CELLULAR IMMUNITY AND THE IMMUNOTHERAPY OF CANCER

Organizers: Michael T. Lotze and Olivera J. Finn

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Cellular Immunity and the Immunotherapy of Cancer

T Cell Recognition of Antigen and the T Cell Receptor

CE 001 ORIGIN AND REPERTOIRE OF EPITHELIUM-ASSOCIATED T CELLS. James P. Allison, David Asarnow, Mark Bonyhadi and Wendy Havran, Dept. of Molecular and Cell Biology and Cancer Research Laboratory, University of California, Berkeley, CA 94720, and Makio Iwashima and Yueh-hsiu Chien, Stanford University School of Medicine, Stanford, CA 94305

Thymocytes bearing T cell antigen receptors (TCR) with different Vγ segments appear in an ordered manner during fetal development in the mouse. Vγ3, borne by the first wave of TCR+ cells to appear in ontogeny, is in the adult mouse found only in the skin, where it is expressed by the vast majority of the T cells. Thymus grafting experiments and in utero antibody treatment confirm that the epidermis-associated T cells arise from precursors found only in the fetal thymus. A consequence of this early fetal origin is a restricted TCR repertoire of the epidermal T cells. Each of several clones as well as polyclonal cell preparations were found to have identical TCR composed of Vγ3Jγ1Cγ1 and Vδ1Dδ2Cδ gene segments, with essentially no junctional diversity. This observation suggests that these cells might function in trauma signal surveillance, rather than surveillance for foreign antigen. T cells associated with another epithelial tissue, the gut, were found to be similarly restricted in Vγ usage to Vγ5, but expressed several different Vδ segments. The gut associated TCR were found to have extreme junctional variability, suggesting that they arose later in ontogeny and are suited for foreign antigen surveillance. In mice transgenic for the skin TCR, gut T cells expressed only the transgenic TCR, indicating that the TCR is not involved in tissue homing.

CE 002 IMMUNOGENICITY OF ANTIGENS RECOGNIZED BY T CELLS, Jay A. Berzofsky, Molecular Immunogenetics and Vaccine Research Section, Metabolism Branch,National Cancer Institute, National Institutes of Health, Bethesda, MD 20892

Both helper and cytotoxic T lympohocytes generally recognize protein antigens not in their intact form, as antibodies do, but on the surface of another cell, after "processing" by that cell to unfold or cleave the protein into fragments and association of the processed antigen with major histocompatibility complex (MHC) molecules on that cell. This complex process leads to immunodominance of certain segments from the protein. Immunodominance depends not only on structural features intrinsic to the antigenic segment itself, but also on antigen processing and on the structure of the MHC molecules of the responding individual. We have explored all three of these factors, including the enzymes involved in processing, the way peptides bind to MHC molecules, and structural features such as helical amphipathicity that seem to favor T-cell recognition. Evidence indicates that a single intracellular thiol protease, such as cathepsin B, may be both necessary and sufficient for processing of a model protein, myoglobin, to present most or all epitopes in association with class II MHC molecules. Studies of mutant class II MHC molecules and substituted antigenic peptides indicate that a peptide may bind to a single MHC molecule in more than one way. Thus, a given residue on an antigenic peptide may not always play the same role in binding to the MHC molecule or to the T-cell receptor, and any given residue in the peptide-binding groove of the MHC molecule may serve as a contact residue when the peptide binds in some configurations but not others. Third, a majority of immunodominant determinants recognized by either CD4 or CD8 T cells have the property that if folded as an alpha helix, that helix would be amphipathic, i.e., would have one side predominantly hydrophilic and the other side predominantly hydrophobic. We have used the property of helical amphipathicity prospectively to successfully locate antigenic sites of proteins from the malaria parasite, the AIDS virus, HIV, and the human leukemia/lymphoma virus, HTLV-I. For HIV and malaria, we have identified both helper and cytotoxic T cell sites and coupled a helper site to a B-cell site to produce a synthetic immunogen that elicits neutralizing antibodies. In the case of HIV, we have studied the effect of viral sequence variation on cytotoxic T cell recognition and binding of the immunodominant peptide to MHC molecules, with possible implications for viral escape from immune control. This infomation suggests strategies for the rational design of synthetic or recombinant vaccines.

CE 003 GENES ENCODING T CELL DEFINED TRANSPLANTATION ANTIGENS EXPRESSED BY TUMOR CELLS.

Thierry Boon, Aline Van Pel, Etienne De Plaen, Christophe Lurquin, Patrick Chomez, Jean-Pierre Szikora, Catherine Sibille, Benoît Van den Eynde and Bernard Lethé.
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Mutagen treatment of mouse P815 tumor cells produces tum- variants that are rejected by the syngeneic DBA/2 mice. These variants express new transplantation antigens (tum- antigens) that are recognized by cytolytic T lymphocytes (CTL) but are unable to induce an antibody response. Transfection of P815 cell line P1.HTR with DNA from tum- variants yielded transfectants expressing the tum- antigen that were detected by their ability to stimulate CTL proliferation. Transfectants were also obtained with cosmid libraries. By packaging directly the DNA of these transfectants into lambda phage heads, we isolated cosmids carrying sequences coding for three tum- antigens : P91A, P35B and P198 (1, 2). No sequence similarity was found between the three tum- genes and they appear to be unrelated to any known mouse or human gene. For the three genes, the normal and tum- alleles of the gene differ by a point mutation located in an exon (3). Synthetic peptides encoded by the region surrounding the mutation rendered P815 cells susceptible to killing by the relevant specific CTL. The P91A tum- mutation creates an aggretope, whereas the P198 mutation creates a new epitope.

We have now also cloned the gene that encodes the major antigen recognized on tumor P815 by DBA/2 mice.

(1) De Plaen E, Lurquin C, Van Pel A, Mariamé B, Szikora JP, Wölfel T, Sibille C, Chomez P , Boon T. Immunogenic (tum-) variants of mouse tumor P815: cloning of the gene of tum$^-$ antigen P91A and identification of the tum$^-$ mutation. Proc Natl Acad Sci USA, 1988, 85 : 2274-2278
(2) Boon T , Szikora JP, De Plaen E, Wölfel T and Van Pel A. Cloning and characterization of genes coding for tum- transplantation antigens. Journal of Autoimmunity, 1989, 2 : 109-114.
(3) Lurquin C, Van Pel A, Mariamé B, De Plaen E, Szikora JP, Janssens C, Lejeune J, Boon T. Structure of the gene of tum$^-$ transplantion antigen P91A: the mutated exon encodes a peptide recognized with L^d by cytolytic T cells. Cell, 1989, 58.

CE 004 HOW TUMOR CELLS TALK DIRECTLY TO T CELLS: ANTIGEN PROCESSING AND PRESENTATION BY MELANOMA CELLS, DuPont Guerry, Michael Alexander and Jeannette Bennicelli, University of Pennsylvania Pigmented Lesion Study Group and Cancer Center, Hospital of the U of PA, Philadelphia, PA 19104

Melanocytic neoplasia is a paradigm for tumor progression. The generic lesions in this tumor progression system include non-obligate precursors (common acquired and dysplastic nevi), primary melanomas without competence for metastasis (in situ and invasive radial growth phase melanomas), primary melanomas with competence for metastasis (vertical growth phase melanomas), and metastatic disease. In immunohistologic analyses steps early in this pathway are associated with an activated T cell infiltrate made up of both CD4+ and CD8+ cells. Interferon-γ appears in the interstitium and the melanocytic cells express MHC class I and II restriction elements. In counterpoint, advanced primary and metastatic disease has an attenuated T cell infiltrate. These in situ findings are paralleled by the following in vitro observations: 1) melanoma cells cultured from biologically early disease provoke the MHC class II-dependent proliferation of autologous T cells, while tumor cells from advanced disease do not; 2) T cells stimulated by melanoma cells produce interferon-γ, which increases MHC class I and II antigen expression by tumor cells; 3) melanoma cells produce IL-1, but do not themselves produce interferon-γ or IL-2; 4) melanoma cells process a model antigen, tetanus toxoid (TT); 5) melanoma cells from early disease present TT to antigen-specific T cell clones, but cells derived from late disease do not; 6) the incapacity of cells from advanced disease to present antigen is shared by lipidized glass beads bearing processed antigen and MHC class II molecules derived from such cells; and 7) the proliferation of T cells provoked by autologous melanoma cells is inhibited by treating the tumor cells with inhibitors of antigen processing. These data suggest that evolving melanoma is both immunogenic and immunomodulatory (producing cytokines and processing and presenting tumor-associated antigens). Advanced melanoma likely escapes immune control through a variety of pathways, one of which is to fail to productively associate tumor-associated antigens and MHC molecules.

Effector Cell Activation and Target Cell Binding

CE 005 NK CELLS AND THEIR ACTIVATION: EXPRESSION OF NK-SPECIFIC GENES, Fritz H. Bach, Susan Fuad, Jean Witson, Cynthia McSherry, Toshio Yabe, and J.P. Houchins, Immunobiology Research Center, Department of Laboratory Medicine and Pathology, University of Minnesota Medical School, Minneapolis, MN 55455

We have attempted to evaluate which cells potentially mediate NK activity in addition to the classically described large granular lymphocytes (LGL). As such, we have isolated CD3- small lymphocytes from PBL both by FACS sorting (selecting for size and the lack of granularity) as well as Percoll gradients (isolating cells from the densest fraction and eliminating T cells). We find that small lymphocytes that do not express the CD3 marker mediate high-level NK activity. These small agranular lymphocytes (SAL) not only mediate NK activity at levels comparable to those mediated by LGL, but both populations also mediate low-level LAK activity without overt activation

In an attempt to activate NK cells, we have found a role for a two-signal model in stimulating proliferation in such cells. We find that K562, which in our hands by itself does not stimulate proliferation of CD3- NK cells, will do so in the presence of low concentrations of T cell growth factor preparations (TCGF). This combined signal, not unlike that reported by Warren and colleagues in the generation of cytotoxicity, suggest that NK cells, like T and B cells, can use a two-signal system for their activation, including stimulation to proliferate. Our data do not allow us to evaluate which population(s) is responding, the SAL or LGL or both.

Using both subtractive hybridization and differential probing, we have looked extensively for genes that are expressed in NK cells and not in B cells (Epstein-Barr Virus-transformed lymphoblastoid cell lines (LCL)). Differential hybridization with first-strand cDNA probe was applied to 10,000 plaques from an NK-cell total cDNA library and 1100 plaques from an NK minus B cell subtracted library. We selected 66 clones for further characterization using Northern blots and DNA sequencing. Twelve different cross-hybridizing groups were found that are expressed in three different NK cells but not in B cells. DNA sequencing revealed that eight of these were previously described including Tiβ, Tiδ, metallothionein, two serine proteases, hsis γ, 519, and 25H8. One of the new groups is expressed only in NK cells. The other three are expressed at similar levels in NK and T cells. Some of these genes are up-regulated at 48-72 hrs after IL2 stimulation of NK cells.

CE 006 NK AND T LYMPHOCYTES: MEMBRANE STRUCTURES TRIGGERING CELL-MEDIATED CYTOTOXICITY, Lewis L. Lanier, Becton Dickinson Monoclonal Center, Inc., 2375 Garcia Avenue, Mountain View, CA 94043

Cell-mediated cytotoxicity is triggered by stimulation throught certain membrane receptors. On T lymphcytes and NK cells CD2, CD3, and CD16 function in this capacity. CD16 is a low affinity IgG Fc receptor that is expressed on NK cells, granulocytes, activated macrophages, and a minor subset of T lymphocytes. CD16 is the only IgG Fc receptor express by NK cells and is solely responsible for antibody-dependent cellular cytotoxicity. Binding of immune complexes or monoclonal antibodies against CD16 to NK cells causes a rapid increase in intracellular Ca^{++} and IP_3 generation, with subsequent transcription of lymphokines and triggering of cell-mediated cytotoxicity. By contrast, on granulocytes CD16 may predominantly function simply as a binding structure for IgG complexes. Biochemical differences between CD16 on NK cells and granulocytes may account for these functional distinctions. Immunoprecipitation demonstrates the presence of a ~30 kD CD16 polypeptide on granulocytes, but ~36 and ~38 kD on NK cells. CD16 on granulocytes is susceptible to cleavage with phosphatidylinositol phospholipase C (PI-PLC), indicating PI-glycan linkage, while CD16 on NK cells is resistant to PI-PLC. cDNA encoding the NK and neutrophil forms of CD16 have been cloned and their structural properties compared.

CE 007 THE ROLE OF CD4 AND CD8 IN T CELL FUNCTION. Jane R. Parnes, M. Carrie Miceli and Paul von Hoegen, Division of Immununology, Stanford University Medical Center, Stanford, CA 94305.

The CD4 and CD8 T cell "accessory molecules" play important roles in T cell function. CD4 is expressed on T cells that recognize class II major histocompatibility complex (MHC) proteins, while CD8 is expressed on T cells that recognize class I MHC proteins. There is now evidence that CD4 and CD8 can bind to nonpolymorphic regions on class II and class I MHC proteins, respectively. The ability of CD4 and CD8 to enhance T cell responses is likely to result from a role in adhesion and/or signal transduction. Recent experiments have shown that accessory molecules can function independently of the T cell receptor complex; however, other experiments demonstrating an intimate association between the T cell receptor complex and accessory molecules have led to the suggestion that this association may be key in CD4 and CD8 function. We have examined the function of CD4 and CD8 after transfection into a mouse T cell hybridoma specific for beef insulin in association with the mouse class II molecule $A\alpha^bA\beta^k$. We had previously shown that release of interleukin-2 (IL-2) by this hybridoma in response to beef insulin is markedly enhanced by cell surface expression of mouse CD4 on the hybridoma cells. Expression of CD4 also resulted in a new response to pork insulin. We have now generated transfectants bearing either CD8 alone or both CD4 and CD8. While CD8 is capable of binding to class I molecules on the antigen presenting cells, it is not able to bind the same MHC molecule to which the T cell receptor binds. Expression of CD8 on the surface of the hybridoma clearly enhances IL-2 production in response to beef insulin, though to a significantly lesser degree than expression of CD4. Furthermore, the additional response to pork insulin is only detectable in CD8 bearing transfectants when very high antigen concentrations are used. These responses could be shown to be specific since they are blocked by monoclonal antibodies specific for CD8. Transfectants expressing the α' form of CD8, which lacks most of the cytoplasmic tail and does not associate with the tyrosine kinase $p56^{lck}$ were found to behave comparably to those expressing the full-length CD8α chain. Transfectants bearing both CD4 and CD8 produce IL-2 in response to beef and pork at levels comparable to those transfected with CD4 alone. The responses of these transfectants are blocked completely by anti-CD4 but only partially by anti-CD8. Comparison of the enhancement of responses with CD8 versus CD4 demonstrates a dichotomy in the ability of these two molecules to stimulate responses to beef and pork insulin and provides evidence for at least two mechanisms by which such accessory molecules can enhance T cell responses.

CE 008 T CELL "ADHESION" MOLECULES - ROLES IN ADHESION, ACTIVATION AND DIFFERENTIATION. Stephen Shaw, Gijs van Seventer, Kevin Horgan, Yoji Shimizu. Experimental Immunology Branch, NCI, NIH, Bethesda, MD 20892.

Molecules often described as "adhesion" molecules are proving to have multifunctional roles in T cell recognition and differentiation. Analysis of these roles has been undertaken in simplified model systems of human T cell interaction with purified adhesion ligands: LFA-1-mediated adhesion to purified ICAM-1 (pICAM-1) and VLA-4- & VLA-5-mediated adhesion to fibronectin (FN). In both of these model systems receptor-mediated adhesion is regulated by the process of cell activation. Resting peripheral T cells bind minimally to immobilized ligand but brief treatment of T cells with CD3 mAb to aggregate the T-cell receptor (or with PMA) results in strong receptor-mediated binding (without an increase in surface expression of the receptors). Furthermore, immobilized ligand (FN or ICAM-1) enables T cell proliferation to immobilized CD3 mAb, which provides one "signal" but is insufficient to activate on its own. The costimulus provided by immobilized ligand must be delivered in proximity to the aggregated CD3, since separate immobilization does not induce proliferation. The ability of P-ICAM-1 or FN to costimulate with immobilized CD3 contrasts with minimal costimulatory activity of cytokines IL-1ß, IL-2 and IL-6. The proliferative response to co-immobilized OKT3 and pICAM-1 is dependent on the IL-2R, which is induced only in the presence of both OKT3 and immobilized ligand (pICAM-1 or FN). Our working model is that when T cells interact with other cells: 1) engagement is initiated by antigen-independent adhesion whose strength depends on the target cell type and the activation state of the effector and target; 2) subsequent activation requires not only T cell receptor occupancy but also permissive signals from accessory interactions including those provided by LFA-1 and VLA-4/VLA-5; 3) adhesion thereafter is actively regulated by the biochemical events which result from activation.

Molecules which are functionally important to T cells often undergo regulation of their expression during T cell differentiation. This is borne out by studies of ourselves and others on receptors/ligands involved in T cell adhesion. Recent studies with VLA-4 and VLA-5 indicate that their expression (as well as LFA-1) is generally enhanced on subsets of T cells which we interpret to be memory cells. Furthermore, VLA-4/VLA-5-mediated (as well as LFA-1-mediated) adhesion to ligand (FN or pICAM-1) is augmented in memory cells. This enhanced adhesion capacity of memory cells may account for their preferential localization in tissue and at epithelial surfaces. Ongoing studies are directed at exploring other functional consequences of the differential expression of adhesion molecules on subsets of T cells.

Animal Models of Adoptive Therapy-I

CE 009 SPECIFIC ADOPTIVE IMMUNOTHERAPY WITH CULTURED T CELLS, Martin A. Cheever, Wei Chen, Kathryn D. Crossland, David J. Peace, Victor K. Lee and Philip D. Greenberg. Oncology/ Medicine, RM-17, University of Washington, Seattle, WA 98195. The goal of developing specific T cell therapy for human malignancy is predicated in part on animal studies showing that T cell therapy can be effective and curative and on human studies demonstrating that T cells specifically reactive to tumor can be isolated and grown from individuals with a variety of cancers. In animal models T cell therapy is quantitative with larger doses of T cells inducing longer survival times and greater percentages of cure. However, the limited therapeutic efficacy of small numbers of tumor-reactive T cells can be greatly augmented by growing the T cells to larger numbers *in vitro* and treating with the increased numbers of T cells. Our laboratory has utilized murine models for the therapy of disseminated erythroleukemia to develop the principles requisite for the use of cultured T cells to augment specific T cell immunity and to mediate specific tumor therapy. Both the *in vitro* culture conditions utilized for generating T cells and the *in vivo* adoptive transfer regimens utilized have been shown to be critical.

In vitro, optimal generation of effector T cells requires stimulation with specific antigen. T cells cultured with IL-2 as the major stimulus for proliferation tend to become dependent upon exogenous IL-2 and fail to provide for a long-lived ongoing immune response or immunologic memory--properties requisite for optimal efficacy in therapy. By marked contrast, tumor-reactive T cells grown long-term *in vitro* in response to intermittent restimulation by specific tumor antigen can function normally *in vivo* as evidenced by retention of the ability to distribute widely, proliferate in response to specific stimulation by tumor, mediate specific tumor therapy and survive long-term to provide specific anti-tumor immunologic memory. Even the progeny of a single tumor-reactive T cell--if grown to large numbers *in vitro* under conditions that allow subsequent ability to respond to antigen--can eradicate disseminated tumor and survive long-term *in vivo* in large enough numbers to provide for detectable specific anti-tumor immunologic memory long after adoptive transfer. In circumstances in which the use of specific antigen in culture is problematic, the use of antigen can be circumvented by the use of non-specific stimulation of the T cell receptor/CD3 complex with anti-CD3 antibody or alternatively by specific stimulation with anti-clonotype antibody. Heretofore, the establishment of tumor-reactive T cells in culture has required initial priming *in vivo*. The prospects for developing priming *in vitro* to generate antitumor effector T cells will be discussed.

In vivo, aspects of adoptive transfer regimens found to be critical include host immunosuppression and IL-2 regimens. Transfer of cultured T cells into normal mice results in rapid rejection of transferred cells. Rejection can be circumvented by prior immunosuppression and may be mediated in part by a host anticlonotype response. IL-2 regimens using a short course of IL-2 (1 to 7 days) have been shown to increase the number and thus function of donor T cells present *in vivo*. However, prolonging the course of IL-2 further (14 to 21 days) is ineffective and often detrimental. By contrast, intermittent courses of IL-2 following intermittent injection of specific antigen utilized to upregulate IL-2 receptors on donor T cells induces intermittent regrowth and substantial increases in the long-term survival of donor T cells *in vivo* with maintenance of therapeutic function.

CE 010 EFFECTS OF UV RADIATION ON IMMUNITY TO MURINE MELANOMAS, Margaret L. Kripke and Cherrie Donawho, Department of Immunology, The University of Texas M. D. Anderson Cancer Center, Houston, Texas 77030
Exposure of the skin to UV radiation alters the appearance and function of cutaneous antigen presenting cells. These alterations are accompanied by the induction of immunologic tolerance to haptens applied to the UV-irradiated site. We are interested in determining whether UV irradiation might also alter immunity to skin tumors by means of its local effects on cutaneous immunity. To investigate this question, we have used several melanomas induced in C3H mice by combinations of chemical carcinogens and UV radiation. These melanomas are highly immunogenic, and in spite of the fact that they are chemically/UV-induced, they are immunologically crossreactive in in vivo assays. When transplanted into UV-irradiated skin, the melanoma cells develop into tumors earlier and with a higher incidence than in irradiated skin. By examining systemic immunity to the developing melanomas and the activity of tumor infiltrating lymphocytes, we hope to determine whether the effects of UV irradiation on melanoma growth are immunologically mediated, and more specifically whether they are mediated by the local effects of UV radiation on cutaneous antigen presenting cells.

