell line, and NS.1 myeloma cells. These monoclonal antibodies, i.e., two IgM(k) clones (A1.201 and A1.245) and four IgG3(k) clones (A1.267, A1.287, A1.410 and A1.425), did not react with normal mouse lymphoid tissues including spleen, lymph node, thymus, and bone marrow, but reacted with a few murine T-cell leukemias (EL4, RBL5 and BALBRVE) as well as human tumor cells of neural crest origin, i.e., melanoma, neuroblastoma, glioma, and small cell carcinoma of the lung. Biochemical characterization of these tumor-associated antigens revealed that all six monoclonal antibodies reacted with ganglioside GD2. Further characterization of the antigenic specificities detected by monoclonal antibodies are described elsewhere. These mouse monoclonal antibodies to ganglioside GD2 were used for the experimental serotherapy of mouse leukemia. EL4-cells were inoculated intraperitoneally or subcutaneously into C57BL/6 mice, and 24 hours later, various doses of the purified monoclonal antibodies were administered intravenously or intraperitoneally. Four IgG3 antibodies showed a marked suppressive effect on the tumor growth and, in some experimental conditions, complete regressions were also observed. On the other hand, IgM antibodies did not show any anti-tumor effects. These reagents would be useful for the therapeutic as well as diagnostic purposes.

T 316 **TUMOR-ASSOCIATED MEMBRANE SIALOGLYCOPROTEIN ANTIGENS ON HUMAN SMALL CELL LUNG CARCINOMA AS TARGETS FOR SPECIFIC THERAPY.** R. Waibel, A. Smith, A. Barth, C.J. O'Hara, A. Maier, U. Schmidt, and R.A. Stahel, Division of Oncology, University Hospital, CH-8091 Zurich, Switzerland, Department of Pathology, New England Deaconess Hospital, Boston, MA 02115 and Institute of Pathology, Ruhr University, D-463 Bochum, West Germany.

Cell surface antigens of small cell carcinoma identified by monoclonal antibodies can be grouped according to tissue reactivity into epithelial antigens, neuroendocrine antigens, antigens shared with white blood cells and tumor-associated antigens. We have focused our attention on the identification of tumor-associated antigens which because of lack of expression in normal tissues represent superior targets for specific tumor therapy. Two such antigens, identified by the monoclonal antibodies LAM8 (IgM) and SWA20 (IgG2a), were characterized as membrane sialoglycoproteins. Despite similarities in antigen size with distinct bands at around 100kD and 180kD and similarities in composition of the epitopes with neuraminic acid as dominant carbohydrate, competition radioimmunoassays and immunprecipitation experiments demonstrated LAM8 and SWA20 to recognize two distinct antigens. Also, only antibody LAM8, but not SWA20 reacted with Le^a positive saliva. The restriction of antigen expression to small cell carcinoma was demonstrated in cell lines and in tissues. Strong antigen expression was present in 4/8 small cell carcinoma cell lines. Of 155 small cell carcinoma biopsies 49% (LAM8) and 45% (SWA20) stained strongly or moderately positive by immunoperoxidase technique. No reactivity was seen in other cell lines and large numbers of pulmonary and extrapulmonary tumor tissues. Anti-idiotypic antibodies mimicking the antigen identified by antibody LAM8 were generated and are used for vaccination experiments. Immunotargeting experiments with radiolabeled antibody SWA20 have shown selective tumor uptake in heterotransplants.

T 317 AN IMMUNOTOXIN SELECTIVE FOR DIVIDING ENDOTHELIAL CELLS, Philip M. Wallace, Regina E. Knyba, Alex N.F.Brown, *Patricia J. Brown and Philip E. Thorpe, Drug Targeting Laboratory, Imperial Cancer Research Fund, P.O.Box 123, London, U.K. and *University of Texas Medical School, Texas 77025.

The value of immunotoxins in treating solid tumours may be reduced by their limited ability to diffuse into the tumour and by antigenic heterogeneity of the tumour cells. These problems might be overcome by targeting the immunotoxin not to the tumour cells themselves, but to vascular endothelial cells within the tumour. Destruction of the vascular endothelial cells should occlude the blood supply to the tumour, deprive it of nutrients and oxygen, and cause an avalanche of cell death within the tumour.