CE 011 TUMOR-BEARING ANIMALS REJECT NORMAL BUT NOT MALIGNANT GRAFTS TRANSFECTED TO EXPRESS THE SAME TARGET ANTIGEN, Hans Schreiber, George A. Perdrizet, Hans J. Stauss, Hartmut Koeppen and Susan R. Ross, Department of Pathology, The University of Chicago, and the Department of Biological Chemistry, The University of Illinois.
A prerequisite for active or passive tumor-specific immunotherapy is breaking the state of immunological unresponsiveness of tumor-bearing individuals. Cancers can be established in the host by various mechanisms and such tumor-bearing hosts fail to mount specific responses to these established tumors even when they are highly immunogenic. Using the highly immunogenic MHC class I antigen K^{216} as a model tumor antigen, we asked whether tumor-bearing mice could respond to the same antigen when presented to the host on nonmalignant cells. The encoding gene for the K^{216} antigen when transfected into progressor tumors resulted in regressor tumors that expressed the K^{216} antigen and these tumors were rejected regularly by normal mice but grew progressively in tumor-bearing mice. Transgenic mice were derived in order to obtain normal cells and tissues expressing the same gene product. We found that normal mice rejected K^{216}-positive normal or malignant tissue grafts and generated K^{216}-specific CTL *in vitro* and *in vivo* in response to these challenges. The immune responses of tumor-bearing mice were different. Such mice failed to reject K^{216}-positive tumors nor did they generate K^{216}-specific CTL. Such mice, however, rejected nonmalignant K^{216}-positive tissue grafts and generated K^{216}-specific $CD8^{+}$ CTL *in vivo* and *in vitro* after stimulation with nonmalignant K^{216}-positive tissues. Surprisingly, while mice bearing K^{216}-positive tumors rejected K^{216}-positive normal tissue grafts the response of such an animal failed to lead to rejection of the simultaneously present tumor graft expressing the same antigen. In fact, this immunity had no measureable effects whatsoever on tumor size or incidence and caused no selection for antigen loss variants. These results, taken together, suggest that transfer of expression of a target antigen into nonmalignant cells provides a way for obtaining an effective induction of antigen-specific CTL in the tumor-bearing host, but it is also demonstrated that additional therapeutic manipulations are required to cause immunological rejection of established tumors.

Idiotypes and T Cell Recognition of Tumor Antigens

CE 012 MURINE ANTIIDIOTYPIC MONOCLONAL ANTIBODIES IN MELANOMA. IMMUNOCHEMICAL CHARACTERIZATION AND CLINICAL APPLICATIONS.
S. Ferrone, G. Chen, H. Yang, M. Yamada, Y. Zheng, and A. Mittelman. Departments of Microbiology and Immunology and of Medicine, New York Medical College, Valhalla, NY 10595 (USA)
Murine antiidiotypic monoclonal antibodies have been developed to syngeneic monoclonal antibodies which recognize distinct determinants of the human high molecular weight–melanoma associated antigen (HMW–MAA). Studies in animal model systems have shown that three antiidiotypic monoclonal antibodies are the mirror image of the HMW–MAA, since they can induce humoral and cell mediated immunity to HMW–MAA. Two of the antiidiotypic monoclonal antibodies have been used in a phase I clinical trial in patients with stage IV melanoma. In spite of the development of anti mouse Ig antibodies repeated injections of murine antiidiotypic monoclonal antibodies were not associated with allergic or anaphilactic reactions. In at least two patients immunization with antiidiotypic monoclonal antibodies induced anti HMW–MAA antibodies. These results suggest that murine antiidiotypic monoclonal antibodies to syngeneic anti HMW–MAA monoclonal antibodies represent useful reagents to implement active specific immunotherapy in melanoma.

CE 013 ANTI-IDIOTYPES IN CANCER PATIENTS, Dorothee Herlyn, The Wistar Institute of Anatomy and Biology, Philadelphia, PA 19104

The immunomodulatory role of anti-idiotypic antibodies (Ab2) in patients with gastrointestinal cancer has been demonstrated in two types of clinical trials[1,2]. In the first, cancer patients were treated with a monoclonal antibody CO17-1A (Ab1) defining a 30/40 kDa gastrointestinal cancer-associated antigen. Ab1 administration initiated an idiotypic network as demonstrated by the induction of both Ab2 and anti-anti-idiotypic antibodies (Ab3) in the treated patients. The Ab2 were directed against the antigen-combining site of Ab1 and therefore may bear the internal image of the antigen defined by Ab1. The Ab3 inhibited binding of autologous Ab2 to Ab1. The Ab3 bound to tumor cells and isolated tumor antigen with the same specificity as the Ab1 at the beginning of the idiotypic cascade. A beneficial role of the Ab3 is postulated for patients showing delayed clinical responses to Ab1 therapy.

In another trial, patients with advanced colorectal cancer responded to immunization with polyclonal goat Ab2 that functionally mimicked *in vitro* and *in vivo* (animals) the CO17-1A antigen by developing highly specific Ab3 with anti-tumor binding reactivities. The Ab3 competed with Ab1 for binding to the same epitope. The Ab3 responses may underlie the clinical responses observed in some of the Ab2-treated patients. Thus, Ab2 are promising agents in immunotherapy approaches to cancer.

Recently, monoclonal Ab2 have been developed against Ab1 CO17-1A in rats. In preclinical studies, the monoclonal Ab2 were superior to the polyclonal goat Ab2 in their capacity to induce antigen-specific Ab3. These monoclonal Ab2 therefore are candidates for treatment of cancer patients (Supported by NIH grant CA43735).

1. M. Wettendorff et al. *Proc. Natl. Acad. Sci. USA* 86:3787 (1989)
2. D. Herlyn et al. *Proc. Natl. Acad. Sci. USA* 84:8055 (1987)

CE 014 ANTI-IDIOTYPES AS THERAPEUTIC REAGENTS, **Heinz Kohler, J-J Chen, F. Norton, A. Mittelman, S. Ferrone and Y. Saeki. IDEC Pharmaceuticals, La Jolla CA 92037 and New York Medical College, Walhalla, NY.**

Monoclonal anti-idiotypic antibodies have been used as "Network Antigens" to induce immunity against tumor-associated antigens in animals and humans. We have evidence in the murine L1210 tumor system that the response to anti-ids is under the regulation by T cells. To investigate these mechanisms, T cell clones were established which respond to anti-ids. The response is MHC restricted and requires processing and presentation. Two kinds of Th cell clones were characterized: one recognizes Fc determinants and the other Fab idiotopes. Both kinds of clones can play a role in the induction of anti-TAA antibodies.

In a parallel study on the effectiveness of anti-id in patients we established a SCID mouse model. SCID mice were reconstituted with peripheral blood monocytes (PBMs) from normal controls and melanoma patients. These Human/SCID mice were immunized with murine anti-idiotypic monoclonal antibody (Ab2). We have detected human anti-anti-Id antibodies (Ab3) in some SCID mice reconstituted with PBMs from Ab2-immunized melanoma patients. These data suggest that SCID mice reconstituted with PBMs from tumor patients may be a viable model for evaluating the immune response of the tumor patients to therapeutic anti-idiotype therapy.

Human Tumor-Specific T Cell Lines and Clones

CE 015 T CELLS RESPONSIVE TO HUMAN TUMOR ANTIGENS, Olivera J. Finn, Department of Microbiology and Immunology, Duke University Medical Center, Durham, NC 27710.
Tumors of epithelial cell origin secrete large, highly glycosylated molecules of the mucin family. Produced by epithelial cells lining the ducts, mucins are normal components of ductal secretions. During the malignant transformation of one of these cells the normal tissue architecture is disrupted and mucins are secreted into the peripheral circulation. We have obtained evidence that pancreatic and breast cancer patients have T cells which proliferate in vitro when specifically stimulated with purified mucins, and/or kill mucin producing pancreatic and breast tumor targets. Cloning and sequencing of the breast mucin cDNA reveals a polypeptide consisting of 20 amino acid, tandemly repeated, conserved peptides. Each peptide contains one T cell stimulating epitope which can be blocked by a specific monoclonal antibody. This antibody also blocks target cell lysis. The multivalent nature of the whole mucin molecule allows its recognition by the T cells in the absence of antigen presenting cells. Recognition of the single peptide, however, requires autologous antigen presentation. The same peptide can evidently bind to ClassII molecules and preferentially stimulate CD4+ T cells, or to the ClassI molecules giving rise to cytotoxic T cells. The mode of presentation may depend on the specific antigen presenting pathway. Mucins on the tumor cell itself may be preferentially presented as endogenous antigens bound to the ClassI molecules. On the other hand, mucins secreted in circulation may be processed by the antigen presenting cells via the exogenous antigen presenting pathway, bound to the ClassII molecules. Considering that different immune responses are generated in each case it may be useful to assure that the majority of the mucin is presented as an endogenous antigen, giving rise to a potentially very efficient cytolytic anti-tumor response. We are attempting to test this approach in vitro by transfecting autologous antigen presenting cells, EBV transformed B cells, with an expression vector containing mucin cDNA sequences, and capable of high level of replication and expression in EBV transformed B cells. We are examining the T cell response generated by stimulation of tumor draining lymph node cells with the transfected antigen presenting cells, and comparing it with the T cells generated in response to the mucin peptide fed to the antigen presenting cells.

CE 016 MELANOMA ANTIGENS RECOGNIZED BY HUMAN T CELLS, Peter Hersey, Clifford J. Meldrum, Chen Qiyuan, Vincent Daniel and Harko J.B. Werkman, Immunology and Oncology Unit, Royal Newcastle Hospital, Newcastle, NSW 2300, Australia.
Antigens recognized by cloned cytotoxic T lymphocytes (CTLs) from patients with melanoma were examined by methods based on the ability of antigens immobilized on nitrocellulose paper to stimulate proliferation of the CTLs. The proliferative response depended on the presence of histocompatible antigen-presenting cells (APCs) in the cultures in the form of either autologous lymphoid cell lines (Epstein-Barr virus-transformed B cells) or histocompatible peripheral blood lymphocytes and was maximal at 3 days. Presentation appeared to be via class II major histocompatibility complex antigens, in that monoclonal antibodies (MAbs) against the class II antigens, but not the class I antigens, on the APCs inhibited the proliferative responses. Extracts from several nonmelanoma 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+ CTLs appeared to be similar to their cytotoxic activity, but CTLs with the CD3+ CD16+ CD8+- phenotype had wider cytotoxic activity against target cells not stimulating proliferative responses. The antigen(s) responsible for the stimulation were shown to migrate on the gels in the mol wt ranges of 46-50 and 35-40kD. Their biochemical nature and relation of these fractions to those identified by serological responses of the patients are under investigation.

Reference: J Natl Cancer Inst 1988;80:826-835.

CE 017 FUNCTION, RECOGNITION STRUCTURE AND ACTIVATION OF AUTOLOGOUS TUMOR-SPECIFIC T CELL CLONES IN HUMAN METASTATIC MELANOMA TUMOR-INFILTRATING LYMPHOCYTES, Kyogo Itoh, Marie A. Salmeron and Paul F. Mansfield, Departments of Surgery and Immunology, The University of Texas, M.D. Anderson Cancer Center, Houston, TX 77030. T cell clones established from tumor-infiltrating lymphocytes (TIL) of human metastatic melanoma were investigated for surface phenotypes, functions (cytotoxicity and interleukin 2 production), recognition structures and activation-requirements. T cell clones from melanoma TIL primarily displayed cytotoxicity restricted to autologous tumor cells, although percentages of those autologous tumor-specific CTL among total established T cell clones varies with individual patients, ranging from 10% to 80%. Some of these autologous-specific CTL produced IL-2 in response to autologous, but not allogeneic tumor cells. No classic markers for T cell subsets correlated with either cytotoxicity or IL-2 production. Anti-CD3 mAb and anti-TCR$\alpha\beta$ mAb inhibited the cytotoxicity at the effector phase, while primarily enhancing IL-2 production in response to autologous tumor cells. Heteroconjugates of anti-CD3 mAb to anti-melanoma mAb increased both the cytotoxicity and IL-2 production mediated by autologous tumor-specific CTL clones. Treatment of tumor cells with anti-MHC Class I mAb inhibited the cytotoxicity. These results suggest that TCR$\alpha\beta$ on effectors and MHC Class I antigens on target cells are involved in the recognition of autologous melanoma cells by T cell clones. All these clones required IL-2 in culture for the proliferation and differentiation. IL-4, but not IL-6, or autologous tumor cells facilitated the activation of T cell clones.

CE 018 CELLULAR IMMUNITY IN SARCOMAS: THE ROLE OF AUTOLOGOUS TUMOR CELLS IN PREVENTING LAK CELL INDUCTION IN VITRO, Susan F. Slovin and Michael J. Mastrangelo, Division of Medical Oncology, Dept. of Medicine, Jefferson Medical College, Philadelphia, PA 19106 Peripheral blood lymphocytes (PBLs), upon stimulation with recombinant interleukin-2 (r-IL-2), acquire the capacity to kill a variety of tumor targets in a 4-hour ^{51}chromium-release assay. The common marker on the target tumor cells that is recognized by these lymphokine activated killer (LAK) cells has yet to be identified; however, the ability of the cells to kill most fresh human tumors, as well as tumor cells lines *in vitro*, has encouraged their use in clinical trials. We have studied the generation of cytotoxic T lymphocytes (CTLs) using PBLs from patients with a variety of soft tissue and bone sarcomas. PBLs incubated with r-IL-2 plus autologous tumor cells killed sarcoma tumor targets in a more restricted manner similar to that of tumor infiltrating lymphocytes, than did LAK cells developed in parallel using PBLs from the same patients. The only distinction between the two induction methods was the addition of autologus irradiated tumor (AIT) cells at the initiation of cultures that produced "educated" lymphocytes. The immediate addition of autologous tumor cells yields lymphocytes whose cytotoxic activity is limited to autologous tumor and to MHC concordant allogeneic tumor of similar histologic types. The delayed addition of autologous tumor to LAK cell cultures also modifies the cytotoxic response of the cultured lymphocytes. Preliminary experiments suggest that modulation of a particular cell population occurs in AIT/lymphocyte cultures and that this event can occur as early as one hour after this culture is initiated. The modulation of culture conditions to yield highly specific cytotoxic cells for tumor targets may, in fact, also be HLA-A2 subtype related, as part of the cytotoxic response appears to be governed by the HLA-A2 haplotype.

Animal Models of Adoptive Therapy-II

CE 019 CD4+ AND CD8+ T CELLS EFFECTIVE IN THERAPY OF A DISSEMINATED RETROVIRAL LEUKEMIA EXHIBIT DISTINCT SPECIFICITIES FOR TUMOR ANTIGEN AND REQUIREMENTS FOR ACTIVATION AND PROLIFERATION, Philip D. Greenberg, Jay P. Klarnet, Kirk R. Schultz, Donald E. Kern and Martin A. Cheever, Division of Oncology, Department of Medicine, RM-17, University of Washington, Seattle, WA 98195. A murine adoptive therapy model has been developed to examine the requirements for T cells to promote eradication of a disseminated, retrovirus-induced syngeneic leukemia. Complete elimination of the FBL leukemia requires that the transferred T cells proliferate in the host and mediate an anti-tumor effect for more than 30 days. Non-cytolytic CD4+ T cells, which cannot directly recognize this class II-negative tumor, can promote complete tumor eradication without participation of CD8+ T cells. This anti-tumor activity apparently results from lymphokine secretion that can activate macrophages to a tumoricidal state. Similarly, cytolytic CD8+ T cells can promote complete tumor elimination in the absence of CD4+ T cells, but this activity potentially reflects both direct cytolysis and secretion of lymphokines that activate tumoricidal macrophages.

These studies suggested that the disparate efficacy of individual T cell subsets observed in therapy of particular tumors might reflect the efficiency with which a T cell subset recognizes a tumor antigen and is specifically activated in response to the tumor, rather than reflect susceptibility of the tumor as a target to a single effector mechanism. Therefore, *in vitro* studies were performed to define the requirements for activation and proliferation of each T cell subset in response to FBL tumor. Activation of primed CD4+ T cells was dependent upon macrophages to degrade tumor antigens in the lysozomal compartment and present the antigens in the context of class II molecules, and proliferation occurred in response to production of IL-2 and IL-4 as endogenous growth factors. Although primed CD8+ T cells recognized the tumor cells directly, activation still required the production of IL-1 by accessory cells, and proliferation resulted from the production of IL-2 but not IL-4 as an endogenous growth factor. Additional analyses were performed *in vivo* to define the requirements for priming to the tumor antigens. Efficient induction of T cell responses required the presence of host B cells, presumably to serve as an APC. Moreover, during *in vivo* priming to tumor antigens, CD8+ T cells could not be adequately primed in the absence of concurrent activation of CD4+ T cells.

The identity of the retroviral FBL tumor antigens recognized by the T cell subsets was assessed using transfected cell lines and recombinant vaccinia viruses containing retroviral *envelope* and/or *gag* genes. Despite presentation of these large unique retroviral antigens in several potentially immunogenic matrices, each subset demonstrated highly selective antigen recognition. Class II-restricted CD4+ T cells recognized *envelope*- and not *gag*-encoded antigens independent of presentation by the tumor cell or a recombinant vaccinia virus, and class I-restricted CD8+ T cells conversely recognized *gag*- and not *envelope*-encoded antigens. By analogy, it can be predicted that human tumors found to express a single unique and potentially immunogenic tumor antigen may not efficiently induce both CD4+ and CD8+ T cell responses, and analysis of candidate antigens must employ methods that activate and induce responses by both subsets.

Abstract Withdrawn

CE 021 SPECIFIC ADOPTIVE IMMUNOTHERAPY MEDIATED BY IN VITRO ACTIVATED, TUMOR-PRIMED T LYMPHOCYTES, Suyu Shu and Alfred E. Chang, Department of Surgery, University of Michigan School of Medicine, Ann Arbor, MI 48109

The adoptive transfer of tumor-sensitized T lymphocytes is an effective means to mediate the regression of a variety of established experimental tumors. A critical component of successful adoptive immunotherapy is the availability of large numbers of properly sensitized antitumor T cells. In animal studies, lymphoid cells from highly immunized syngeneic animals demonstrate potent antitumor effects. Similar effector cells are difficult to obtain from animals with progressively growing tumors. Since the ability of generating effector cells from cancer patients is an inherent requirement for cellular therapy, our goal has been to define principles and to develop necessary techniques for isolating and propagating tumor-reactive lymphocytes from tumor-bearing animals. With the use of the weakly immunogenic MCA 105 murine sarcoma, we demonstrated that, although lacking in vivo antitumor effects, lymph nodes draining a growing tumor contained tumor-primed pre-effector cells. These cells could be stimulated and expanded to acquire full immunologic function by an in vitro sensitization (IVS) procedure. The IVS required antigenic stimulation provided by tumor cells and the presence of IL-2 for cellular proliferation. The ability to elicit pre-effector cells during tumor progression appeared to reflect the intrinsic immunogenicity of the tumor because the growth of a defined nonimmunogenic tumor, MCA 102 failed to stimulate a pre-effector cell response. However, tumor-sensitized pre-effector cells were detected in the lymph nodes draining an inoculation of tumor cells admixed with a bacterial adjuvant, Corynebacterium parvum. Effector cells generated following IVS mediated the regression of established MCA 102 metastases and prolonged survival. Considering the potential clinical application of the IVS procedure, the need for large numbers of autologous tumor cells may present a serious impediment. In an attempt to identify methods of IVS utilizing reagents other than viable tumor cells, we have recently found that tumor-primed pre-effector cells could be activated with anti-CD3 monoclonal antibodies to acquire antitumor efficacy. This activation procedure brought about an 8-fold cell expansion in 5 days. In adoptive immunotherapy experiments, these cells mediated the regression of established tumors in an immunologically specific manner. Based on these observations, clinical trials are being initiated to test the therapeutic efficacy of similarly activated T lymphocytes in the treatment of humans with advanced cancer.

CE 022 IMMUNIZATION WITH SOLUBLE GP96 ANTIGENS ELICITS TUMOR - SPECIFIC CELLULAR IMMUNITY, Pramod K Srivastava, Department of Pharmacology, Box 1215, Mount Sinai School of Medicine, City University of New York, New York, NY 10029

Cell surface glycoproteins of 96,000 d (gp96) have been implicated in the individually distinct immunogenicity of chemically induced tumors of rats (1) and mice (2,3). Mouse gp96 genes and cDNAs have been isolated, characterized (4) and mapped to chromosome 10 (5). More than one type of gp96 - related transcripts and genes have been identified in mice and humans (6,7). However, tumor - specific alterations have not been detected so far. Sequencing of gp96 genes reveals that they bear homology with stress - induced proteins of the hsp90 family and are closely related to hsp100 family (8). The basis of immunogenicity of these highly conserved proteins is unclear.

We have demonstrated that the individually specific immunogenicity of methylcholanthrene - induced BALB/c mouse sarcomas is T cell mediated (9). In order to explore the role of soluble gp96 antigens in eliciting tumor - specific T cell immunity, mice were immunized with purified gp96 derived from Meth A sarcoma. Splenic T lymphocytes from gp96 - immunized mice were adoptively transferred to naive mice, which were then challenged with live Meth A and CMS5 sarcoma cells : mice were resistant to Meth A, but not CMS5 challenge. Normal splenic T lymphocytes did not impart resistance to either tumor. Adoptive immunotherapy of mice with *pre-existing tumors* was also successful and showed a similar specificity : 5 day old Meth A tumors, but not CMS5 tumors underwent partial to complete regression after transfer of splenic T lymphocytes from Meth A gp96 - immunized donors. Purified soluble gp96 antigens thus elicit T cell immunity which is active, not only prophylactically, but also in a curative manner. Supported by a FIRST Award (CA44786) and Irma T Hirschl Award.

(1) Int J Cancer 1984 *33*, 407. (2) PNAS 1986 *83*, 3407. (3) Immunol Today 1989 *9*, 78. (4) PNAS 1987 *84*, 3807. (5) Immunogenet 1988 *28*,205. (6) VII Int Congress of Immunology, 1989 Abst 127-13. (7) Cancer Res 1989 *49*, 1341. (8) VII Int Congress of Immunology, 1989Abst 101-28. (9) Cancer Res 1987 *47*, 5074.

T Cell Growth Factors

CE 023 USE OF RECOMBINANT HUMAN IL-2 AND IL-4 IN VITRO AND IN VIVO TO EXPAND TIL'S.
Michael T. Lotze, Surgery Branch, National Cancer Institute, Bethesda, MD 20892.
Approximately 30-50% of patients with melanoma can have autologous MHC restricted TIL's with cytolytic activity preferential for the autologous tumor expanded with IL-2. Expansion to numbers sufficient for adoptive immunotherapy and for prolonged periods for immunologic studies is impossible in many cases. We have shown that IL-4 promotes the growth of TIL's specific for human autologous melanoma (J. Exp. Med. 168:2183-2191, 1988) and that TNFα, γ-IFN and IL-6 enhance TIL growth, especially at low concentrations of IL-2, but nonspecifically. Antigenic restimulation, provision of other growth factors, host pretreatment in vivo with cytokines including alpha interferon and IL-2 prior to tumor harvest, addition of antibodies to TGFβ and other inhibitory factors and provision of substrate molecules including laminin, collagen, and fibronectin (ligands of some members of the integrin family) to late cultures are all studies currently in progress to allow further expansion of antigen reactive TIL's. In addition, studies of antigen processing and identification of unique T cell receptor rearrangements important in melanoma recognition are in progress and will be discussed. The administration of rIL-2 to patients with cancer has now been extended to many patients at this and other centers. At least 25-30% percent of patients with melanoma and renal cell carcinoma respond with objective regression of cancer. Well defined toxicities of treatment include primarily those associated with a vascular leak syndrome with endothelial activation occurring in all treated patients. This is presumably mediated by the secondary elaboration of other cytokines which include TNF , interferon-α and IL-6. Peripheral lymphoid cells expand in vivo with IL-2 treatment to 2-20 fold greater numbers than prior to therapy. Far less is known about the in vivo role of IL-4. We have treated a total of 44 patients on an escalating dose protocol and have now defined a maximum tolerated dose of 20 ug/kg given thrice daily. Toxicity has included fever, chills, nausea/vomiting, diarrhea, epigastric distress, peripheral edema and malaise. Weight gain, sinus and nasal congestion, and gastritis have been the dose limiting toxicities. Using either a bioassay based on the induction of CD23 or an ELISA, a half life of 8 minutes (alpha distribution) and a second slower clearance (β phase) of 48 minutes is found. Profound lymphopenia, relative decreases in CD4 positive cells, induction of detectable (110-200 units) levels of soluble CD23, and lack of major changes in immunoglobulin levels or type were noted. Future plans include early initiation of IL-2/IL-4 combination trials.

CE 024 INVERSE IMMUNOREGULATORY EFFECTS OF TNF-α AND TGF-β ON T-CELL PROLIFERATION. Michael A. Palladino, Irene S. Figari, Glenn C. Rice, Gerald, E. Ranges and Paula M. Jardieu. Department of Immunology Research and Assay Technologies, Genentech Inc., South San Francisco, CA 94080
Recent studies have demonstrated that both TNF-α and TGF-β are potent regulators of a variety of T-cell functions *in vitro*. We have defined in greater detail the range of activities for TNF-α and TGF-β on a murine cytotoxic T-cell clone CT-6. This cell line is unique as it will proliferate specifically in response to a variety of agents including IL-2, IL-4, TNF-α and PMA. At concentrations ranging from 0.01 to 10.0 ng/ml, TGF-β_1 significantly inhibited IL-2 and IL-4 induced proliferation of CT-6 cells: IL-2 responses were maximally inhibited approximately 50% and IL-4 responses 90%. However, even at concentrations as high as 25 ng/ml, TGF-β failed to inhibit TNF-α induced proliferation. In fact TGF-β treated CT-6 cells showed slightly increased proliferative responses to TNF-α. To determine whether these effects were the result of receptor down regulation by TGF-β, IL-4 and TNF-α receptor expression was determined by flow cytometric techniques using directly biotinylated IL-4 and TNF-α and the IL-2 receptor by an IL-2 p55 receptor monoclonal antibody. The results demonstrated that TGF-β did inhibit the expression of the IL-2 p55 receptor which may account for the decrease in proliferation to IL-2. However, no effect was detectable on the expression of either the IL-4 or TNF-α receptor suggesting that the inhibitory effect of TGF-β was not mediated by receptor downregulation. In addition, the proliferative responses of CT-6 cells to PMA was also significantly inhibited by TGF-β: PMA responses were maximally inhibited approximately 90% at 1ng/ml TGF-β. Interestingly, the inhibitory effects of TGF-β on IL-4 induced proliferation were not seen in the presence of TNF-α. On the contrary, the inhibitory effects of TGF-β on PMA induced proliferation of CT-6 cells were observed even when TNF-α was present in the cultures. Our results suggest that the inhibitory effects of TGF-β on T-cell proliferation are specific for the agent providing the proliferative signal. Given the well established effects of PMA on protein kinase C, the findings suggest that one mechanism by which TGF-β suppresses T-cell proliferation is through inhibition of an intermediate in the PKC activation pathway.

Cellular Immunity and the Immunotherapy of Cancer

CE 025 INTERLEUKIN-6 IN HOST-TUMOR INTERACTION AND IN T CELL ACTIVATION,
Pravinkumar B. Sehgal, Laboratory of Cell Physiology and Virology, The Rockefeller University, New York, NY 10021.
The cytokine interleukin-6 (IL-6) is emerging as an important participant in the host-tumor interaction. Strong-to-moderate IL-6 immunoreactivity is observed in the neoplastic and stromal cellular elements present in a variety of solid tumors (primary squamous cell carcinomas, adenocarcinomas of mammary, colonic, ovarian, and endometrial origin, various adenocarcinomas metastatic to lymph nodes, soft tissues tumors including leimyosarcoma and neurofibrosarcoma). Furthermore, a substantial proportion of cancer patients have elevated levels of circulating IL-6. On the one hand, IL-6 inhibits the proliferation of tumor cells derived from certain carcinomas, whereas on the other hand, IL-6 enhances the proliferation of myeloma cells. High levels of IL-6 are observed in patients administered various immunomodulators (administration of IL-2, TNF or endotoxin). Although in peripheral blood mononuclear cell cultures OKT3 enhances production of IL-6, this phenomenon still remains to be investigated in a clinical setting. It is likely that the induction of circulating endogenous IL-6 contributes to the altered immunological and biochemical status of the host. IL-6 strongly synergizes with cytokines like IL-1 to enhance T cell activation in a variety of different experimental systems (conA-activated thymocytes and mitogen- but not antigen receptor-activated D10.G4.1 cells). Although IL-6 clearly stimulates the cytotoxic activity of NK cells (via an IL-2-dependent pathway), its effect on LAK cells is less clear. An important facet of the role of IL-6 in the host-tumor interaction is highlighted by the following new observation (in collaboration with Dr. Igor Tamm): although IL-6 inhibits the proliferation of T47D and ZR-75-1 breast carcinoma cells, it increases the motility of these cells (assayed by time-lapse cine microphotography) and decreases adherens junctions in these breast carcinoma cell lines, raising the possibility that IL-6 may play a role in enhancing the invasiveness of certain tumors. On balance, it is not yet clear whether IL-6 is of net "benefit" to the host or the tumor.

Related Systems: Allograft Rejection and Autoimmunity

CE 026 Cellular Mechanisms Involved in the Rejection of Murine Allografts,
A.S. Rosenberg, Division of Cytokine Biology, F.D.A. Bethesda, MD 20892.
Allograft rejection is a complex process that is contingent on crucial cellular elements of both the donor tissue and responsive host T cell populations. Tissue grafts are a collection of cells, heterogeneous both in function and in expression of histocompatibility (H) antigens. For rejection to occur, tissue grafts must 1) contain cells with antigen presenting activity (APCs) that can activate host T cell populations and 2) express cell surface alloantigens capable of serving as target ligands for cytolytic effector T cells. The host requirement for rejection of a tissue graft is the presence of adequate populations of antigen specific T helper (T_h) and T effector (T_{eff}) cells that can be activated by APCs of the graft. Upon activation by graft APCs, T_h secrete a variety of cytokines which not only activate effector T cell populations, but also modulate the expression of Major Histocompatibility Complex (MHC) antigens on graft cells. The effector mechanism mediating rejection of both skin and pancreatic islet grafts is exquisitely antigen specific, destroying only cells which express allogeneic H antigens but not those expressing syngeneic H antigens. This pattern is consistent with the activity of antigen specific cytolytic T_{eff}.

To gain insight into the cellular interactions involved in graft rejection responses, we studied two systems in which skin grafts expressing MHC disparities fail to be rejected. The response of B6 mice to both Qa-1^a and bm6 alloantigens is characterized by a deficiency of antigen specific T helper cells, resulting in long term maintenance of the skin grafts. However, B6 mice uniformly reject these grafts if simultaneously engrafted with a second skin graft expressing bm6 or Qa-1 determinants plus additional H disparities. In this circumstance, $CD4^+$ T_h specific for the additional determinants provide requisite second signals for activation of Qa-1^a or bm6 effector cells. In the case of Qa-1 disparate grafts, however, initial exposure of the host to Qa-1^a skin grafts in the absence of these additional helper signals leads to a state of specific immunologic tolerance. The immunogenicity versus tolerogenicity of tissue grafts will be discussed.

CE 027 THE USE OF MONOCLONAL ANTIBODIES FOR TREATMENT OF AUTOIMMUNE DISEASE, Lawrence Steinman, Departments of Neurology, Pediatrics and Genetics, Stanford University School of Medicine, Stanford, CA 94305-5235.

Over the past decade monoclonal antibodies have been successfully employed in a number of animal models of autoimmune disease. We have employed antibodies to the class II gene products of the major histocompatibility complex (MHC), the CD4 molecule on helper T cells, and the T cell receptor (TcR).

Monoclonal anti-class II antibodies have been used to reverse paralytic disease in the animal model of multiple sclerosis (MS), experimental autoimmune encephalomyelitis (EAE). These antibodies not only reverse acute paralytic disease but are able to decrease the number of relapses in a model of relapsing/remitting MS when given after the first attack. The advantage of this form of therapy over other therapies is that it is haplotype specific. In otherwords, in a heterozygous individual it is possible to block the MHC gene associated with disease susceptibility while leaving other MHC gene products free for antigen presentation. Thus, animals given this form of immunotherapy are not significant-ly immune suppressed.

Antibodies to the CD4 molecule have been equally effective in treating animal models of autoimmunity. Thus, we and others have reversed ongoing paralysis in EAE. Relapses have been diminished after the administration of anti-CD4 antibodies. Like antibodies to MHC class II molecules, antibodies to anti-CD4 have been successfully used to treat animal models of systemic lupus erythematosus, rheumatoid arthritis, and myasthenia gravis. Recently trials with anti-CD4 antibodies have been successful in the treatment of rheumatoid arthritis and cutaneous T cell lymphoma. The latter trial employed a chimeric human/mouse antibody.

Antibodies to the variable region of the TcR have recently been employed to treat EAE. These antibodies were again effective in both preventing and reversing ongoing disease. These antibodies targeted the variable region gene products of TcR's that were involved in autoimmune disease. It is remarkable that a limited heterogeneity of TcR's is responsible for autoimmune conditions. However, in certain instances the TcR repertoire is more diverse and may require a cocktail of monoclonal antibody reagents. Other approaches to treatment of autoimmune disease based on targeting the variable region of the TcR involve active molecular vaccination. This will also be discussed during the presentation.

CE 028 T CELLS REACTIVE TO HEAT SHOCK PROTEINS IN AUTOIMMUNE DISEASE, S. Strober and J. Holoshitz, Department of Medicine, Division of Immunology and Rheumatology, Stanford University School of Medicine, Stanford, CA 94305.

Heat shock proteins (HSP) are highly conserved among prokaryotic and eukaryotic cells, and their synthesis is increased by stress or injury. Ordinarily HSP are intracytoplasmic, but recent evidence indicates they can function as immunogens on the surface of stressed macrophages or methylcolanthrene induced tumors in mice[1,2]. T cell clones reactive to mycobacterial 65 Kd HSP can induce arthritis after injection into rats. T cells obtained from the joints of patients with rheumatoid arthritis (RA) frequently proliferate in vitro in response to stimulation with mycobacterial protein extracts. We have derived a panel of mycobacterial protein reactive T cell clones from the joints of patients with RA. Approximately half expressed the γ, δ T cell receptor and half expressed the α, β receptor. Studies of γ, δ clones showed that they could respond to the purified 65 Kd HSP without HLA restriction. A single exposure of a mixture of RA synovial T cells and fibroblasts in the continued presence of rIL-2 induces a self-perpetuating proliferation of the cell mixture which can be serially propagated for at least several months. The cultures form organized tissue outgrowths which have the appearance of the fresh inflammatory RA joint tissues.

1. Koga, T. et al. Science 245, 1112-1115, 1989.
2. Ullrich, S.J. et al. Proc. Natl. Acad. Sci. USA 83, 3121-3125, 1986.

Clinical Trials of Adoptive Immunotherapy

CE 029 TUMOR INFILTRATING LYMPHOCYTES AND INTERLEUKIN 2 IN THE TREATMENT OF HUMAN CANCER. James T. Kurnick, Lenora Boyle, Franco Pandolfi, Beverly Grove, Gail Hawes, William Bennett, Jose Solovera, David Lazarus, Steven Dubinett, Frederic Preffer, Elise Davidson, Ronald Callahan, H. William Strauss, Julie Gifford, Clare Pinto and Richard Kradin, Department of Pathology, Massachusetts General Hospital and Harvard Medical School, Boston, MA 02114.

Tumor-infiltrating lymphocytes (TIL) were propagated in recombinant Interleukin 2 (IL2) directly from small tumor biopsy samples of 28 patients with advanced cancers (13 malignant melanoma, 7 renal cell carcinoma, and 8 non-small cell lung cancers). The patients were treated with autologous expanded TIL (about 10^{10}) and continuous infusions of recombinant IL 2 (1-3 x 10 U/m per 24 hours). 29% of the patients with renal cell cancer and 23% of those with melanoma achieved objective tumor responses lasting 3-14 months. None of the patients with lung cancer responded to this therapy. Toxic side effects were limited, and no patient required intensive care monitoring. An additional 12 patients with malignant melanoma were treated with TIL and IL 2 and cyclophosphamide (15mg/kg), but none of these patients achieved a sustained therapeutic response. The mechanisms by which TIL and IL 2 reduce the tumor burden is uncertain. The distribution of radiolabelled TIL showed that the majority of the injected cells were located in the lung, liver and spleen, but did not concentrate at sites of tumor. While all of the patients showed an increase in NK and LAK activity in the blood during IL 2 therapy, this activity did not correlate with clinical responses. These data suggest that neither the TIL nor LAK cells directly are responsible for the tumor reduction in responding patients, and it seems likely that the therapy relies on amplification of cell-mediated responses as well as production of soluble mediators. The histology of responding tumors demonstrated intense cellular infiltration and also vascular injury. Infiltration of lymphocytes into small tumor-associated vessels may be associated with tumor regression. Additional studies of the T cell receptor repertoire among TIL suggests that limited diversity may be a feature of the host response to tumors. Cloning of T cells from TIL which show specific anti-autologous tumor lysis will help to elucidate this observation.

CE 030 ACTIVE, ADOPTIVE, RESTORATIVE AND PASSIVE IMMMUNOTHERAPY: COMBINED TREATMENTS OF HUMAN MELANOMAS, Malcolm S. Mitchell, U.S.C. Cancer Center, Los Angeles, CA 90033. Most classifications recognize four principal types of immunotherapy: 1) **active immunotherapy**, in which the immune system of the tumor-bearing host is specifically and/or nonspecifically stimulated to cause rejection of the tumor; 2) **adoptive immunotherapy**, where immune cells or immunological information is administered; **restorative immunotherapy**, which attempts to replete deficient immunological subpopulations or inhibit suppressor influences; and **passive immunotherapy**, which implies the transfer of antibodies, either as antisera or now more commonly monoclonal antibodies (MAbs). All of these categories are subsumed under the heading of biomodulation (biological response modification), which also includes such things as modification of the behavior of the tumor cell, improvement of the host's ability to tolerate cytotoxic therapy and alteration of tumor cell membranes to make them more easily killed by cytotoxic or immunologic mechanisms. Examples of the effectiveness of all of these categories in at least one type of human tumor exist, and combination regimens of agents in different categories concomitantly or sequentially have been devised. In particular treatment with low-dose cyclophosphamide (**restorative**) and **adoptive** immunotherapy (interleukin-2 ± LAK cells) has been effective in melanoma. **Active** immunotherapy with melanoma "vaccines" and **adoptive** immunotherapy with IL-2 have been combined, and trials of **passive** immunotherapy with MAb after **adoptive immunotherapy** with IL-2 ± LAK cells, to improve the specificity of the LAK cells are in progress. It is theoretically possible to combine all four forms of immunotherapy because they should be complementary to one another but lack additive toxicity. In addition, multifaceted immunotherapy can be used following cytoreductive chemotherapy, to improve further its efficacy in advanced disease.

CE 031 PROSPECTS FOR COMBINATION THERAPIES WITH IL-2: PRECLINICAL IN VITRO ANALYSES WITH CELLS FROM PATIENTS RECEIVING IN VIVO IL-2 TREATMENT.

Sondel P.M. Departments of Pediatrics, Human Oncology and Genetics, University of Wisconsin, Madison, WI 53792.

IL-2 treatment of human immune cells in vitro or in vivo results in activation of the LAK phenomenon, cytokine release, and destruction of both neoplastic and normal tissue. While increasing the intensity of IL-2 therapy may potentially provide a greater anti-tumor effect, it also clearly causes a greater toxic effect to normal tissues. The mechanisms of these toxicities in vivo may include both LAK activity and indirect effects through cytokine release. Because the limiting factor in these treatments is the toxicity generated, improved anti-tumor effects will only be obtained when this same immunologic activation can be focused with greater specificity on tumor tissue. A number of approaches are now being investigated. First, T cells can show greater specificity in vitro than do LAK cells. Potential in vivo induction of such cells might be generated by "artificial" activation of T cell receptors with anti-CD3 monoclonal antibody. Such studies are in progress. A modified phenotype of LAK cells is observed following more than one week of in vivo IL-2 treatment. Sustained prolonged IL-2 treatment might enhance clinical effects. Lymphocytes can mediate augmented tumor destruction if they have Fc receptors and bind to antibody labelled tumor cells. This ADCC reaction is dramatically augmented for cancer patients when PBL are used immediately following in vivo IL-2 therapy. Attempts to induce in vivo ADCC are underway. Finally, the major population of cells activated in vivo to mediate the LAK phenomenon in vitro are CD3- and CD16- cells which are unable to mediate ADCC with conventional antibody. This in vivo activated population of cells might be best brought to tumor cells to mediate their destruction by a bifunctional antibody recognizing triggering structures on the effector and target structures on the tumor.

Cellular Immunity and the Immunotherapy of Cancer

T Cell Activation and Antigen Recognition

CE 100 EXPRESSION OF β1-INTEGRIN RECEPTORS ON A SUBSET OF MELANOMA CLONES WITH ENHANCED SUSCEPTIBILITY TO CELL-MEDIATED LYSIS.
Andrea Anichini, Roberta Mortarini and Giorgio Parmiani, Division of Experimental Oncology D, Istituto Nazionale Tumori, 20133 Milan, Italy.
Marked heterogeneity for susceptibility to lysis by IL-2-activated $CD3^-$ and $CD3^+$-effectors was found among tumor clones from a human metastatic melanoma. Tumor clones with enhanced susceptibility to lysis could also be lysed by $CD3^-$ effectors in Ca^{2+}-free medium and expressed elevated levels of ICAM-1 antigens. Phenotype analysis for β1- and β3-integrins revealed that the same clones with enhanced susceptibility to lysis expressed also a peculiar VLA phenotype, characterized by expression of 4 or 5 distinct β1 integrins including VLA-1, VLA-2, VLA-3, VLA-4 and VLA-6. The phenotypic and functional associations detected at the clonal level were confirmed at the bulk population level by immunoselection experiments which showed that within a heterogeneous neoplastic population the same subset of cells may express: 1) enhanced susceptibility to lysis, 2) increased levels of ICAM-1 and, 3) a peculiar VLA phenotype.

CE 101 SEARCHING FOR A SORTING SIGNAL OF THE MHC CLASS II MOLECULE ASSOCIATED INVARIANT CHAIN (Ii).
Oddmund Bakke and Bernhard Dobberstein. EMBL, Heidelberg, FRG.
The MHC molecules are intracellularily associated with the invariant chain (Ii). This polypeptide has recently been shown to be required for processing/ presentation of native antigen to T helper cells (Stockinger et al. Cell, 56, 683-89, 1989). We have investigated intracellular transport and location of Ii. Ii spans the ER membrane once and exposes its N-terminal on the cytoplasmic side. The assembly of Ii with class II molecules occurs shortly after insertion into the ER membrane. Ii dissociates from the class II molecules in a post Golgi compartment. Preliminary data shows that the invariant chain is found in a post Golgi, mannose-6-phophate receptor rich compartment (Lipp et al., unpublished). Ii must therefore contain sorting information which directs it to this location, or be connected with molecules containing this information. To search for a sorting signal on Ii we have made progressive deletions from the N-terminus and expressed the mutated proteins in CV1 cells. The intracellular location was studied by microscopy. The Ii mutant lacking 11 aa was found in cytoplasmic vesicles like the native protein, whereas deletion of 15 aa resulted in a surface membrane protein. This indicate that intracellular sorting information of Ii is located within the cytoplasmic tail and that aa 12-15 is important for sorting.

CE 102 INDUCTION OF HEAT SHOCK (HSP70) IN HUMAN LYMPHOID CELLS BY CYCLOSPORINE A,
Laurine M. Bow, Michelle E. Pleau, Barbara A. Reynolds and Robert T. Schweizer, Department of Surgical Research, Hartford Hospital, Hartford, CT 06115
Cyclosporine A (CyA) has been shown to be a powerful immunosuppressant when used as therapy to prevent organ transplant rejection. The exact mechanism of action of this drug has yet to be defined, though recent reports have identified its ligand to be a cis trans isomerase involved in protein folding. As members of the HSP70 family have also been associated with folding of damaged proteins, we sought to investigate what effect if any CyA would have on the induction of the heat shock response. Human peripheral blood lymphocytes were isolated and incubated overnight in two concentrations of cyclosporine A. Cells were then heat shocked at 43°C or left at 37°. After four hours, protein extracts of the cells were prepared and separated by SDS gel electrophoresis, followed by standard Western blot to determine the amounts of constitutive and induced HSP70 in each assay. Cells treated with CyA and not subjected to 43°C (heat shock), show a much pronounced level of HSP70 synthesis, as compared to control cells. At low levels of CyA, the increase appears to be due to an increase in synthesis of the constitutive form of HSP70 (HSP73). At higher levels the inducible form (HSP72) is synthesized, indicating that the cells may be stressed when the concentration is increased. Cytospins of the cells were also stained at various intervals to determine which cells were induced. These results may aid in understanding the mechanism of immunosuppression by cyclosporine A. The HSP70 gene has been mapped to the human MHC and HSP70 constitutive protein may be involved in class I protein assembly and peptide presentation, as these processes involve extensive protein folding and transport pathways.