The approach adopted was to prepare an immunotoxin from an antibody directed against a fibronectin receptor which in quiescent endothelium, such as is present in normal tissue, is localised to the inacessible abluminal surface of the cell. It was reasoned that the immunotoxin would bind selectively to dividing endothelial cells, such as are present in tumour tissue, because these cells would depolarise during division and allow the fibronectin receptor to redistribute to the accessible luminal surface.

Capillary endothelial cells were isolated from the Chinese Hamster and the immunotoxin tested for toxicity to confluent and sub-confluent cell cultures. The immunotoxin was selectively toxic to sub-confluent (dividing) endothelial cells and reduced protein synthesis by 50% at a concentration(IC_{50}) of 10^{-10}M. In contrast the immunotoxin was more than a thousand times less toxic to confluent endothelial cells($IC_{50}>10^{-7}$).

T 318 **Expression in COS cells of a mouse-human chimaeric B72.3 antibody**

Nigel Whittle, John Adair, Chris Lloyd, Jeffrey Schlom* & Mark Bodmer. Dept. of Molecular Immunology, Celltech Ltd, Slough, UK and *NCI, NIH, USA.

B72.3 is a mouse hybridoma cell-line secreting an IgG1 antibody which recognizes an epitope on a tumour associated antigen, TAG-72. This high molecular weight mucin-like molecule is found on a variety of human neoplasms, including colon, breast and ovarian carcinomas. Chimaeric immunoglobulin genes with the B72.3 specificity have been constructed by joining the mouse variable regions from cDNA clones to human genomic constant regions using recombinant DNA techniques. The chimaeric heavy and light chain immunoglobulin genes were placed under the control of a strong viral promoter, and transfected into COS-1 cells. SDS-PAGE analysis of the ^{35}S-labelled products demonstrated that the transiently expressed antibodies were correctly synthesised, assembled and secreted. The specific binding characterisics of the parent B72.3 antibody were retained by the chimaeric antibody in an antigen-based ELISA. This system generated sufficiently high transient expression of the chimaeric antibody molecules to allow rapid physical and immunological characterisation of the engineered gene products. [Protein Engineering (1987) Vol 1, No. 6.]

T 319 CONJUGATE OF MONOCLONAL ANTIBODY ANTI-CCT2 AND BLEOMYCIN A6 SHOWS SELECTIVE CYTOTOXICITY TO LEUKEMIA CELLS, Yong-su Zhen, Yu-xian Chen, Bin Yu and Pei-yu Tian, Institute of Medicinal Biotechnology, Chinese Academy of Medical Sciences, Tiantan, Beijing 100050, China

Monoclonal antibody anti-CCT2 which was directed against human fetal thymocytes showed high reactivity with cells derived from some cases of lymphocytic leukemia and low reactivity with peripheral T cells. Anti-CCT2 was not reactive with B cells and myeloid cells. Bleomycin A6 was covalently conjugated with anti-CCT2 by using SPDP as the cross-linking agent. The growth of human leukemia CEM cells was inhibited much stronger by the immunoconjugate than by free bleomycin A6 with the IC50 values of 0.14 uM and 1.56 uM respectively. For irrelevant SP2/0 cells no significant difference in cytotoxicity between the immunoconjugate and free bleomycin A6 was found. The internalization of colloidal gold particles adsorbed with immunoconjugate was observed with electron microscope. After 4 h incubation 72% of CEM cells contained Au particles whereas only 14% of the irrelevant U937 cells showed the same appearance. Preincubation with unconjugated anti-CCT2 blocked the Au uptake into CEM cells. As early as 15 min incubation 56% of CEM cells had internalized Au particles and interestingly some particles were found in the cell nucleus. Results indicate that the immunoconjugate shows selective cytotoxicity and it enters into the target cells specifically and rapidly. Since bleomycin A6 is less toxic to hemopoiesis and immune system, this immunoconjugate may be more safe and useful in leukemia chemotherapy.