CE 103 LFA-1/ICAM-1 INTERACTION COACTIVATES THE CD3/T CELL RECEPTOR COMPLEX IN TRIGGERING CELL MEDIATED LYSIS. E.Braakman*, P.S. Goedegebuure, R.J. Vreugdenhil, D.M. Segal, S. Shaw and R.L.H. Bolhuis. Dept. of Immunology, Daniel den Hoed Cancer Center, Rotterdam, The Netherlands. Dept. of Tumor Immunology, Radio Biological Institute, TNO Health Organization, Rijswijk, The Netherlands. We examined the contribution of the LFA-1/ICAM-1 interaction to CD3/TCR mediated lysis by CTL. We used ICAM-$1^{- or +}$ tumor cell lines as target cells and anti-CD3- and anti-LFA-1 containing hetero-crosslinked mAb to bridge CTL and targets and to activate CTL. The ICAM-1^- melanoma derived cell line IgR39 was relatively resistant to CD3 mediated lysis by both $TCR\alpha\beta^+$ and $TCR\gamma\delta^+$ CTL, when compared with ICAM-1^+ cell lines. Induction of ICAM-1 on the membrane of IgR39 cells by TNF rendered these cells more susceptible to CD3 mediated lysis. Anti-ICAM-1 mAb inhibited this TNF enhanced susceptibility to lysis, directly demonstrating that the induction of ICAM-1 was critical in the TNF induced increase in susceptibility to lysis of IgR39 cells. CTL formed less efficient conjugates with the ICAM-1^- cells as compared to ICAM-1^+ cells. Both spontaneous and CD3 induced conjugate formation as well as CD3 mediated lysis of ICAM-1^- target cells by CTL were enhanced by the addition of anti-LFA-1 containing hetero-crosslinked mAb thereby mimicking the LFA-1/ICAM-1 interaction between CTL and target cells. Soluble anti-CD18 mAb inhibited CD3 mediated lysis of ICAM-1^- target cells by CTL without affecting their conjugate formation. Taken together, anti-LFA-1 mAb added postconjugate formation still inhibited lysis of both ICAM-$1^{+ or -}$ tumor cells. These findings suggest that the LFA-1/ICAM-1 interaction coactivates CD3/TCR mediated lysis by CTL through both an enhanced CTL-target cell binding and the delivery of postconjugate costimulatory signals.

CE 104 MONOCLONAL ANTIBODIES TO THE HUMAN T LYMPHOCYTE ACTIVATION ANTIGEN DIPEPTIDYL PEPTIDASE IV (CDw26) STIMULATES PROLIFERATION, Susan M. Carroll, John L. Bednarczyk, and Bradley W. McIntyre, Department of Immunology, The University of Texas M.D. Anderson Cancer Center, Houston, TX 77030

Monoclonal antibody (mAb) AC7 was derived from a hybridoma designed to produce antibodies directed against T lymphocyte activation antigens. MAb AC7 and mAb to Ta1 (CDw26) were shown to bind to the same 105,000 kD cell surface protein by reciprocal immunodepletion studies. Solid phase double determinant binding assays demonstrate that these mAbs recognize the same molecule but at spatially distinct sites. The enzymatic activity of DPP IV is depleted by mAb AC7 from lysates of IL2/OKT3 activated PBL. DPP IV activity was detected in lysates from the T cell tumor HSB and normal PBL activated by alloantigen/IL2 or OKT3/IL2 but was not found in the T cell lines HPB-ALL and Jurkat or the B cell lines Pally, Toto, and SGAR. MAb AC7 alone does not induce a proliferative response in PBL. However, proliferation does occur when mAb AC7 is added to PBL in conjunction with PMA.

CE 105 REGULATION OF TNF-α AND IFN-γ SECRETION IN LYMPHOKINE ACTIVATED KILLER (LAK) CELLS, Anita S.-F. Chong, William J. Grimes, Evan M. Hersh and Philip Scuderi, Department of General Surgery, Rush Medical College, Chicago, IL 60612, and Departments of Biochemistry and Microbiology/Immunology, University of Arizona, Tucson, AZ 85724. We recently reported that interleukin-2 (IL-2) activated peripheral blood lymphocytes and $CD3^+$, LAK cell clones release tumor necrosis factor-α (TNF-α) and interferon-γ (IFN-γ) when stimulated with K562, erythroleukemia cells. These cytokines released by LAK cells may play a significant role in mediating tumor regression *in vivo*. We examined the phenotype of IL-2 activated peripheral blood leukocytes that secrete TNF-α and IFN-γ when stimulated with K562 cells and demonstrated that TNF-α secretion is not due to the presence of contaminating mononuclear phagocytes. Further, we demonstrate that activated T-lymphocytes exposed to K562 cells secrete both TNF-α and IFN-γ, while natural killer cells release only IFN-γ under the same conditions. Preliminary attempts at a biochemical definition of the tumor-associated molecules stimulating cytokine release implicate membrane glycoproteins. Our results are discussed in terms of a recently proposed hypothesis that T-lymphocytes require two signals for the optimum induction of TNF-α release.

CE 106 RECOGNITION BY THE CD8 RECEPTOR IS ABLATED BY SUBSTITUTION AT SEVERAL ACID RESIDUES OF THE CLASS I $\alpha 3$ DOMAIN, Janet M. Connolly, Ted H. Hansen, Amie L. Ingold and Terry A. Potter. Department of Genetics, Washington University School of Medicine, St. Louis, MO 63110 and Department of Medicine, National Jewish Center, Denver, CO 80206. The CD8 molecule on class I-reactive cytotoxic T lymphocytes (CTL) is believed to function as a coreceptor along with the $\alpha\beta$ TCR. Whereas the $\alpha\beta$ TCR recognizes polymorphic residues in the $\alpha 1/\alpha 2$ domains of the class I molecule, the CD8 molecule is believed to recognize monomorphic class I residues. Our previous experiments suggested that residue 227 in the $\alpha 3$ domain of MHC class I molecules contributes to the determinant recognized by CD8. Using a panel of site-directed mutants of H-2D^d, this observation has been extended. Our findings indicate that for recognition by CD8-dependent CTL, residue 227 must be either glutamic acid or aspartic acid and cannot be either basic or uncharged. However, the recognition by CD8-independent CTL is unaffected by any of the substitutions at position 227 of H-2D^d. Similarly, alterations of other charged residues at positions 222, 223 and 229 have an analogous effect to substitution at residue 227, whereas substitutions at residues 192 and 232 do not. In addition, mutant H-2D^d molecules that are not recognized by CD8-dependent CTL are unable to stimulate a primary CTL response, yet they can stimulate a secondary CD8-independent H-2D^d-specific CTL response. These findings suggest that CD8 recognition is obligatory for the priming of class I-dependent CTL responses. Since endogenous class I molecules were expressed by all of the transfected cell lines, these findings provide direct genetic evidence that CD8 and the $\alpha\beta$ TCR must interact with the same class I molecule.

CE 107 EXPRESSION AND FUNCTION OF MUTANT Ia ANTIGEN IN TRANSGENIC MICE. Chella David, Bing-Yuan Wei, Javier Martin, Roger Little, Gary Anderson, Suresh Savarirayan, Jean-Marie Buerstedde and David McKean; Department of Immunology, Mayo Clinic and Medical School, Rochester, MN 55905.
For cell surface expression of Ia antigens, assembly of α and β heterodimers is necessary. Previous studies of in vitro transfections have shown that while second polymorphic region (region "B") of A_β polypeptide is a serodominant epitope determining the recognition of A_β by most allele-specific mAbs, it does not affect the α/β chain pairing. We have produced a double transgenic mouse with a wild form A^k_α gene and a mutant A^k_β (A^k_β MB) gene with a d-allele substitution at position 63 and 65-67. Although the data from transfection studies have indicated that A^k_α and A^k_β MB pair with each other and are expressed on the cell surface, the A^k_α and/or A^k_β MB polypeptides were not detected on the surface of lymphoid cells of the transgenic mice prior to LPS stimulation. However, with LPS stimulation, A^k_α/A^k_β MB assembly and subsequent surface expression was induced. The tail skin from transgenic founder mice were rejected by the parental mice indicating a role for the mutant antigen on the allograft. MLR between the founder mice and parental mice also showed that the transgenic product can serve as an alloantigen for lymphocyte proliferation. In addition, the A^k transgenic mice on H-2$^{q/q}$ background can partially delete $V_\beta 6^+$ T cells suggesting the presence of the transgene product in the thymus.

CE 108 CHARACTERIZATION OF A NATURALLY PROCESSED MHC CLASS II-RESTRICTED T CELL DETERMINANT OF HEN EGG LYSOZYME, Stéphane Demotz,* Howard M. Grey,* Ettore Appella† and Alessandro Sette*, *Cytel, 11099 N. Torrey Pines Road, La Jolla, CA 92037, †National Cancer Institute, NIH, Bethesda, MD 20892
Compelling evidence suggests that T cells recognize a complex formed by MHC molecules and antigenic peptide fragments. Such fragments are thought to arise from limited proteolytic degradation of native antigen inside acidic compartments of the antigen-presenting cells. Then, complexes between MHC molecules and antigenic peptides are expressed on the surface of the antigen-presenting cell and recognized by class II-restricted T cells. This concept is based largely on the ability of small synthetic peptides to stimulate T cells without any processing requirement. However, up to now no direct chemical characterization of a physiologically processed antigen has been presented. We have characterized a physiologically processed antigen corresponding to a previously described IE^d-restricted T cell antigenic determinant of hen egg lysozyme (HEL 107-116). The molecular weight of this antigenic material was about 2 kDa. HPLC analysis identified at least three separate molecular species, suggesting limited, albeit significant, heterogeneity of naturally processed peptides. Murine B lymphomas, IE^d-transfected L cells, as well as DR2-EBV B cells, appeared to similarly process this HEL determinant. Finally, under the experimental conditions employed it was calculated that a substantial proportion (10-40%) of IE^d molecules were occupied by these HEL-derived antigenic determinants.

CE 109 MAST CELL PROTEASES LIBERATE STABLE ENCEPHALITOGENIC FRAGMENTS FROM INTACT MYELIN. Gregory N. Dietsch, Dejan Dordevich and David J. Hinrichs. Chiles Research Insitute, Providence Medical Center, Portland, OR 97213 and Immunology Research, Veterans Administration Medical Center, Portland, OR. 97207.
Protease containing supernatants from activated rat mast cells were found to degrade and to release encephalitogenic peptides from purified myelin preparations. The two most abundant peptides were identified as portions of myelin basic protein (MBP) consisting of residues 69-87 (GSLPQKTQRSQDENPVV) and resides 69-88 (GSLPQKTQRSQDENPVVH). Purification of different mast cell proteases determined that chymase, a "chymotrypsin like" enzyme, cleaves MBP to release the encephalitogenic portion of the molecule consisting of residues 69-89. Mast cell carboxypeptidase activity then degrades the peptide from the COOH terminus yield peptides 69-88 and 69-87. Additional proteolytic degradation of the 69-87 peptide could not be detected following exposure to high concentrations of crude supernatants. When Lewis rats were immunized with the purified rat peptide, residues 69-87, clinically apparent experimental allergic encephalomyelitis (EAE) developed in a significant percentage of the treated rats. The rat MBP sequence 69-87 was also found to activate encephalitogenic MBP reactive T cell lines to adoptively transfer clinical EAE. The release of encephalitogenic peptides from the myelin sheath by mast cell proteases, may play a role in activation of encephalitogen-specific T-cells during the progression of EAE.
Supported by NIH #24130

CE 110 USE OF HUMAN CLASS I MOLECULES AS RESTRICTING ELEMENTS IN HUMAN CLASS I TRANSGENIC MICE, Victor H. Engelhard, John P. Ridge, and Elizabeth Lacy, Department of Microbiology, University of Virginia, Charlottesville, VA 22901 and Dewitt Wallace Research Laboratory, Program in Molecular Biology, Memorial Sloan-Kettering Cancer Center, New York, NY 10021.
Transgenic mice have been constructed that express the human class I MHC molecules HLA-A2.1 and HLA-B7. The expression of these molecules in lymphoid tissues and organs was comparable to that of normal H-2 molecules. However, we have previously shown that the frequency of cytotoxic precursors that could recognize other human class I molecules as alloantigens, or utilize the transgene products as restriction elements, was very low. Nonetheless, it has been possible to isolate long-term lines and clones of influenza-specific, HLA-A2.1 or HLA-B7 restricted CTL from these mice after repeated stimulation with influenza A/PR8 infected-cells. The epitope recognized by all of the HLA-A2.1 restricted clones could be formed by incubating uninfected HLA-A2.1$^+$ cells with a peptide spanning residues 55-73 of the influenza M1 protein. This is the same epitope recognized by HLA-A2.1 restricted influenza-specific human CTL. The influence of a series of HLA-A2.1 mutants on recognition was also similar to the effects previously observed on recognition by human CTL. These results indicate that the major epitope recognized by HLA-A2.1 restricted influenza specific CTL is not altered by species-specific differences in T cell receptor gene structure, antigen processing, or the influence of other self-molecules that modify the expressed T-cell repertiore. The results are consistent with the idea that that few, if any, other influenza-derived peptides bind to the HLA-A2.1 molecule, and suggest the utility of these mice to define other viral and pathogen-specific epitopes presented by HLA-A2.1. (Supported by PHS grants AI20963, AI21393, AI24815, and American Cancer Society Grant ACS-IM-457. E. Lacy is the recipient of an American Heart Association Established Investigatorship.)

CE 111 TUMOR SELECTIVE CYTOLYTIC ACTIVITY EXECUTED BY CD5$^+$CD8$^+$CD45RB$^+$ BUT NOT BY CD5$^+$CD8$^+$CD45RB$^-$ RAT T LYMPHOCYTES Per-Olof Ericsson, Gunnar Hedlund, Johan Hansson, Mikael Dohlsten and Hans-Olov Sjögren, The Wallenberg Laboratory, Department of Tumor Immunology, University of Lund, Box 7031, S-220 07 Lund, Sweden.
CD4$^+$ T helper cells can be divided into naive and activated (memory) cells by expression of different isoforms of the CD45 molecule. The question arises whether this applies also for CD8$^+$ cells and in this study we have investigated the expression of the CD45RB molecule on CD8$^+$ T cells and the cytolytic activity of peritoneal CD8$^+$CD45RB$^+$ and CD8$^+$CD45RB$^-$ T cells from tumor- and allo-immunized rats. WF rats were sensitized by intraperitoneal inoculations of irradiated syngeneic W439 lymphoma cells or BN allogeneic spleen cells. Peritoneal cells were analyzed and sorted in a flow cytometer. 90% of peripheral blood and 40-70% of peritoneal CD8$^+$ T cells expressed the CD45RB molecule. Analysis of cytotoxicity of sorted cells showed selective cytolytic activity to W439 lymphoma cells in the CD5$^+$CD4$^-$CD8$^+$CD45RB$^+$ but not in the CD5$^+$CD4$^-$CD8$^+$CD45RB$^-$ lymphocyte population. None of these populations exhibited cytolytic activity to the NK sensitive cell-line YAC-1, whereas the CD5$^-$CD45RB$^+$ population showed cytotoxicity to YAC-1 but not to W439 cells. In allo-sensitized animals both CD5$^+$CD4$^-$CD8$^+$CD45RB$^+$ and CD5$^+$CD4$^-$CD8$^+$CD45RB$^-$ peritoneal cells exhibited strong cytolytic activity to BN cells. The expression of the CD45RB molecule on CD45RB$^+$ cells was found to be down-regulated after 4 days of activation with anti-TCR mAb. Whether molecules of the CD45 family play a role in recognition and regulation of tumor selective cytotoxic T cells remains to be investigated.

CE 112 DESIGN OF CYTOTOXIC T LYMPHOCYTES WITH ANTIBODY-TYPE SPECIFICITY AGAINST TUMOR CELLS USING CHIMERIC TcR, Zelig Eshhar, Tova Waks, Guy Gorochov, Joseph Lustgarten and Gideon Gross, Department of Chemical Immunology, The Weizmann Institute of Science, Rehovot 76100, Israel.
The use of anti-tumor antibodies for immunotherapy is limited by the inaccessibility of many solid tumors, primarily because of poor vascularization. T cells, on the other hand, because of their ability to extravasate can serve as efficient mediators to reject solid tumors. In an attempt to combine the specificity of antibodies with the efficient lytic potential of T cells, we developed a novel approach in which we constructed and expressed in T cells chimeric T cell receptor (TcR) genes composed of the variable domain of antibody and constant region of the TcR. In a model system, we used the variable domains of anti-TNP heavy and light chains in order to establish the optimal conditions for expression of the chimeric Ab/TcR genes. Employing different transfection techniques we could express these genes as functional receptors in murine CTL hybridomas and T-cell lines and in Jurkat, a human T cell line. The chimeric TcR endowed the recipient T cells with anti-TNP specificity that was non-MHC restricted and that could transmit a signal for T cell activation and specific target cell killing. We used synthetic primers and PCR technology to establish a universal cloning and a shuttle vector system that allows the rapid construction of chimeric TcR genes containing V-domain derived directly from any hybridoma cell. This method has been recently used to derive chimeric TcR of anti-tumor specificity for possible use in anti-cancer immunotherapy.

CE 113 CYTOTOXIC ACTIVITY OF LFA-1 DEFICIENT HUMAN T CELL CLONES. *Carl G. Figdor, Anje A. te Velde, Yvette van Kooyk, Cornelis J.M. Melief, and Elly v.d. Wiel-van Kemenade.* Division of Immunology, The Netherlands Cancer Institute, 1066CX, Amsterdam.

Lymphocyte function associated antigen-1 (LFA-1) is involved in the adhesion of cytotoxic effector cells to target cells. To investigate the role of adhesion molecules other than LFA-1 we raised cytotoxic T cell clones from peripheral blood lymphocytes of a patient with a leukocyte adhesion deficiency (LAD). The lymphoid cells of this patient did not express CD11/CD18 on their cell surface. Furthermore they lacked CD18 mRNA expression, but exhibited normal quantities of CD11a mRNA. A $CD4^- 8^+$ (LAD1) and a $CD4^+ 8^-$ (LAD6) clone out of 21 clones, showed cytotoxicity against various targets, including the stimulator cell JY, K562 and several melanoma cells, but not autologous EBV transformed B cells. The cytotoxic activity of LAD1 is high compared to LAD6 except for JY, which was lysed equally well by both clones. Cytotoxic activity against K562 ($LFA1^-$) can be completely blocked by anti-LFA-3 antibodies, whereas total inhibition of the cytotoxic activity against JY ($LFA1^+$) requires both anti-LFA-3 and anti-LFA-1 antibodies.
From these results we conclude that LFA-1 deficient T cells can exhibit cytotoxic activity independent from LFA-1 and that cellular interactions are most likely mediated by the CD2/LFA3 pathway.

CE 114 The influence of MHC Qa antigens on shaping of T cell repertoire. Cohava Gelber, Roxanne Morse and Robert S. Goodenow
Molecular and Cell Biology Dept. U.C. Berkeley, California 94720 U.S.A The class I family of the murine major histocompatibility complex consists of the H-2 loci, encoding the highly polymorphic classical transplantation antigens, as well as the Q and Tla loci, encoding the relatively non-polymorphic hematapoietic differentiation antigens, whose function is unknown. In order to begin to elucidate the potential function of the Qa antigens, we have been analyzing the TCR V gene usage of Qa reactive T cells together with the influence of Qa expression on the clonal elimination of specific T cell subsets. We have observed marked differences in the peripheral T cells of Qa negative B6.K1 versus Qa positive B6.K2 mice which might be attributed to the induction of tolerance to the non-classical transplantation antigens. Whereas B6.K2 mice exhibit an overall reduction in CD8 Vb5,6,9,11,13,14 and17a positive T cells relative to B6.K1 mice, there is a pronounced decrease in the number of CD4 Vb13 bearing T cells in the Qa positive versus Qa negative B6 recombinant strain. Since this correlates with the profile of Qa reactive T cells in B6.K1 versus B6.K2 mixed lymphocyte reactions, we believe the diversity of Qa reactive T cells reflects the immune recognition of heterogeneous self-peptides presented by Qa restriction elements.

CE 115 HUMAN CD4 EXPRESSION IN TRANSGENIC MICE, David S. Grass[1], Dan Littman[2], Kay M. Higgins[1], Alex Garvin[3], Roger Perlmutter[3], and Elizabeth Lacy[1], [1]Department of Molecular Embryology, Sloan-Kettering Institute for Cancer Research, 1275 York Ave., New York, N.Y. 10021; [2]Department of Microbiology and Immunology and Howard Hughes Medical Institute, UCSF, San Francisco, CA 94143; [3]Department of Medicine and Howard Hughes Medical Institute, University of Washington, Seattle, Washington 98195.

We have produced several lines of transgenic mice expressing the human CD4 (T4) gene. T4 expression in these various lines is driven by either the *lck* promoter or the murine CD3 δ promoter. Three lines have been produced carrying the *lck*-T4 construct, lines 37, 55, and 57. Northern blot analysis shows T4 expression in line 55 is restricted to lymphoid tissue. In addition, in lines 37 and 55, 80-100% of both peripheral T cells and thymocytes express human T4 on the cell surface. In line 57, only 15% of thymocytes and peripheral T cells express T4 on the cell surface. In all of these lines the transgene is expressed equally well on L3T4 positive T cells and Lyt-2 positive T cells. Three transgenic lines have also been produced with the CD3 δ-T4 construct. Analysis of these lines will be discussed. In addition, further analysis of the *lck*-T4 lines to determine the role of T4 during thymic development and T cell activation will be presented.

CE 116 **T CELL RECEPTOR GENE USAGE AND EXPRESSION IN RENAL ALLOGRAFT-DERIVED T CELL LINES.** S.L. Hand, B.L. Hall, and O.J. Finn, Department of Microbiology and Immunology, Duke University Medical Center, Durham, NC 27710.
Southern blot analyses indicate that the T cell receptors (TCRs) of alloreactive T cell lines derived from needle biopsies of human kidney allografts are selected based on β chain usage. In order to examine this selection at the level of TCR expression, we have generated monoclonal antibodies directed toward the TCRs of three allograft-derived T cell lines; MH3, WP3, and EH3. Monoclonal antibodies have been isolated which appear to react specifically with each of these three T cell lines. One anti-MH3 antibody precipitates a molecule from the surface of MH3 cells that co-migrates with the α/β TCR on a polyacrylamide gel. Ten WP3-reactive monoclonal antibodies were identified which cause a modulation of CD3 from the surface of WP3 T cells. We are in the process of characterizing the anti-EH3 monoclonal antibodies. Since Northern blot analysis of EH3 RNA has revealed that a member of the Vβ6 gene family is expressed by this T cell line, we are attempting to identify a monoclonal antibody reactive with this Vβ6 gene product. In order to establish the identities, on the sequence level, of variable gene segments predominantly involved in these responses, we are constructing TCR specific, primed cDNA libraries of each of these allograft-derived cell lines, and cloning and sequencing these segments.

CE 117 THE T CELL ANTIGEN RECEPTOR ζ CHAIN GENE: REGULATION OF EXPRESSION BY DNA METHYLATION, Victor W. Hsu, Richard D. Klausner and Michal Baniyash, Cell Biology and Metabolism Branch, National Institute of Child Health and Human Development, Bethesda, MD 20892
The T cell, thru the T cell antigen receptor (TCR), recognizes antigen in the context of the major histocompatibility antigen complex. ζ, one of the subunits of the TCR, has been demonstrated to be critically involved in transducing the signal of receptor-ligand binding to T cell activation. In searching for clues to the regulation of transcription of the ζ gene, we have begun to examine at the DNA level differences between ζ-expressing versus ζ-non-expressing cell lines. One difference that has emerged is the degree of methylation at the 5' end of the gene. We have used methylation-sensitive restriction endonucleases to study this area. Using *HhaI* on DNA from ζ-expressing T cell lines, we have detected 2 sites that are cut by this enzyme at the junction of the first exon and intron, but none in ζ-non-expressing cell lines. Interestingly, using *HpaII*, we found the converse result; that is, this enzyme recognizes two sites, one in the 5' flanking region and another in the first intron, in DNA in ζ-non-expressing cells and none in ζ-expressing cells. We see this similar trend when examining the initial expression of ζ during thymocyte development. To reconcile these differences we propose that methylation can result in stimulation or repression of transcription depending on whether activators or repressors are bound to a DNA region. The net interaction of these regions ultimately determines the fate of ζ gene expression. Studies are now underway to better define the extent of these regions and factors that may be binding to them in mediating the observed effects.

CE 118 ENHANCED EXPANSION OF CELLS WITH LYMPHOKINE ACTIVATED KILLER (LAK) ACTIVITY FROM HUMAN BONE MARROW AND PERIPHERAL BLOOD-IMPLICATIONS FOR IMMUNOTHERAPY, David M. Jablons, Michael T. Lotze, Surgery Branch, NCI, Bethesda, MD 20892. We investigated the ability of a unique, immortalized feeder cell line, BSM, to support the growth of cells with LAK activity from various sources of human lymphoid cells. Irradiated BSM (iBSM) or BSM cultured supernatants'effect was greatest in bone marrow (BM) cell cultures where IL-2 (10^3 U/cc) alone usually failed to generate lytic cells (BM mean LAK activity, n=3, in lytic units (LU): IL2 0.6±0.3; BSM alone 101.8±52.5; BSM + IL-2 78.9±54.2, $p_2<0.05$). iBSM induced LAK activity from human splenocytes (mean LU, n=3, IL-2 (10^3U) 76.8±7; BSM 19.8±2.9; BSM + IL-2 173.9±38.5, $p_2<0.05$). In PBMC culture (7 day), allogeneic PBMC feeder vs BSM feeders generated 0.2 and 41.3 LU LAK activity, respectively. BSM conditioned media (CM) was able to generate LAK activity in PBMC cultures (mean LU, n=2, media control <0.1; CM 9.1±8.9: IL-2 (10^3U) 36.6±4.7). CM did not contain IL-1, IL-4, IL-6, or TNF. BSM constitutively produced IL-2, detectable by CTLL assay and Northern blot analysis. When hybridized with an IL-2 probe, a single band was seen. Flow cytometric analysis of BSM demonstrated 3A1 (a pre-T cell marker) and transferrin receptor only. Southern blot analysis revealed rearrangement of the T cell receptor chain. We conclude that BSM is a unique feeder cell capable of generating and supporting human LAK effectors from a variety of sources. Furthermore, BSM derives from an immature T cell lineage and constitutively produces IL-2 and possibly other factors useful for the growth of lytic cells.

CE 119 FUNCTIONAL AND SEROLOGICAL ANALYSIS OF MIXED ISOTYPE CLASS II MOLECULE IN Eαd GENE INTRODUCED C57BL/6 TRANSGENIC MICE. **Masao Kimoto, Moronari Matsunaga, Toshihiro Mineta and Katsuhiko Seki, Department of Immunology, Saga Medical School**, Nabeshima, Saga 840-01, Japan. We have demonstrated the existence of mixed isotype AβbEαd class II molecule in Eαd gene introduced B6 transgenic mice. Immunoprecipitation and two dimensional (NEPHGE/SDS-PAGE) gel electrophoresis analysis using monoclonal anti-Aβb antibody showed Eαd spots as well as Aβb and Aαb spots. We were able to obtain an alloreactive T cell clone (BE1.12) from B6 anti-B6Eαd long term cultures that recognize AβbEαd molecule. Clone BE1.12 was stimulated by B6Eαd and (BALB/c x B6Eαd)F1 but not by (BALB/c x B6)F1 (CBF1) stimulators. Since the quality of class II molecule expressed on (BALB/c x B6Eαd)F1 and CBF1 stimulator cells should theoretically be the same and the only difference is the presence or absence of the Eαd transgene, the specificity of BE1.12 might be dependent on the Eαd transgene products. This was confirmed by mAb blocking experiments in which both anti-Aβb and anti-Eαd mAb blocked the proliferative response of clone BE1.12. We were also able to raise mAb (SEA40) that recognize specifically AβbEαd molecule. Interestingly, CBF1 spleen cells that do not react with SEA40 were induced to express AβbEαd molecule (recognized by SEA40) when cultured with IL-4 for 24 hours. Our results would provide important implications for the complexity of class II mediated immune responses including positive and negative selection in the thymus

CE 120 HUMAN $\gamma^{+}\delta^{+}$ T CELLS SPECIFIC FOR TETANUS TOXOID, Danuta Kozbor[1] and Giorgio Trinchieri[2], Fels Institute for Cancer Research, Philadelphia, PA 19140, The Wistar Institute, Philadelphia, PA 19104
$\gamma^{+}\delta^{+}$ T cell lines with specificity for tetanus toxoid (TT) were established from two immunized donors by stimulation with antigen and autologous antigen presenting cells (APC). In both cell lines, of 100% T cells reacting with framework specific anti-TCR-δ1 monoclonal antibodies (mAbs), over 90% cells expressed Vδ1 gene segments recognized by TCS-δ1 mAb. Majority of these $\gamma^{+}\delta^{+}$ cells expressed CD8 antigen. Phenotypic analysis of several $\gamma^{+}\delta^{+}$ T cell clones with mAbs specific for V region of TCR-γδ complex indicated presence of three different groups of clones: TCS-$\delta1^{+}$,TiγA^{-}; TCS-$\delta1^{+}$,TiγA^{+}; TCS-$\delta1^{-}$,TiγA^{+}. Response of various $\gamma^{+}\delta^{+}$ T cell clones to TT alone or presented on autologous APC was analyzed in the presence of exogenous IL-2 (30 U/ml). Although production of IFN-γ (50-110 U/ml) was detected when T cells were stimulated with TT and autologous APC, some $\gamma^{+}\delta^{+}$ clones were able to respond to TT in the absence of APC (9-50 U/ml of IFN-γ). To functionally immortalize the TCR-γδ complex, we have constructed $\gamma^{+}\delta^{+}$ somatic T cell hybrids with TT specific T cells and TCR^{-} lymphoma, Molt-4. Results of these experiments should provide clues to identify the ligand(s) recognized by the TT-specific $\gamma^{+}\delta^{+}$ T cells, and to determine contribution of these cells to immune defense mechanism in humans

CE 121 LYMPHOKINE SECRETION BY INFILTRATING T CELLS PROMOTES ALLOGENEIC TUMOR REJECTION BY CYTOTOXIC T CELLS. Bruce R. Ksander, Y. Bando, M. Mammolenti, and J.W. Streilein, Dept of Microbiology and Immunology, Univ of Miami Sch of Med, Miami, Florida 33101.
P815 tumor cells inoculated into the anterior chamber (AC) or into the subconjunctival (SC) space of BALB/c mouse eyes experience different fates and generate different systemic immune responses. In the AC, an immunologically privileged site, progressive tumor growth eventually kills the recipients, whereas in the SC, an alloimmune response rejects the tumor. Despite these different clinical outcomes, both sets of recipients display comparable frequencies of primed cytotoxic T cell precursors (pTc) in draining lymph nodes, in spleens and at the tumor graft site. However, fully functional cytotoxic T cells (Tc) can only be detected at the SC. Since DBA/2-specific delayed hypersensitivity (DH) is induced by SC-injected tumors, but not by AC-injected tumors, we speculated that terminal differentiation of pTc into Tc may occur in the former because of local infiltration with T_{DH} effectors, functioning as "helper" cells. To test this hypothesis, infiltrating lymphocytes were recovered from AC and SC tumor-containing eyes by enucleation and collagenase treatment. Frequencies of T helper cells (IL-2- and IL-4-secreting) were measured using limit dilution analysis. The results indicate that IL-2-producing T cells appear as early as 8 days after P815 injection into the SC space (frequency = $81/10^6$ lymphocytes recovered), a time prior to onset of rejection. Three days later, when rejection is underway and Tc can be identified, T helper cells that produce IL-2 and IL-4 are detectable (frequency = $74/10^6$ lymphocytes recovered). By contrast, lymphokine-secreting T cells were never harvested from AC tumor-bearing eyes at any time point. These results suggest that tumor rejection is mediated by Tc, and that these cells are generated from infiltrating pTc that must undergo the terminal stages of differentiation in situ. The differentiation stimuli, comprised of a stereotypic pattern of lymphokines, are secreted by recirculating T_{DH} cells that also infiltrate the graft site. Immune privilege in the AC, and perhaps privilege established by successful tumor growth at non-ocular sites, may mitigate against rejection by preventing the local delivery of "help".

CE 122 HUMAN INFLUENZA VIRUS-SPECIFIC T-CELL CLONE RESTRICTED BY HLA-DR1 CROSSREACTS WITH HLA-DR1Dw1, Karel C. Kuijpers, Pammy Treep-Van Leeuwen, and Cees J. Lucas, Centr.Lab.Bloodtransf.Serv.,Lab. Exp. and Clin.Immunol.,University of Amsterdam, the Netherlands
Human CD4+ influenza virus-specific T-cell clones were studied with regard to their antigen-specificity and their HLA-restriction element. Twelve out of 14 clones studied were neuraminidase N2-specific. In the presence of influenza virus, 11 neuraminidase N2-specific T-cell clones responded to a panel of DR1+ EBV-transformed B-cell lines (B-LCL). In the absence of virus, however, one T-cell clone (6H5) also responded to DR1Dw1+, but not to DR1Dw20+ B-LCL. Cold target inhibition experiments and the rearrangement pattern of the T-cell receptor β-chain revealed that 6H5 was a monoclonal T-cell population most likely using one T-cell receptor for both responses. In the absence of virus, T-cell clone 6H5 reacted to DR1Dw1+ B-LCL, but, surprisingly, not to other cell types expressing DR1Dw1+.
These experiments further support the concept that recognition of allogeneic MHC (in this case DR1Dw1) may result from a cross-reactivity of T cells specific for a foreign antigen in the context of self-MHC (neuraminidase N2 + DR1Dw20). Furthermore, this allorecognition appears to depend on the recognition of a complex of allogeneic MHC and a (self)peptide present on certain cell types.

CE 123 ONTOGENY OF T CELL RECEPTOR (TCR) α-β+ and γ-δ+ INTRAEPITHELIAL LYMPHOCYTES (IEL), Leo LeFrancois, Judy Mayo and Thomas Goodman, Department of Cell Biology, The Upjohn Co., Kalamazoo, MI 49001
The influence of the thymus on the development of IEL of the small intestine was examined using thymectomized, bone marrow reconstituted, irradiated CD8 congenic mice. Eight weeks post-reconstitution IEL were isolated and tested for TCR expression and Lyt2 phenotype. Normal numbers of IEL developed in the absence of a thymus and the majority of TCR γ-δ+ cells were of donor origin. Moreover, a significant proportion of TCR α-β+ IEL were donor-derived. A population of long-lived radiation resistant TCR α-β+ IEL was also detected. IEL from normal I-E+ mice expressed $V_{\beta 11}$+ TCRs while lymph node T cells did not, indicating that TCR α-β+ IEL had not undergone negative selection in the thymus. TCRs utilizing $V_{\beta 6}$ were absent in lymph node T cells as well as IEL suggesting that deletion of this TCR did not require passage through the thymus or that both thymic and extrathymic selection occurred. Further proof of the intestine as a site of T cell differentiation came from the demonstration that both CD4-8- and CD4+8+ IEL could be detected. The results indicate that IEL are thymic independent regardless of TCR type and that intestinal TCR α-β repertoire selection is distinct from that of the thymus.

CE 124 IMMUNE RESPONSE TO *PLASMODIUM BERGHEI* SPOROZOITE ANTIGEN(S): T CELL ACTIVATION DOES NOT REQUIRE SPOROZOITE ANTIGEN PROCESSING. Heidi T. Link and Urszula Krzych. Department of Biology, The Catholic University of America and Dept. Immunology, Walter Reed Army Inst. Research. Washington, D.C. 20307.

Vaccine development against malaria requires a thorough understanding of the mechanism involved in protective immunity. Previous studies have shown that protection against the infective stage of *Plasmodium berghei* can be induced by prior immunization with irradiated sporozoites. We have previously shown that Balb/c mice are completely protected following one dose of irradiated sporozoites and are responsive to sporozoite antigen(s) in T cell proliferative assays following two doses of irradiated sporozoites. The mechanism of T cell activation by sporozoite antigen(s) was analyzed in an antigen presentation assay indicating activated B cells are the primary antigen presenting cells. Furthermore, sporozoite antigen presentation is Ia dependent in that monoclonal antibodies directed against anti-Ia^d but not anti-Ia^k block anti-sporozoite T cell responses both during antigen pulsing and throughout the lymphocyte culture. Finally, the sporozoite-antigen(s) do not require internalization and processing as demonstrated by gluteraldehyde or monensin treatment of B cells prior to sporozoite antigen pulsing. Our findings suggest the sporozoite antigen(s) activate T cells without the requirement of extensive antigen processing by the antigen presenting cell.

CE 125 HUMAN T CELL RESPONSES TO MURINE ANTIGENS REQUIRE SELF-APC. Philip J. Lucas, Gene M. Shearer, Ronald E. Gress. Experimental Immunology Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892
Human cytotoxic T lymphocytes (CTL) specifically recognize polymorphic determinants of murine MHC molecules. These interactions closely resemble human allogeneic interactions involved in transplantation rejection. The generation of CTL is dependent on T helper cell function. In allogeneic responses such T cell help may be provided by CD4$^+$ or CD8$^+$ helper cells interacting with allogeneic antigen presenting cells (APC) or responder APC which have processed allogeneic MHC antigens. We have found that in a human anti-murine response, the generation of CTL is dependent on responder APC. The inability of the murine APC to stimulate human cells is not caused by a precursor CTL defect since human recombinant IL-2 can replace the human APC. Instead, it appears that there is an inability of murine APC to interact with human helper T cells. Depletion studies show that the defective interaction is between human CD4$^+$ T cells and murine APC. This generation of human xenogeneic T cell effectors therefore represents a primary, in vitro, CD4$^+$ helper T cell response in which allogeneic MHC molecules are recognized as processed antigen. This response can be used to study CD4$^+$--APC interaction requirements and should be useful in monitoring CD4$^+$ helper function in T cell immunodeficient patients or patients receiving immunosuppression.

CE 126 ACTIVATION OF RESTING T CELLS IN HUMAN MELANOMA WITH ANTI-CD3 MAB OR AN ANTI-CD3/ ANTI-MELANOMA HETEROCONJUGATE FOLLOWED BY IL-2, Paul F. Mansfield, Michael G. Rosenblum and Kyogo Itoh, Depts. of Gen. Surg. and C.I.B.T., The Univ. of Texas, M.D. Anderson Cancer Center, Houston, TX 77030. We investigated if anti-CD3 mAb or a hetero-conjugate (HC) of anti-CD3 mAb, anti-melanoma antigen mAb (96.5 mAb), could activate T cells from human metastatic melanomas. Treatment with anti-CD3 mAb or HC alone was insufficient to induce proliferation. Culture with IL-2 was required for proliferation. In all 15 cases tested, pretreatment with anti-CD3 or HC followed by incubation with IL-2 induced the greatest proliferation (vs. 96.5 mAb, a 96.5 mAb homoconjugate, or IL-2 alone; $p<.05$). Pretreatment of cells with as little as 1 ng/ml of anti-CD3 mAb or HC significantly enhanced proliferation, with the highest proliferation at concentrations of 0.1 to 1.0 μg/ml. One hour treatment was as (or more) effective as longer periods (up to 48 h) in enhancing proliferation. CD3$^+$ CD16$^-$ T cells predominated in all cases, regardless of pretreatment schedule, dose, or source of lymphocytes. Proliferated lymphocytes from non-LN metastases and some LN metastases regardless of pretreatment, displayed cytotoxicity primarily restricted to autologous tumor cells. Supernatants from pretreatment of fresh lymphocytes from non-LN or LN metastases with anti-CD3 mAb or HC did not contain significant amounts of IL-2 activity, whereas those from PBMC did, indicating that lymphocytes in proximity of tumors may consist primarily of resting T cells. In summary, resting T cells in proximity of metastatic tumors could be activated with greater efficiency in vitro by anti-CD3 mAb or HC followed by IL-2 without changing their immunological properties. Utilization of anti-tumor cell mAb in vivo in the HC form may serve as a vehicle to deliver anti-CD3 mAb to lymphocytes at tumor sites.

CE 127 ANALYSIS OF T CELL REPERTOIRE DIVERSITY BY LINKER FACILITATED PCR,
Kannan Natarajan, Dianne Edgar, Barbara Hibner and Maurice Zauderer, Cancer Center, University of Rochester Medical Center, Rochester, NY 14642.
Current methods for T cell repertoire analysis depend on generating numerous cloned lines and laboriously screening multiple libraries for infrequent T cell receptor cDNA clones. In all except the most simple responses, a relatively large number of specific clones would have to be analyzed in order to arrive at a meaningful estimate for diversity of V-gene usage. Repertoire analysis by these means is always tedious and often impossible. This is particularly the case for T cell populations with potentially interesting repertoires but ill-defined antigenic specificity. We have employed a linker facilitated polymerase chain reaction (PCR) as a means of circumventing these problems. This modification of PCR makes it possible to construct libraries in which 30 to 60% of cDNA clones include $V\alpha$ or $V\beta$ sequences. We have applied this method to analysis of the T cell response to a synthetic peptide, YYEELLKYYEELLK, which is related to the venerable antigen (Y,E)-A--K, the response to which is Ir gene regulated. Our results demonstrate a striking restriction in $V\alpha$ utilization in association with relatively unrestricted $J\alpha$ expression among YYEELLKYYEELLK-specific T cells.

CE 128 CLASS II MHC UTILIZES THE ENDOGENOUS PATHWAY OF ANTIGEN PRESENTATION, Jed G. Nuchtern*, William E. Biddison+, & Richard D. Klausner*, *Cell Biology and Metabolism Branch, National Institute of Child Health and Human Development and +Neuroimmunology Branch, National Institute of Neurologic Disorders and Stroke, NIH, Bethesda, MD 20892. Cytosolic antigens must be partially degraded, transported into the lumen of the vacuolar system and form complexes with MHC molecules before they can be presented to the antigen receptor on cytolytic T lymphocytes (CTL). For class I restricted antigens this process is insensitive to inhibitors of lysosomal degradation, such as chloroquine, and blocked by inhibitors of protein synthesis, thus it has been called endogenous to distinguish it from the exogenous pathway in which soluble protein antigens are taken up into endosomes where they are partially degraded and form complexes with MHC molecules. Using the drug Brefeldin A we have previously shown that the endogenous pathway of class I restricted presentation is dependent on protein transport out of endoplasmic reticulum (ER). These results imply that the ER may be the site where class I MHC and processed antigen first combine. For the class II restricted cytoplasmic antigen, Influenza A matrix protein, we find two separate pathways for antigen presentation. The exogenous pathway is capable of presenting matrix antigen both from intact virus and from a formalin-inactivated vaccine preparation. This pathway is completely blocked by chloroquine, pointing to the likely importance of endosomal processing in this system. In order to delineate a non-endosomal, endogenous pathway for presenting this cytosolic antigen, we used a technique which allows direct fusion of virus particles with the presenting cell's plasma membrane. Under these conditions matrix protein is presented to class II restricted CTL in a chloroquine-insensitive manner. This process is blocked by Brefeldin A, implying a strict requirement for protein transport from the ER in this process and suggesting that the ER may in fact be the site of antigen-MHC assembly in class II restricted endogenous antigen presentation as well.

CE 129 CD8 REGULATION OF HUMAN T-CELL ACTIVATION : ANTI-CD8 MABS INHIBIT CD2 TRIGGERING IN AN EPITOPE DEPENDENT FASHION, Daniel Olive, Marie-Dominique Franco, Jacques Nunès, Marc Lopez, Carol Lipcey and Claude Mawas, INSERM U.119, 27 Bd Leï Roure, 13009 Marseille, France.
CD8 surface molecule is a T cell subset marker involved in MHC class-I binding. $CD8^+$ cells are mainly cytotoxic T lymphocytes restricted to class I MHC Molecules.
In addition, CD8 has been implied in the regulation of CD3, CD2, Tp103 or CD28 mAbs induced T cell activation. Thus anti-CD8 mAbs can inhibit or enhance CD3-triggering, in this latter case the simultaneous cross-linking of CD8 and CD3 molecules is required. These results have led to the hypothesis that some degree of interaction could exist between CD3-TcR and CD8 molecules.
We have studied the interaction of a panel of anti-CD8 mAbs with CD2 stimulation of a $CD8^+$ cytotoxic clone. We demonstrate that anti-CD8 mAbs inhibit in an epitope dependent fashion and with the same dose-response curves CD2-mediated Ca^{2+} response, non MHC specific cytotoxicity and IL-2 release of this $CD8^+$ clone.
These results demonstrate a regulatory role for CD8 in the CD2 pathway that could stand at the level of the transduction machinery i.e. possibly PLC-activity associated with CD2.

CE 130 BEHAVIOR OF ANTIGEN-CONDITIONED T CELLS IN VIVO, C.G. Orosz and D.K.Bishop, Ohio State University College of Medicine, Columbus, OH 43210, and University of Utah, Salt Lake City, UT 84132.
We have used limiting dilution analysis (LDA) to define the patterns of T cell redistribution observed in vivo after antigen deposition. Separate LDA techniques were used to enumerate antigen-specific cytotoxic T cells (CTL) or antigen-specific helper T cells (HTL). Critical to this analysis was the development of modified LDA techniques which selectively enumerate only those T cells with prior in vivo contact with antigen. We refer to such cells as antigen-conditioned T cells, or cT cells. These modified LDAs ignore all other precursor T cells, even those with appropriate antigen specificity. We have observed that cCTL and cHTL appear only after antigen deposition, but are distributed differently among the various lymphoid compartments. This suggests that the lymphocyte-endothelial interactions associated with lymphocyte migration may be different for cCTL and cHTL. Further, cHTL are considerably more persistent than cCTL, suggesting that immunologic memory may be a property of HTL rather than CTL. Finally, treatment with anti-CD4 monoclonal antibody interferes with the appearance of cHTL, but not cCTL at the site of antigen deposition, indicating the independence of these two functional T cell subpopulations in vivo. Since most of these observations were could not have been made with conventional LDAs, they demonstrate the experimental utility of the modified LDA techniques. In general, these studies illustrate the importance of selectively monitoring antigen-conditioned T cells, rather than the entire population of antigen-reactive T cells, for in vivo studies of cellular immunity.

CE 131 CLONAL DELETION AND CLONAL ANERGY IN THE THYMUS INDUCED BY DIFFERENT CELL TYPES, Joseph L. Roberts, Susan O. Sharrow, and Alfred Singer, Experimental Immunology Branch, National Cancer Institute, National Institutes of Health, Bethesda MD 20892. The present study demonstrates that immune tolerance can be achieved in the thymus both by clonal deletion and by clonal inactivation, but that the two tolerant states are induced by different cellular elements. T cell receptor (TCR) engagement of self antigens on bone marrow derived, radiation-sensitive (presumably dendritic) cells induces clonal deletion of developing thymocytes, whereas TCR engagement of self antigens on radiation-resistant thymic epithelium induces clonal anergy. The non-deleted, anergic thymocytes are unable to proliferate in response to either specific antigen or anti-TCR antibodies, but do develop into phenotypically mature cells that emigrate out of the thymus and into the periphery.

CE 132 CD4 EXTERNAL DOMAINS ARE SUFFICIENT FOR BINDING TO MHC CLASS II MOLECULES; BLOCKAGE OF SUCH INTERACTION BY SOLUBLE HIV-gp120. Yvonne Rosenstein, Barry Sleckman, Steven H. Herrmann and Steven J. Burakoff. Division of Pediatric Oncology, Dana-Farber Cancer Institute, 44 Binney Street, Boston, MA 02115.
T lymphocyte adhesion is essential for antigen recognition and lymphocyte activation. CD4 is a cell surface glycoprotein that has been shown to mediate the adhesion between T cells and antigen presenting cells (APC) expressing MHC class II Ags. We constructed two different forms of the CD4 molecule, CD4Δ in which 31 of the 38 amino acids of the cytoplasmic domain were deleted and CD4Pi which lacks the membrane spanning portion of CD4 and is anchored to the cell membrane via phosphatidylinositol. To further define the structural requirements for the CD4-MHC class II interaction we assessed the ability of CD4, CD4Δ and CD4Pi molecules, as expressed in a murine T cell hybridoma, to recognize affinity purified human MHC class II Ags by forming conjugates with cell sized lipid vesicles (artificial target cells, ATC) bearing MHC class II proteins. Conjugate formation was a transient process with the greatest number of specific cell-ATC conjugates occuring after ~30 minutes of incubation at 37°C. No appreciable difference was noted in the ability of the CD4Δ and CD4Pi mutants to mediate conjugate formation as compared to the wild-type CD4 molecule. In all cases, anti-CD4 mAb as well as soluble HIV gp120 specifically blocked conjugates between $CD4^+$ cells and MHC class II ATCs. These results demonstrate that the extracellular domains of the CD4 molecule are sufficient to mediate adhesion. Furthermore they suggest that in vivo binding of shed HIV-gp120 to CD4 might disrupt the immune response by blocking the interaction between $CD4^+$ cells and class II^+ APCs.

CE 133 EVIDENCE THAT LFA-1/ICAM-1 INTERACTION PROVIDES A PHYSIOLOGICALLY IMPORTANT "SECOND SIGNAL" FOR T CELL RECEPTOR-MEDIATED ACTIVATION OF RESTING T CELLS.

* Stephen Shaw, G.A. van Seventer, Yoji Shimizu, and Kevin J. Horgan. Experimental Immunology Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD.

Functional studies demonstrate that T cell activation often requires not only occupancy of the T cell receptor but costimulatory interactions of other molecules, which remain largely undefined. We have tested the hypothesis that LFA-1 interaction with its ligand ICAM-1 is such a costimulatory interaction in a model system using biochemically purified ICAM-1 (P-ICAM-1) and T cell receptor cross-linking by anti-CD3 mAb OKT3 immobilized on plastic. Resting T cells adhere minimally to P-ICAM-1 immobilized on plastic. Nevertheless, P-ICAM-1 deposited on plastic together with non-mitogenic immobilized OKT3 results in a potent activating stimulus. This costimulation cannot be readily accounted for by ICAM-1-mediated adhesion but is consistent with a role in signalling, which can be directly demonstrated in ICAM-1-mediated augmentation of activation induced by PMA/ionomycin.

The ability of P-ICAM-1 to costimulate with immobilized CD3 contrasts with minimal costimulatory activity of cytokines IL-1β, IL-2 and IL-6. The proliferative response to co-immobilized OKT3 and P-ICAM-1 is dependent on the IL-2R, which is induced only in the presence of both OKT3 and P-ICAM-1. The specificity of co-stimulatory activity is emphasized by the finding that P-ICAM-1 minimally augments activation by mitogenic pairs of CD2 mAb. The present data demonstrate that LFA-1/ICAM-1 interaction is a potent costimulus for T cell receptor-mediated activation; this observation, interpreted in light of previous reports, suggests that LFA-1/ICAM-1 is of major physiologic importance as a "second signal".

CE 134 IL1 AND IL6 PROVIDE SECOND SIGNALS FOR LYMPHOKINE-SECRETING CD8+ T LYMPHOCYTES, Peter H. Stein and Alfred Singer, Experimental Immunology Branch, NCI, NIH, Bethesda, MD 20892. Activation of T lymphocytes occurs upon transmission of at least two signals. The first involves cross-linking of the TcR-CD3 complex. The second is less well understood but may be mediated by lymphokines and/or cell surface adhesion molecules. We have developed a system in which second signals for lymphokine-secreting CD8+ cells can be investigated in the absence of stimulator cell populations. Highly purified CD8+ cells are obtained by passage over nylon wool, Ab and complement treatment, and overnight culture. Cross-linking of the TcR-CD3 complex with the anti-CD3 Ab 2C11 bound to microtiter wells does not result in IL2 secretion or proliferation. Similarly, stimulation with IL1 and/or IL6 does not result in T cell activation. However, stimulation with a combination of 2C11 and either IL1 or IL6 results in both IL2 secretion and proliferation. The effects of IL1 and IL6 appear to be additive. The anti-IL2 Ab S4B6 blocks proliferation in response to anti-CD3 plus IL1 and/or IL6, indicating that the proliferation is mediated by IL2. We conclude that IL1 and IL6, acting alone or in combination, are capable of providing second signals for IL2 secretion and subsequent proliferation by CD8+ T cells. IL1 and IL6 act directly on CD8+ cells as there are no stimulator cell populations added to the cultures. Further investigation will focus on the role of accessory molecules in T cell activation and the ability of various cell populations to provide second signals.

CE 135 CD28, AN ALTERNATIVE T-CELL DIFFERENTIATION PATHWAY?, René A.W. van Lier, Pieter C.F. Stokkers, Frank J.Borst, Miranda Brouwer and Rolien de Jong, Central Lab. Neth. Red Cross Blood Transf. Service and Lab. Exp. & Clin. Immunol., University of Amsterdam, Plesmanlaan 125, 1066 CX Amsterdam, The Netherlands.

We previously reported that CD3/T-cell receptor (TCR) and CD28 differ in their coupling to intracellular signal transduction pathways. In contrast to T-cell stimulation via CD3/TCR, ligand binding to CD28 is not accompanied by formation of diacylglycerol, mobilization of intracellular Ca^{2+} or activation of PKC. Moreover, CD28-mediated activation in the presence of anti-CD2 mAb is relatively insensitive to the action of both PKC inhibitors and the immunosuppressive drug Cyclosporin A. Given this apparent distinction in usage of second messenger systems, we analyzed whether functional differences could be demonstrated between T cells stimulated via either CD3/TCR or CD28/CD2. To allow a quantitative comparison between these systems, monocyte-depleted lymphocyte suspensions were stimulated with either graded quantities of the CD28 mAb CLB-CD28/1 in the presence of anti-CD2 antibodies or with plastic immobilized anti-CD3 mAb in varying densities. In both systems lymphokine production, proliferation, CTL differentiation and helper activity for IgM production could be induced. These data suggest that T-cell differentiation in vitro can be induced via both CD3/TCR-dependent as well as -independent pathways.

CE 136 IMMUNITY AGAINST SYNTHETIC T CELL RECEPTOR PEPTIDES, Vandenbark A.A. Hashim G.A. and H. Offner, Neuroimmunology Research, V.A. Med. Ctr., Portland, OR 97221. In Lewis rats, T cells that recognize the immunodominant and encephalitogenic 72-89 peptide of guinea pig basic protein (GP-BP) utilize common T cell receptor (TCR) variable region genes. From the deduced amino acid sequence of the TCR ß chain from a single encephalitogenic T cell clone, we identified and synthesized peptides that included either the CDR1 or the CDR2 region of the Vß8 gene family. As control peptides, we synthesized the corresponding sequences from the Vß14 gene family. Immunization with the Vß8 but not the Vß14 CDR2 peptide prevented completely the subsequent induction of experimental autoimmune encephalomyelitis (EAE). T cell lines and antibodies selected from Vß8 CDR2 peptide-immunized rats responded specifically to the immunizing peptide, but not to any of the other TCR peptides or to any peptides from GP-BP. Moreover, either TCR Vß8-CDR2 reactive T cells or antibodies could protect naive rats against EAE. The anti-TCR Vß8 reactive T cells expressed both CD4 and CD8 molecules, but the response to TCR peptide was restricted only by MHC Class I molecules. These regulatory anti-Vß8 reactive T cells could be stimulated selectively by Vß8+ T cells, resulting in a non-cytolytic inhibition of response by encephalitogenic T cells to the 72-89 epitope. The antibodies also interacted directly with the Vß8+ T cells, causing increased immunofluorescence but not cytolysis. These data demonstrate that both cellular and humoral immunity to defined TCR sequences in the CDR2 region can modulate in vivo and in vitro the activity of VB8+ encephalitogenic T cells.

CE 137 SELF-TOLERANCE CAN CRIPPLE IMMUNE SYSTEM, Damir Vidović, Basel Institute for Immunology, CH-4005 Basel, Switzerland.
Two sets of genes control the immune response of H-2^d mice to the synthetic antigen GT. One set involves class II MHC loci encoding an A^d product that serves as a recognition context to GT-reactive T_h cells. The other one is a background gene, the product of which, in association with the same MHC-restricting element, mimics the GT/A^d complex. Mice expressing the GT-mimicking background encoded structure (Im^{gt}), which is preferentially displayed on B lymphoblasts, do not respond to GT as a consequence of self-tolerance. On the other hand, elimination of cells bearing Im^{gt} renders these mice responsive to GT. Analysis of expression and structure of Im^{gt}, using specific T cell clones and monoclonal antibodies, revealed that Im^{gt} is probably not identical to GT, but resembles it in the way if forms complexes with A^d molecules of MHC.

Cell Trafficking and Endothelial Interactions; Non-Specific Immunity and Other Topics

CE 200 SELECTION OF TUMOR-INFILTRATING LYMPHOCYTES TRANSDUCED WITH THE NEOMYCIN PHOSPHOTRANSFERASE GENE, Paul Aebersold, Attan Kasid, R. Michael Blaese, W. French Anderson and Steven A. Rosenberg, Surgery and Metabolism Branches, National Cancer Institute and the Laboratory of Molecular Hematology, National Heart, Lung, and Blood Institute, Bethesda, MD 20892.
Lymphocytes infiltrating surgically resected nodules of metastatic malignant melanoma have been expanded in vitro with interleukin-2 for adoptive immunotherapy. Portions of such tumor-infiltrating lymphocytes (TILs) have been transduced with the bacterial gene for neomycin phosphotransferase (NPT) using the retroviral vector LNL6, a modified N2 constructed by A. D. Miller. TILs expressing NPT can in principle be selected with toxic concentrations of the neomycin analog G418. However, when TILs expressing NPT are present at low levels in a population of T cells, straightforward selection fails apparently because a majority of dying cells renders the medium unsuitable for growth of the G418 resistant cells. Selection of small proportions of resistant cells can be accomplished either by low density culture utilizing conditioned medium from interleukin-2 stimulated peripheral blood mononuclear cells or by gradual increases in G418 starting with marginally toxic concentrations. Using these methodologies, G418 resistant TILs have been selected from biopsies from patients treated with transduced TIL populations.

CE 201 B220 EXPRESSION BY LYMPHOKINE-ACTIVATED KILLER (LAK) SUBSETS, Zuhair K. Ballas and Wendy L. Rasmussen, Department of Internal Medicine, and the Iowa City VA Medical Center, University of Iowa, Iowa City, Iowa 52242. The precursors of murine LAK can be divided into two major subsets: NK-like ($CD8^-$, $NK1.1^+$, asialo $GM1^+$) and T-like ($CD8^+$, $NK1.1^-$, asialo $GM1^+$). LAK effectors have generally been characterized as being either $CD8^+$ or $NK1.1^+$. In this study, we examined the expression of the following determinants on LAK effectors: B220, as defined by the monoclonal antibody 6B2, and Ly24 (Pgp-1), as defined by the monoclonal antibody 9F3. Freshly obtained $CD8^+$ and $NK1.1^+$ cells were $B220^-$. Most of the fresh $NK1.1^+$ cells and 40% of the $CD8^+$ cells were $Ly24^+$. 76% of the lymphocytes obtained from 6 day IL2 cultures were $B220^+$, $Ly24^+$; this subset contained most of the LAK lytic activity. Further fluorescence analysis revealed that the CD8 and NK1.1 markers were expressed on mutually exclusive subsets but each subset could be further subdivided into $B220^+$, $Ly24^+$ and $B220^-$, $Ly24^-$ subsets with the lytic activity confined to the double positive subset. A distinct $NK1.1^+$, $B220^-$, $Ly24^+$ subset existed but had minimal lytic activity, suggesting that some but not all NK cells can develop into LAK.

The acquisition of the B220 marker by the $CD8^+$ subset closely paralleled its expression of lytic activity. The $CD8^+$ lymphocytes were $B220^-$ (and had minimal lytic activity) on day 3 of culture but a distinct subset acquired this marker (and lytic activity) by day 6 of culture. On the other hand, classical MHC-restricted $CD8^+$ CTL were $Ly24^+$ but remained $B220^-$, suggesting that the acquisition of B220 may serve as a marker of MHC-non-restricted killers.

CE 202 ZINC INHIBITION OF DELAYED CUTANEOUS HYPERSENSITIVITY TO RECALL ANTIGENS IN THE ELDERLY, John D. Bogden, Francis W. Kemp and Kay S. Bruening, Department of Preventive Medicine, UMDNJ-New Jersey Medical School, Newark, NJ 07103-2757.
There is convincing evidence that moderate to severe zinc deficiency inhibits cellular immune functions in humans and animals. These effects may be mediated by zinc interactions with cytokines. Most elderly United States residents have zinc intakes well below the RDA and frequently have impaired cellular immunity, but it is not known if zinc administration will improve immune functions in older adults. Subjects (n=63) aged 60-89 were given a placebo, 15 mg Zn, or 100 mg Zn daily for 12 months. All subjects also received daily a multivitamin/mineral supplement containing low to moderate doses of 21 other micronutrients. Delayed cutaneous hypersensitivity (DCH) to a panel of 7 recall antigens was assessed prior to and at 3, 6, 12, and 16 months after beginning Zn supplementation by measuring positive responses and induration. There was a progressive improvement in DCH in all treatment groups; this may have been due to one or more components of the multivitamin/mineral supplement administered to all study subjects. For example, in the placebo group the mean ($\pm$ SE) number of positive responses to the 7 skin test antigens was 1.58 ± 0.30, 2.17 ± 0.32, 2.71 ± 0.37, 2.95 ± 0.46, and 3.53 ± 0.41 at the 0, 3, 6, 12, and 16 month visits, respectively. The enhancement in DCH was significantly greater in the placebo group than in either Zn treatment group, particularly the group receiving the highest dose of zinc. The data suggest caution in the use of even moderate doses of Zn supplements in the elderly, since they may adversely influence cellular immunity. (Supported by NIH grant AG04612 and the New Jersey State Commission on Cancer Research.)

CE 203 KINETICS OF ACTIVATION AND LYTIC ACTIVITY OF BISPECIFIC mAb TARGETED LYMPHOCYTES. R.L.H. Bolhuis, C.P.M. Ronteltap, N.E.B.A.M. van Esch, E. Braakman and S. Warnaar, Dept. of Immunology, Daniel den Hoed Cancer Center, Rotterdan, The Netherlands, *Dept. of Tumor Immunology, Radio Biological Institute,* TNO Health Organization, Rijswijk, The Netherlands. Purified bispecific monoclonal antibody (bsAb) against CD3 and human ovarian carcinoma (OvCa) induce lysis of OvCa cells by cloned CTLs. In view of immunotherapeutic in vivo application we examined whether bsAb targeted T cells could recycle, i.e. perform more than 1 lytic cycle. Therefore, T cells were incubated with bsAb, washed and cultured with or without OvCa cells for various periods of time (0-24 hrs). Subsequently, T cells were tested for their ability to lyse 51Cr labeled OvCa cells. Targeted CTL maintain their functional activity for several days. However, upon interaction with OvCa cells bsAb targeted CTL lost their lytic activity after a relatively short period of time, although bsAb could still be detected on their cell surface. Addition of "fresh" bsAb reconstituted their cytotoxic activity against OvCa cells, demonstrating that their cytotoxic potential was still intact. These in vitro findings suggest that repeated in vivo bsAb targeting of activated T cells after the "transfer of bsAb targeted T cells will prolong the time period of CTL activity.

CE 204 A PARACRINE CIRCUIT IN THE REGULATION OF THE PROLIFERATION OF MACROPHAGES INFILTRATING MURINE SARCOMAS, Barbara Bottazzi, Eugenio Erba, Nadia Nobili, Francesca Fazioli Alessandro Rambaldi, Alberto Mantovani, Istituto di Ricerche Farmacologiche "MARIO NEGRI", Via Eritrea 62, 20157 Milan, Italy.
Tumor-associated macrophages (TAM) are important in the immunobiology of neoplasia and as targets for therapy. TAM are recruited from the blood compartment via a novel cytokine chemotactic for monocytes (Bottazzi, Science 220, 210,1983). Moreover, we recently observed that TAM from 2 murine sarcomas have high levels of proliferative activity (7-11% of cells in S phase) compared to peritoneal macrophages (1-2% of cells in S phase). In an effort to elucidate the mechanisms responsible for the proliferative activity of TAM, expression of c-fms and M-CSF was investigated in TAM and sarcoma cells. TAM had high levels of mRNA transcripts of the c-fms protooncogene, which encodes a tyrosine kinase probably identical to the M-CSF receptor, but did not express M-CSF transcripts while sarcoma cells had high levels of M-CSF mRNA. Sarcoma cell conditioned medium had M-CSF activity on bone marrow cells and induced proliferation of peritoneal exudate and bone marrow-derived macrophages. These activities were blocked by anti-M-CSF antibodies. These findings outline a paracrine circuit in the regulation of TAM proliferation, involving M-CSF, secreted by sarcoma cells and acting on c-fms expressing TAM. Since TAM from these murine sarcomas have tumor growth promoting activity, a "ping pong" reciprocal feeding interaction may occur between macrophages and neoplastic cells in these tumors.

CE 205 **Expression of Lymphokine Genes Using Retroviral Mediated Gene Transfer**
Yawen L. Chiang[1], Stanley Friedman[1], Marlene Hammer[1],Daniel T. Kuebbing[1], Jeanne L. McLachlin[1], Martin A. Eglitis[1], Robert C. Moen[1], P. Tolstoshev[1], Attan Kasid[2], Steven A. Rosenberg[2], and W. French Anderson[3]. 1: Genetic Therapy, Inc., Gaithersburg, Maryland; 2: Surgery Branch, NCI, NIH; 3: Laboratory of Molecular Hematology, NHLBI, NIH, Bethesda, Maryland
To evaluate the utility of somatic cell gene transfer in adoptive immunotherapy for certain cancers, lymphokine genes incorporated into retroviral vectors have been constructed and are being evaluated. It has previously been shown that tumor infiltrating lymphocytes ("TIL") can be transduced by retroviral vectors containing the bacterial neomycin resistance gene and maintain their phenotypes and specific cytotoxicity for tumor cells. Certain lymphokines such as human interleukin-2 ("IL-2") and tumor necrosis factor ("TNF-α") were selected to be expressed using retroviral vectors. Vector backbones were of the LN series previously used to gene-mark human TIL, as well as a new G1 vector backbone recently developed to facilitate analysis of regulatory elements in the vector. Expression of the lymphokines is being examined under the influence of viral (i.e. LTR, CMV, and SV40) or other internal promoters. High titer producer clones derived from the PA317 packaging line have been isolated. Significant quantities of human TNF-α were detected in the supernatant of the producer lines. IL-2 expression through retroviral-mediated gene transfer, is currently under evaluation, as is the ability to express both of these genes in human TIL. Expression of relevant lymphokines and the ability to deliver them directly to the tumor site via gene-engineered TIL may improve the effectiveness of TIL adoptive immunotherapy for cancer.

CE 206 **MURINE MACROPHAGE CELL LINE J774 IS PRIMED FOR ANTIBODY DEPENDENT CELL MEDIATED CYTOTOXICITY (ADCC) BY GAMMA-IRRADIATION**, Reggie Duerst and Kathryn Werberig, Department of Pediatrics, University of Rochester, Rochester, NY, 14642. Macrophage activation for cytotoxicity follows a sequence of priming and triggering signals that result in production and release of cytotoxic molecules that mediate target cell killing. J774 macrophage cells, 3H-thymidine labeled SW1116 human colon carcinoma cells and monoclonal antibody (mAb) 17-1-A (murine IgG2a subtype) were used to examine ADCC in vitro. SW1116 cells +/- mAb 17-1-A were added to J774 macrophages, incubated for 4-48 hr and 3H released into the supernatants was measured. Unirradiated J774 cells did not mediate ADCC. ADCC was observed if the J774 cells were first exposed to gamma-irradiation (5-70 Gy) and then incubated at least 6 hr prior to addition of SW1116 cells. Full development of the primed state for ADCC requires incubation for 24 hr from the time of irradiation exposure. The primed state following irradiation is stable for at least 24 hr. ADCC was detectable by 16 hr after addition of SW1116 cells and mAb 17-1-A and occured at mAb concentrations $\geq$ 1 mcg/ml and effector/target ratios $\geq$2. Recombinant murine interferon-gamma (rmIFN-gamma, $\geq$5 U/ml) enhances priming of irradiated J774 cells. rmIFN-gamma (10 U/ml) alone also primes the macrophages for ADCC but the extent of target cell death that results is 20-30% less than that following gamma-irradiation alone. MAb to IFN-gamma partially inhibits gamma-irradiation induction of the primed state for ADCC. Thus, priming of macrophages for ADCC by gamma-irradiation is mediated in part by IFN-gamma.

CE 207 PHENOTYPE AND FUNCTIONAL ALTERATIONS OF MACROPHAGES BY TUMOR DERIVED GM-CSF, Yangxin Fu, Yang Wang, Mayra Lopez-Cepero, Diane Y. Mochizuki* and Diana M. Lopez, Department of Microbiology and Immunology, University of Miami School of Medicine, P. O. Box 016960 (R-138) Miami, FL. 33101, and *Immunex Corporation, Seattle, Washington 98101.
Mac-1^+2^+ macrophages induced by mammary tumors are capable of downgrading lymphocyte responses to mitogens and tumor associated antigens by a cell to cell contact dependent mechanism as well as by increase of PGE_2 production. We have previously shown that this tumor constitutively releases rGM-CSF *in vivo* and *in vivo*. This factor may be responsible for the systemic increase of cells of the macrophage lineage in the tumor bearing mice. Daily injections of tumor cystic fluid into normal mice i.p. for 3 weeks mimicked the hemopoietic and immunologic alterations observed in tumor bearers. Furthermore, injection of rGM-CSF into normal mice induced similar changes as those observed with tumor associated factors. An increase of Mac-1^+2^+ macrophages in spleen and bone marrow can also be detected in the mice treated with rGM-CSF. In addition, rGM-CSF was able to enhance spleen, bone marrow and peritoneal cells from normal mice to release PGE_2 *in vitro*. These data indicate that the high levels of rGM-CSF contitutively produced by the tumor may be responsible for the hemopoietic changes and immunologic alterations observed in tumor bearing mice.

CE 208 AN *IN VITRO* METHODOLOGY FOR THE GENERATION OF ACTIVATED HUMAN B CELLS PRODUCING ANTIBODY TO CARCINOEMBRYONIC ANTIGEN (CEA). Neil I. Goldstein, ImClone Systems Incorporated 180 Varick Street, New York, New York 10014

We have developed an *in vitro* stimulation protocol for the isolation of antigen-specific B cells for use in the generation of human monoclonal antibodies. The procedure involves the stimulation of Ficoll-purified PBLs with polystyrene beads coated with antigen in the presence of IL-1, IL-6 and substituted nucleotides. Cells then undergo a secondary stimulation in the presence of IL-6 or IL-6 plus doxorubicin. We have used CEA as one of our stimulatory antigens and have been able to generate a specific antibody response. The activated B cells are now being used to develop human MABs.

CE 209 IRREVERSIBLE MODULATION OF STEADY STATE PROTEIN SYNTHESIS IN HUMAN NEUROBLASTOMA CELLS INDUCED BY NERVE GROWTH FACTOR IS COINCIDENT WITH TERMINAL DIFFERENTIATION AND LOSS OF MITOTIC ACTIVITY, Lee Jensen, Eric M. Shooter*, and Robert S. Goodenow, Department of Molecular and Cellular Biology, University of California, Berkeley, CA 94720; *Department of Neurobiology, Stanford University School of Medicine, Stanford, CA 94305. Human SY5Y neuroblastoma cells can be terminally and irreversibly differentiated to a nonproliferative state by long-term exposure to nerve growth factor (NGF) and selection with aphidicolin and exhibit additional properties indicative of mature peripheral neurons including long neurite structures, increased membrane resting potentials, NSE and monoamine staining, and a survival dependence upon NGF (Jensen, Dev. Biol. 120:56; 1987). We have monitored the steady state levels of over 1000 proteins via computer-aided 2D equilibrium and nonequilibrium PAGE as a function of NGF-induced differentiation. Two proteins were found to be restricted to the undifferentiated phenotype and 2 were restricted to the nonproliferative, differentiated phenotype. Levels of 8 detected proteins increased 5-fold or more as a consequence of differentiation while 4 decreased. Significant changes were not detected in the majority of proteins assessed. Withdrawal of NGF from differentiated cultures caused the disappearance of one and expression of another protein unique to NGF withdrawal; alterations in protein synthesis patterns that occur as a consequence of differentiation are not reversed upon withdrawal of NGF. The induction of MHC expression by NGF, γ-interferon, and retinoic acid is currently being assessed.

CE 210 MONOCLONAL ANTI-IL-1 EFFECTS ON IL-1 SYNERGY WITH LT IN HUMAN ISLET CYTOTOXICITY, AND IL-1 SUPPRESSION OF INSULIN SECRETION BY PERIFUSED HUMAN ISLETS. Douglas J. Kawahara, Diabetes Research Laboratory, Childrens Hospital of Orange County, Orange, CA 92668.

We studied human recombinant IL-1 alpha (rIL-1a) and IL-1 beta (rIL-1b) suppression of glucose-stimulated insulin secretion by perifused human islets, and the cytotoxic effect of human recombinant lymphotoxin (rLT) with either rIL-1a or rIL-1b on isolated human islets. Human islets were kindly provided by Dr. D. Scharp (Washington University, St. Louis, MO). Insulin secretion was sup-pressed by either rIL-1a or rIL-1b. In addition, while rIL-1a, rIL-1b or rLT alone were not cytotoxic, rLT in combination with either rIL-1a or rIL-1b was cytotoxic for cultured human islets.

Monoclonal antibodies (mAb) specific for non-crossreactive epitopes on IL-1a (H43 & H12) and rIL-1b (H34 & H21) were kindly provided by J. Kenney (Syntex Research, Palo Alto, CA). All 4 mAb neutralize thymocyte stimulation by IL-1, and the islet cytotoxic synergy between IL-1 and LT. Preincubation of rIL-1b with either H34 or H21 neutralized rIL-1b suppression of insulin release. Suppression of insulin secretion by rIL-1a was neutralized by H43, but not, however, by H12.

These results suggest that rIL-1a and rIL-1b act on human islets via different sets of receptors and/or mechanisms. One set for the suppression of insulin release is apparently different than that utilized for thymocyte stimulation. Another, apparently similar to that in thymocyte stimulation, is involved in islet cytotoxic synergy between IL-1 and rLT.

CE 211 **LAK CELL EFFECTORS FROM LONG-TERM CULTURES OF HUMAN PERIPHERAL BLOOD LYMPHOCYTES WITH rhINTERLEUKIN-2 ARE A VIRTUALLY PURE POPULATION OF $CD3^+$,$CD16^-$,$CD56^-$ LARGE GRANULAR LYMPHOCYTES,** **Eugene Roussel and Arnold H. Greenberg.**
Manitoba Institute of Cell Biology, University of Manitoba, Winnipeg, Canada, R3E OV9.

Controversy is present in the literature about the $CD3^+$ or $CD16^+$ cell lineage of LAK cell effectors in human PBL. As we developed a two step method to generate LAK cells from human PBL in long-term cultures (10-12 days), we characterized the evolving LAK cell population by testing its phenotype and cytotoxic activity in function of time. After 10-12 days, the expanded cells were essentially a pure (>90%) $CD3^+$,$CD16^-$,$CD56^-$ cell population LGL by morphology that displayed strong non MHC-restricted killing activity (>200 LU). Over the same period of time, the $CD16^+$ cells showed almost complete regression in these cultures. IL-2 high (500 U/ml) and low (10U/ml) dose culture conditions were compared and we found that 10 (U/ml) was totally sufficient to produce these LAK cells. Further characterization of these LAK cells revealed a major population of $CD4^+$ (60%) and $CD8^+$ (30%) with a small minority (<9%) of $\gamma\delta^+$ cells. This study indicates that LAK cell effectors from human PBL stimulated to proliferate with IL-2 in long-term cultures are a LGL population of $CD3^+$,$CD16^-$,$CD56^-$ cells. Taken together the actual work on LAK cells suggest that either $CD16^+$ or $CD3^+$ LAK cells are obtained depending if the PBL have been incubated with IL-2 in short-term culture or have been able to proliferate in long-term culture.

CE 212 **RETROVIRUS-MEDIATED TRANSFER OF HUMAN IL-2 INTO MOUSE HEMATOPOIETIC STEM CELLS,** Dale G. Schaar and Sueihua Pan, Dept. of Pathology,UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ 08854. The lymphokine, interleukin-2 (IL-2), is pivotal in the amplification of activated T-cells and also is involved in the activation of other effector cells, i.e.: NK and LAK cells. Currently, we are studying the effects of constitutive IL-2 expression using retrovirus-mediated gene transfer in mice. The cDNA for human IL-2, under the control of the cytomegalovirus (CMV) immediate early promoter, has been cloned into the N-2 retroviral vector and recombinant retrovirus produced by theΨ-2 ecotropic packaging line.This N2-CMV-IL-2 virus was used to infect CB6F1/J bone marrow cells (enriched for stem cells), which were subsequently used to reconstitute lethally irradiated CB6F1/J recipients. Southern analysis of one recipient, 8 weeks post-reconstitution, revealed a common pattern of proviral integration in the lymphoid tissue indicating the repopulation by a single infected stem cell. Preliminary analysis of IL-2 expression by these recipients was assessed using media conditioned for 24 hours by bone marrow, spleen and thymus cell cultures versus a normal CB6F1/J control. In these assays, conducted over a 4 day period, the bone marrow and spleen cells of the CMV-IL-2 mouse expressed high levels of IL-2 as assayed by the proliferation of HT-2 cells (an IL-2 dependent cell line). Interestingly, the IL-2 activity in the thymocyte-conditioned medium was very low. The consequences of such constitutive IL-2 expression in vivo is being analysed and will be discussed.

CE 213 SOME ASPECTS OF THE CELLULAR IMMUNITY IN THE SELF-LIMITING PLASMODIUM KNOWLESI INFECTION IN BONNET MONKEY (MACACA RADIATA),
Pawan Sharma[1], Mujtaba Husain[2], Anil Kumar[1] and G. P. Dutta[2], [1]International Centre for Genetic Engineering & Biotechnology, New Delhi; [2]Central Drug Research Institute, Lucknow, INDIA.
Bonnet monkey is a good model for self-limiting P. knowlesi (PK) infection. Since CMI presumably contributes significantly to the development of protective immunity against malarial infection, we studied a few correlates of CMI in a group of four monkeys during experimental Pk infection. In a lymphocyte proliferation assay, cultures stimulated with Pk antigen showed 2.5-fold increase in the stimulation index (SI) at the peak parasitemia (about 6.5%) compared to the pre-infection SI values. As animals successfully cleared their parasitemia, elevated SI values remained virtually unaltered. Percent concentration of T and B cells in peripheral blood of these monkeys did not change markedly during or after the infection. Animals which successfully cleared repeated live infections were tested for phagocytic activity of the reticuloendothelial (RE) system. The rate of clearance of the intravenously injected colloidal carbon which we took as measure of the RE activity, was nearly two-fold faster in immune monkeys as compared to that in naive monkeys.

CE 214 MONOLONAL ANTI - IDIOTYPES FOR HERPES SIMPLEX VIRUS AND THEIR ROLE IN SPECIFIC LYMPHOPROLIFERATION
Veenita Sinha and Pradeep Seth, Department of Microbiology, All India Institute of Medical Sciences, Ansari Nagar, New Delhi - 110029, India.

A mouse monoclonal antibody to the neutralizing epitope on the surface glycoprotein (120 KD) of Herpes Simplex Virus (HSV) type I , strain AC , was used to prepare mouse monoclonal anti-idiotypic antibody . In antigen inhibition assay, the specificity and fine specificity of the anti-idiotypic antibodies were determined. In- vitro lymphoproliferative response was studied using splenocytes of mice immunized with anti-idiotypes and it was observed that the specificity of the responsiveness correlated with that of the HSV glycoprotein

CE 215 THE INTEGRIN VLA-4 ON HUMAN LYMPHOCYTES IS INVOLVED IN BOTH INTERCELLULAR AND LYMPHOCYTE TO ENDOTHELIAL CELL ADHESION
Margit C. Szabo, James N. Wygant, Bradley W. McIntyre, Department of Immunology, The University of Texas M.D. Anderson Cancer Center, Houston, TX 77030
Monoclonal antibody L25 recognizes the α chain of human VLA-4, an integrin of the β_1 subfamily. The addition of mAb L25 promotes a divalent cation-, temperature-, and energy- dependent aggregation among lymphocytes. Pretreatment of lymphocytes with cytochalasin B inhibited the formation of mAb L25 induced aggregates. VLA-4 molecules from lymphocytes induced to aggregate by mAb L25 were detected in cell fractions stabilized for cytoskeletal components. These two studies suggest that the cytoskeleton may play an important role in this adhesion event. Endothelial cell lines derived from mouse peripheral lymph node and the human umbilical vein were capable of supporting human lymphocyte adhesion. MAb L25 blocked lymphocyte adhesion to these endothelial cells at 4, 10, 22 and 37°C. Lymphocytes bound to endothelial cells could be detached by addition of mAb L25.

CE 216 HIGH AFFINITY IL-2 RECEPTORS ON A HODGKIN'S DERIVED CELL LINE, Hans Tesch[1], Thomas Herrmann[2], Harry Abts[1], Tibor Diamantstein[3], and Volker Diehl[1], [1] I. Medizinische Klinik, Universitätsklinik Köln, Joseph-Stelzmann-Str.9, D-5000 Köln 41, F.R.G., [2] Ludwig Institute for Cancer research, Epalinges, Switzerland, [3] Institut für Immunologie, Klinikum Steglitz, D-1000 Berlin, F.R.G.
Hodgkin and Sternberg Reed cells, the putative malignant cells of Hodgkin's disease carry regularly T-cell activation antigens, like CD30 and CD25 (low affinity IL-2 receptor). We have investigated the Hodgkin cell line L540, bearing characteristic markers of H and SR cells for its expression of the low affinity IL-2 receptor (IL-2R) and for IL-2. Expression of the low affinity IL-2R was found on mRNA level, by detection of specific 3.5 kb and 1.4 kb mRNA and on the protein level by immunoprecipitation of a 55 kDa molecule from detergent extracts of surface iodinated cells, however IL-2 specific mRNA was not detected. Scatchard plot analysis revealed the presence of 2 x 10^3 high affinity IL-2Rs. Crosslinking experiments directly demonstrated the high affinity IL-2R to consist of the 55kDa light chain (L), and the 70/75 kDa (H1/H2) heavy chains. IL-2 was rapidly internalized by these receptors, suggesting that they can be functional. The expression of functional IL-2Rs might be involved in induction or differentiation of Hodgkin's disease.

CE 217 CHARACTERIZATION AND ACTIVITIES OF MURINE PERIPHERAL LYMPH NODE HOMING RECEPTOR (mPLN-HR) IgG CHIMERAS, Susan R. Watson,Chritopher Fennie,Joyce Geoffrey,Yasuyuki Imai,Steven D. Rosen and Laurence A. Lasky,Dept.,of Cardiovascular Research,Genentech ,Inc.,460,Pt.San Bruno Blvd.,S.S.F.94080 and Dept., of Anatomy,University of California,San Francisco,94143. The cloning of the mPLN-HR has revealed that it is composed of domains - a C-type or calcium dependent lectin-like domain (L),an epidermal growth factor (EGF)-like domain (E) and 2 complement regulatory protein-like domains (C). To examine the relative contributions of each of these domains in PLN high endothelial venule (HEV) binding we have constructed chimaeras containg these protein domains linked to human Ig fragments. These chimeras,L-IgG,LE-IgG and LEC-IgG, have been used as immunohistochemical reagents to study the distribution of the ligand to which this receptor binds,in frozen section cell binding assays to determine the abilty of each of the constructs to block lymphocyte binding and in an ELISA assay to quantitate their relative abilities to bind poly-phosphomannan ester (PPME) and the abiltity of other sugars to compete for binding to these molecules. Current results suggest that all domains are required for the receptor to interact with its ligand.

Immunologic Recognition of Tumor Antigens

CE 300 ENHANCEMENT OF EPIDERMAL GROWTH FACTOR RECEPTOR (EGF-R) EXPRESSION ON GLIOMA CELLS BY RECOMBINANT TUMOR NECROSIS FACTOR (rTNF)-α, *Koji Adachi, *Paul Belser, **Derui Li, **David Woo, ***Michael Varello, ***Barbara Atkinson, *Ulrich Rodeck, *Hilary Koprowski and *Dorothee Herlyn, *The Wistar Institute of Anatomy and Biology, Philadelphia, PA 19104, **Hahnemann University, School of Medicine, Philadelphia, PA 19102, ***Medical College of Pennsylvania, Philadelphia, PA 19129.
rTNF-α (optimal dose 1000 U/ml) significantly increased EGF-R density in 3 of 4 glioma cell lines in culture as determined by Scatchard analysis of binding of anti-EGF-R monoclonal antibody (MAb) 425. This dose of rTNF-α did not show growth inhibitory effects on cultured glioma cells. When cells were treated with rTNF-α in combination with interferon-γ, the optimal rTNF-α dose that induced maximal increase in EGF-R density was 10-fold lower (100 U/ml) compared to treatment with rTNF-α alone. Primarily those cells that were in G2/S phase as opposed to cells in G1 phase showed upregulation of EGF-R by rTNF-α. The phase difference suggests a differential effect of rTNF-α on EGF-R expression on actively dividing cells (such as tumor cells) and resting (normal) cells. Since enhancement of EGF-R expression by rTNF-α was inhibited by pretreatment of the cells with the protein synthesis inhibitor cycloheximide, rTNF-α effects most likely are protein synthesis-dependent.

We investigated whether rTNF-α treatment of glioma cells increases the tumoricidal effects of MAb 425 which correlate with MAb density on tumor cell surfaces. Inhibition of growth of glioma cells *in culture* by ^{125}I-labeled MAb 425 was significantly enhanced after treatment of the cells with rTNF-α. In our previous clinical trials, ^{125}I-labeled MAb 425 has shown immunotherapeutic effects in glioma patients. The present study provides the basis for considerations of combined immunotherapy of glioma patients with ^{125}I-labeled MAb 425 and rTNF-α.

CE 301 COMPARISON OF MONOCLONAL AB1 ANTI GANGLIOSIDE IDIOTYPES WITH ANTI AB1 INDUCED IDIOTYPES FOR MELANOMA TUMOR SPECIFIC TARGET BINDING, Darrell R. Anderson, Robert E. McCoobery and Syamal Raychaudhuri, Department of Tumor Immunology, IDEC Pharmaceuticals Corp., La Jolla, CA 92037. Two monoclonal anti ganglioside antibodies (8A6 and 8C2) were produced against the melanoma associated tumor glycolipid GD3. Both antibodies demonstrated a high specificity for melanoma cells and purified antigen. Digestion of tumor cells with neuraminidase results in loss of antibody binding when measured by flow cytometry.

A number of anti idiotype antibodies were prepared from the two Ab1 idiotype templates and were used to immunize rabbits. The resulting antisera were evaluated for anti id network induced anti tumor responses by direct binding to purified antigen and tumor cells. Comparison of the target response to that of the original Ab1 indicates less fidelity in the AB3 population to the target antigen yet still demonstrates neuraminidase sensitivity.

CE 302 SPECIFIC, MHC-UNRESTRICTED RECOGNITION OF EPITHELIAL TUMOR-ASSOCIATED MUCINS BY HUMAN CYTOTOXIC T CELLS. D. L. Barnd and O.J. Finn, Dept. of Microbiology and Immunology, Duke University Medical Center, Durham, N.C. 27710.

We have previously reported specific, MHC-unrestricted recognition of tumor-associated mucins by a human CD8+ αβTCR+ cytotoxic T cell (CTL) line established by continuous stimulation of pancreatic tumor draining lymph node cells with allogeneic pancreatic tumor cell lines (Proc. Natl. Acad. Sci. USA 86: 7159). The mucin antigen recognized is expressed on pancreatic and breast tumors and tumor cell lines and can directly bind and activate the CTL in the absence of self MHC, presumably via abundant and regularly repeated antigenic epitopes. Sequencing of breast and colon mucin cDNA clones by others has demonstrated that mucin molecules are encoded by a tandemly repeated, highly conserved sequence with each repeat containing a number of immunogenic epitopes. The breast mucin gene and colon mucin gene were found to encode peptides of entirely different sequence, as is reflected by the lack of recognition of colon mucins by our CTL. The predicted CTL epitope, based on blocking studies with a number of anti-mucin peptide antibodies, contains two potential O-linked glycosylation sites. The level of glycosylation at these sites may account for the differential reactivity of these CTL with tumor tissue vs. normal tissue, as has been observed for differential anti-mucin peptide antibody reactivity. In contrast to the CD8+ cytolytic T cell lines expanded when mucin-expressing tumor cell lines are used as stimulating antigen, CD4+ non-cytolytic T cell lines are expanded when mucin peptide-pulsed APC are used as stimulating antigen. We are currently examining the different pathways by which tumor-associated mucin is processed and presented for recognition by these phenotypically and functionally distinct T cell subsets.

CE 303 ONCOGENE-PRODUCT-SPECIFIC T CELL IMMUNITY: IMMUNOGENICITY AND SPECIFICITY

Kemp B. Cease[1], Gwo-Hsiao Chen[1], William S. Probert[1], Jacalyn H. Pierce[2], and Jean Y. J. Wang[3].
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The Abelson oncogene (*v-abl*) of the acutely transforming Abelson Murine Leukemia Virus encodes a recombinant *gag/abl* protein with tyrosine kinase activity and belonging to the *src* family of oncogenes. In human chronic myelogenous leukemia an analagous recombinant *bcr/abl* protein is expressed resulting from the 9:22 chromosomal translocation forming the Philadelphia chromosome.

We have analyzed the T cell response to Abelson oncogene-encoded protein using a recombinant *t/abl* 40a fusion protein containing 80 residues of SV40 small t antigen followed by 351 residues of *v-abl* sequence expressed in bacteria. Using *t/abl* 40a purified by either affinity chromatography or SDS PAGE we have observed a substantial T cell proliferative response in 5 of 6 independent inbred lines of mice examined. A strong, presumably T cell dependent antibody response to *t/abl* 40a is also observed. Less than 1 microgram of *t/abl* 40a is immunogenic *in vivo* and either complete or incomplete Freund's adjuvant can be used with equivalent results, while a significant but lesser response is seen with trehalose dimycolate/ monophosphoryl lipid A and no response is elicited with Alum. We have experimentally excluded confounding mitogenicity of *t/abl* 40a and have found the response not to be focused on the small t segment as we observe no significant response to baculovirus-derived SV40 large T antigen. T cell epitope mapping in *t/abl* 40a is in progress using enzyme cleavage peptides and synthetic peptides selected based on amphipathicity analysis. Current studies are also employing established *t/abl* 40a specific helper T cell lines.

Selection of *abl* as a prototype for detailed characterization allows careful immunological studies in the mouse, including disease challenge and protection studies, which may generate the basic knowledge and reagents necessary for effectively studying the human T cell response to proteins encoded by *abl* and other oncogenes.

CE 304 AMPLIFICATION OF THE IN VITRO, HUMAN, HLA RESTRICTED, MELANOMA SPECIFIC, CYTOTOXIC T-CELL RESPONSE BY PHORBOL DIBUTYRATE AND IONOMYCIN, TIMOTHY L. DARROW, MARY ANN QUINN-ALLEN, NANCY J. CROWLEY AND HILLIARD F. SEIGLER, DEPARTMENT OF SURGERY, DUKE UNIVERSITY MEDICAL CENTER, DURHAM, N.C. 27710. Human melanoma specific, cytotoxic T-cell lines can be generated by in vitro stimulation and culturing of peripheral lymphocytes, or lymph node cells, with autologous or HLA-A region matched melanomas in the presence of a low concentration (5U/ml) of IL-2. Stimulation is followed by a period of clonal expansion and differentiation into cytotoxic T-cells which are HLA-A region restricted and specific for melanoma. We investigated the effect of the PKC modulating drug Phorbol Dibutyrate combined with the calcium ionophore Ionomycin on growth and differentiation of the cell lines. T-cell Growth was substantially augmented in the presence of the drugs with increases of tenfold or more in clonal expansion by three weeks of culture. The cell lines were IL-2 dependent for growth in the presence or absence of the drugs and the phenotypic distribution remained CD3+ T-cells of mixed CD4 and CD8 phenotypes. In spite of the increased rate of growth in the presence of the drugs, autologous melanoma specific cytotoxicity was abrogated in drug treated cultures. The cells were non-specifically lytic in the presence of Concanavalin A. The melanoma specific cytotoxic response was completely restored following culture with IL-2 alone. The results suggest that the tumor specific, HLA restricted cytotoxic T-cell response to melanoma can be induced and amplified in the presence of immune modulating drugs.

CE 305 FLOW CYTOMETRIC ANALYSIS OF TUMOUR INFILTRATING LYMPHOCYTES IN UVEAL MELANOMA. Fiona H. Durie, Ailsa M. Campbell, W.R. Lee and B.E. Damato. Dept of Biochemistry and Opthalmology, University of Glasgow, Glasgow G12 8QQ

Uveal melanoma is a malignant neoplasm which originates in the pigmented tissue of the eye. It is estimated that most uveal melanomas are derived from naevi which are benign melanocytic tumours which occur in 2-6 % of the population. Uveal melanomas, like their cutaneous counterparts, are considered to be relatively susceptible to immunological influences because of reports of spontaneous regression and of the delayed appearance of metastatic disease, sometimes decades after enucleation. In addition, the immunological privilege of the eye may confer special features on such tumours. Tumours, including uveal melanomas, can be infiltrated by lymphocytes consisting mainly of T cells, but despite the evidence that a more favourable prognosis is associated with lymphocytic infiltration, it is uncertain whether TILs play a significant role in tumour immunity.

We have (1) characterised the tumour infiltrating lymphocytes and (2) studied the expression of HLA-DR and IL-2 receptor (Tac) which are markers of activation on the surface of helper and suppressor / cytotoxic subpopulations of tumour infiltrating T lymphocytes. Analysis was carried out by dual fluorescence flow cytometry. Results demonstrate the predominance of T suppressor / cytotoxic lymphocytes and insignificant levels of B cells present in the infiltrate. The T suppressor/ cytotoxic cells are always activated to a higher degree than the T helper cells when considering the levels of HLA-DR. T helper cells also express more IL-2 receptor than T suppressor / cytotoxic cells.

CE 306 **THE EXTRACELLULAR DOMAIN OF P185^{HER2} STIMULATES BOTH A HUMORAL AND CELLULAR RESPONSE IN IMMUNIZED GUINEA PIGS.** Brian M. Fendly, Claire Kotts, Dave Vetterlein, Gail H. Lewis, Susan R. Watson and H. Michael Shepard. Genentech, Inc., South San Francisco, CA 94080.

The protooncogene HER2/*neu* encodes a 1,255 amino acid protein tyrosine kinase (p185^{HER2}) which is homologous to the human epidermal growth factor receptor. Amplification and/or overexpression of HER2/*neu* occurs in multiple human malignancies and appears to be integrally involved in progression of at least breast and ovarian cancers. Because of this, it is a potential target for the design of specific cancer therapeutic strategies. One such strategy is active specific immunotherapy, in which the immune system of the patient is preferentially directed at the tumor burden. In the present experiments we have employed a cell line secreting the extracellular domain of p185^{HER2} (HER2-ECD) as a source of immunogen. Affinity purified HER2-ECD was used to immunize guinea pigs and the resulting antisera tested for the ability to inhibit growth of human cell lines which either do or do not overexpress p185^{HER2}. The results showed that such antisera specifically inhibited the proliferation of breast tumor cells overexpressing this receptor-like protein. HER2-ECD used as an immunogen in this system can also stimulate cellular immunity as demonstrated by a strong delayed-type hypersensitivity response. These data provide support for an immunotherapeutic approach to cancers characterized by overexpression of p185^{HER2}.

CE 307 INDUCTION OF CLASS I HISTOCOMPATIBILITY ANTIGENS IN VIVO ON A CLASS I NEGATIVE MURINE TUMOR, Mark D. Foresman, Edith M. Lord, and John G. Frelinger, Cancer Center and Department of Microbiology and Immunology, University of Rochester Medical Center, Rochester, NY 14642. The line 1 lung carcinoma is a spontaneous BALB/c (H-2^d) tumor which normally fails to express class I histocompatibility antigens. Intramuscular injection into allogeneic (C57BL/6,H-2^b) mice leads to tumor growth but eventual rejection. In naive or primed syngeneic mice the tumors continue to grow. Interestingly, line 1 cells removed from allogeneic animals prior to tumor rejection now express normal levels of class I antigens as analyzed by monoclonal antibodies and flow cytometry. Furthermore, class I antigen is inducible in vivo on line 1 cells in syngeneic mice as early as 3 days after challenge. Previous work in our laboratory has shown that class I antigen was inducible in vitro on line 1 with dimethyl sulfoxide (DMSO), gamma interferon (IFN), or beta IFN. Gamma IFN may play an important role in the induction of class I antigen on line 1 cells in vivo. Potential inducing agents such as retinoic acid, butyric acid, ethanol, and the cytokines interleukin (IL) 1 beta, IL-2, IL-3, IL-4, IL-6, and tumor necrosis factor (TNF) alpha all fail to induce class I antigen expression in vitro. Furthermore, TNF alpha does not appear to synergize with gamma IFN in the induction of class I antigen. Anti-gamma IFN antibodies block gamma IFN induced expression of class I antigen in vitro. The ability of these antibodies to inhibit in vivo induction is presently under investigation.

CE 308 HOST IMMUNE RESPONSE TO IMMUNOGENIC TUMORS: LEUKOCYTES INFILTRATING PRIMARY UV-INDUCED TUMORS AND JB/MS MELANOMA TRANSPLANTS, Helen L. Gensler and Hwudaurw Chen, Department of Radiation Oncology and Cancer Center, University of Arizona College of Medicine, Tucson, AZ 85724. Most UV-induced tumors are highly antigenic and are able to grow in the host of origin because UV irradiation induces an immunosuppression which prevents their rejection. We have hypothesized that this immunosuppression would prevent large numbers of leukocytes from infiltrating into primary UV-induced tumors. To test this hypothesis we have analyzed the phenotypes of leukocytes infiltrating 5 primary tumors in BALB/c mice which had been exposed to 4.9 x 10^5 Jm^{-2} over a 12 week period. The proportions of nucleated cells in primary tumor cell suspensions which reacted with monoclonal antibodies were: 51% Mac-1^+, 21% Lyt-1^+, 13% Lyt-2^+, 7% L3T4^+, and 8% IL-$2R^+$ cells. Thus, UV irradiation of BALB/c mice with 4.9 x 10^5 Jm^{-2} did not prevent the infiltration of leukocytes into primary UV-induced tumors, suggesting that chemotaxis was intact in these mice. The proportions of reactive nucleated cells in antigenic JB/MS melanoma transplants were also analyzed, at 2 weeks after intradermal injection of 5 x 10^5 cells. There were 4.8% Mac-1^+, 9.7% Lyt-1^+, 2.9% Lyt-2^+, 2.9% L3T4^+, 0.5% IL-$2R^+$ cells. These results indicate that there were very low numbers of tumor-infiltrating leukocytes in growing JB/MS melanoma transplants, and that there can be disparate host responses to different immunogenic tumors.

CE 309 RECOGNITION OF MUCIN EPITOPES BY BREAST CANCER SPECIFIC CYTOTOXIC T CELLS. K. Jerome, D. Barnd and O. Finn. Department of Microbiology and Immunology, Duke University Medical Center, Durham, NC 27710.

Tumor-reactive CTL can be isolated from draining lymph nodes of patients with certain cancers. We have demonstrated that a population of cancer-specific T cells can be isolated from lymph nodes of breast cancer patients and propagated in culture using allogeneic tumor lines as antigen and IL-2. These cells exhibit MHC-unrestricted lytic activity against several breast and pancreatic tumor lines, yet show no activity against the NK target K562.

Mucins are large, highly glycosylated molecules expressed on the breast tumor lines used as targets for these CTL. The role of mucins as tumor-associated antigens has been well documented using antibodies, but their role as T cell antigens is less clear. Recently, the protein core of a breast mucin has been sequenced by others and core-specific antibodies raised. The core consists of a 20 amino acid repeat found in both normal and malignant breast tissue. We have shown that 4 different anti-core antibodies bind to the breast tumor lines, and that 2 of these inhibit lysis by the CTL. Knowledge of the binding sites for these antibodies has allowed us to localize the region of the mucin polypeptide core recognized by the T cell receptor

CE 310 LYSIS OF RENAL CELL CARCINOMA USING BISPECIFIC ANTIBODY DIRECTED LYMPHOCYTES: IN VITRO AND IN VIVO STUDIES, Lindsey A. Kerr, Catherine J. Huntoon, Paul J. Leibson, David Segal, John H. Donohue, Sven Warnaar, Reinder Bolhuis, and David J. McKean, Mayo Clinic, Rochester, MN 55905. Tumor cell lysis can be significantly increased when bispecific antibodies are used to redirect CTLs of varying specificities to kill tumor. These novel immune reagents are monomeric IgG molecules with one antigen binding site specific for tumor antigen and the second specific for the T cell antigen receptor complex. CTL activation is an important prerequisite for their use and we have developed a protocol for expansion of activated effectors. Culturing of PBLs in rIL2 and anti-CD3 for 12 days results in expansion as great as 160 fold of suitable effectors. High levels of lysis were elicited by an anti-renal tumor bispecific antibody in combination with rIL2 and anti-CD3 activated patient effector lymphocytes. At an E:T of 6.25:1, specific tumor lysis was 44% with the anti-renal bispecific antibody G250/OKT3 compared to 11% lysis in its absence. Chemically crosslinked antibody heteroaggregates composed of intact G250 and OKT3 monoclonal antibodies were compared to the bispecific G250/OKT3 antibody. At optimal concentrations for tumor lysis, the bispecific antibody was more effective at directing killing than the heteroaggregate (58% vs 49%, $p<0.01$) and at one tenth the concentration. A renal tumor xenograft nude mouse model (TK82) was used to examine in vivo localization of G250/OKT3. When G250/OKT3 was given intravenously, 13% of the injected dose was retained at 24 hours. The biodistribution index (tumor:blood cpm/gm ratio) was 14.6. Together these results suggest that G250/OKT3 bispecific antibody may be a useful immunotherapeutic tool for treatment of renal cell carcinoma.

CE 311 USE OF POLYETHYLENE GLYCOL-6000 (PEG) IN MEDIA CONTAINING TUMOR CELL ANTIGEN AND RECOMBINANT INTERLEUKIN-2 (rIL-2) TO GENERATE CD8+ CELLS WITH ENHANCED ANTI-TUMOR ACTIVITY IN CULTURES OF TUMOR INFILTRATED SPLEEN CELLS (TISpC). Maribeth Laude, Katherine Siessmann, Margalit B. Mokyr and Sheldon Dray, Department of Microbiology and Immunology, University of Illinois at Chicago, Chicago, IL 60680.
TISpC cultured for 5 days with MOPC-315 stimulator tumor cells and 60 IU/ml rIL-2 or 2% PEG are effective in curing a mouse bearing a MOPC-315 tumor after a low subcurative dose of cyclophosphamide (CY). The addition of both PEG and rIL-2 to the culture media enhanced the effectiveness of the TISpC in adoptive chemoimmunotherapy (ACIT) as compared to cells cultured in either PEG or rIL-2 alone. The combination of PEG and rIL-2 in the culture media also increased the cell recovery. TISpC, which are effective in ACIT, exhibited a direct in vitro lytic activity against MOPC-315 tumor cells in an antigen specific manner. TISpC expanded in culture for 27 days were found to maintain their potent cytotoxicity and specificity for MOPC-315 tumor cells. Since the CD8+ cells are responsible for the effectiveness of TISpC in ACIT as well as for the direct specific anti-MOPC-315 lytic activity, we determined whether these culture conditions resulted in a greater increase in the frequency of CD8+ cells. TISpC cultured for 5 days had an increased percentage of CD8+ cells, while, TISpC cultured for a total of 27 days were almost exclusively CD8+ T cells. Experiments are now in progress to compare the effectiveness in ACIT of day 5 cultured TISpC with day 27 cultured TISpC. Thus, the incorporation of PEG in the culture media of TISpC, rIL-2 and antigen leads to a high yield of cells which display an increased effectiveness in ACIT and an enhanced direct lytic activity which can be maintained in culture for at least 27 days.

CE 312 LYMPHOCYTE POPULATIONS MEDIATING REJECTION OF HIGHLY IMMUNOGENIC MURINE TUMOR VARIANT CLONES. Stephen J. LeGrue and William Simcik. Department of Immunology, M. D. Anderson Cancer Center, Houston, TX, 77030. Treatment of weakly immunogenic murine tumors with chemical mutagens, physical mutagens, or hypomethylating agents such as MNNG, UV radiation or 5-azacytidine results in the high-frequency induction of strongly immunogenic (Imm^+) variant clones. Imm^+ clones are rejected by normal mice but grow in immunosuppressed mice, and variant rejection can specifically immunize the host against growth of the weakly immunogenic parental tumor. We investigated the phenotype of the lymphocytes mediating rejection of several Imm^+ variants and the parental MCA-F tumor using the local adoptive transfer assay (LATA) and the *in vitro* CTL assay. Using antibody + complement depletion, anti-parent immunity could only be transferred to naive recipients using nylon-wool purified spleen cells with a $Thy1^+$ $CD4^+$ $CD8^-$ surface phenotype. The immunity was tumor specific, and afforded no protection against challenge with tumors bearing biochemically related but immunologically distinct tumor rejection antigens. The lymphocyte populations capable of transferring anti-variant immunity were dependent upon the dose of immunizing cells used. Under conditions of low immunizing multiplicities (3×10^4 viable Imm^+ cells) where anti-parent immunity was not engendered, Imm^+–rejecting splenocytes with a $Thy1^+$ $CD4^+$ $CD8^-$ phenotype were exclusively obtained. However, both $CD4^+$ and $CD8^+$ populations were generated in mice immunized with high numbers ($\geq 10^5$) of Imm^+. The phenotypes of the populations transferring immunity to the parent and variant were unaffected by the method used to induce the Imm^+ variant, suggesting that the $CD4^+$ cells are the predominant mediators of antitumor immunity regardless of the method used to generate the Imm^+ variants. To investigate the role of CTL in rejection of the Imm^+ clones, mice were hyperimmunized with Imm^+ and CTL effectors obtained after *in vitro* MLTC. The resulting CTL were specific for the Imm^+ in a 4 hr. ^{51}Cr release assay, with no killing of the MCA-F parent or unrelated tumors. Using bulk populations, anti-variant cytotoxicity was partially blocked by addition of anti-L3T4a antibodies, suggesting the presence of $CD4^+$, MHC class 2–restricted CTL in this system. (Supported by the University of Texas Cancer Fdn.)

CE 313 CLONING AND CHRACTERIZATION OF TUMOR INFILTRATING T-CELLS IN HUMAN GLIOMAS, Magnus Lindvall, Leif Salford and Hans Olov Sjögren, Department of Tumor Immunology, Lund University, 220 07 Lund, Sweden. We have cloned tumor infiltrating T-cells from human Astrocytomas grade III-IV. We found such T-cells in about 20% of the patients. Initially we cultured the infiltrating lymphocytes in the preseance of 25u/ml IL-2 and after a week they are cloned with BSM, an EBV transformed human B-cell line and 100u/ml IL-2. The clones were then restimulated every 10th day with SEA (Staphylococcus Enterotoxin A) and BSM (HLA-Dr+) as SEA presenting cell. With this technic we preferentially got CD4- and CD8+ T-cells. We collected those T-cells that lysed the autologous tumor cells but not autologous fibroblasts and priferal blood lymphocytes. As control we also used the NK-cell sensitive cell line K562 and BSM as target cells. One of these patients were investigated more intensively and from this tumor we now got 7 CD8+ clones with tumor selectivity. They are class I restricted. A broader characterization of these clones will be presented on the meeting.

CE 314 PRIMARY VIRUS-INDUCED LYMPHOMAS EVADE T CELL IMMUNITY BY FAILURE TO EXPRESS VIRAL ANTIGENS.
Cornelis Melief, Wies Vasmel, Alice Sijts, Tine Leupers, Elisabeth Matthews. Division of Immunology, The Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands
T-lymphoma induction by MCF 1233 murine leukemia virus in C57BL background mice is strongly influenced by an H-2 I-A restricted anti-viral T cell response. We compared the MHC class I and viral *env/gag* antigenic cell surface profiles of frequent T lymphomas of H-2 I-A non-responder type mice to those of rare T lymphomas of H-2 I-A responder type mice. Surface expression studies with anti-*env* monoclonal antibodies (mAbs), a polyclonal anti-p30 serum and anti-H-2 class I mAbs, showed that all 17 non-responder tumors tested expressed high levels of *env/gag* viral proteins, and 15 of these tumors also expressed high levels of H-2 K/D antigens. In contrast, 10 out of 11 responder tumors lacked *env* and/or *gag* determinants. The only responder tumor with both strong *env* and *gag* expression failed to express H-2 K/D antigens. Both responder- and non-responder T lymphoma DNA contained multiple new proviral integrations. Differences in viral antigen cell surface expression were confirmed at cytoplasmic and RNA levels. The amounts of 8.2 and 3.2 kb viral RNA were greatly reduced in 2 responder tumors when compared to 4 non-responder tumors. In both responder tumors, aberrantly sized viral RNA was found. *In vivo* passage of these responder tumors in either immunocompetent or nu/nu mice showed that various molecular mechanisms underlie the lack of viral antigen expression. One tumor re-expressed viral antigens when transplanted in nu/nu mice, but the other remained *gag*-negative. These findings indicate that an H-2 I-A regulated antiviral immune response not only strongly reduces T-lymphoma incidence, but also forces T lymphomas that still arise to poorly express viral antigens, allowing their escape from immunosurveillance.

CE 315 ANTIGEN REQUIREMENTS FOR *In Vivo* CTL ACTIVATION AND TUMOR REDUCTION IN SYNGENEIC HOSTS. Matthew F. Mescher and Joy Rogers, Medical Biology Inst., La Jolla, CA
Activation of precursor CTL depends critically on a large area of surface interaction with the antigen-bearing cell and a sufficient surface density of antigen. Artificial membranes which satisfy these criteria have been constructed (termed Large Multivalent Immunogen or LMI). *In vivo* administration of LMI bearing purified class I alloantigen dramatically augments murine CTL responses to allogeneic cells. The resulting CTL remain antigen-specific, and augmentation occurs only if the LMI and allogeneic cells bear the same antigen. LMI have also been examined for their effects on responses to tumors in syngeneic hosts, using purified tumor cell plasma membranes as antigen. Administration of LMI to mice bearing P815 (mastocytoma) augments tumor-specific cytolytic activity and results in a dramatic reduction in tumor load. Mice receiving 10^5 P815 (LD_{50} is about 100 cells/mouse) die in about 22 days. A single administration of LMI (day 0) results in significantly prolonged survival. Treatment with neither LMI alone, nor cyclophosphamide (CY) alone results in long term survival. However, treatment with both LMI (day 0) and CY (day 3) results in long term survival of 40 to 60% of the mice. LMI need not be administered at the site of tumor growth to be effective. The physical form of the antigen is critical; treatment with plasma membrane vesicles (antigen) has little or no effect in comparison to the same antigen on LMI. Comparable effects of LMI have been observed for P815, EL4, RDM-4 and BCL-1 tumors in their syngeneic hosts. These findings strongly suggest a therapuetic potential for tumor antigen in the appropriate physical form, alone or in combination with chemotherapy. In addition, this form of antigen administration may offer a means of generating more effective effector populations for use in adoptive transfer protocols.

CE 316 DETECTION OF MEMBRANE PROTEINS IN NK AND IL-2 ACTIVATED KILLER CELL-TUMOR CELL INTERACTIONS. Mary O. Mondragon, Riva Schneck and Allen J. Norin, Departments of Medicine and Anatomy & Cell Biology, SUNY Health Science Center at Brooklyn, New York 11203
Receptor ligand system(s) for tumor destruction by activated and non activated killer lymphocytes have not been characterized. Studies on tumor membrane proteins involved in MHC non restricted cell mediated cytotoxicity using A549, a human lung carcinoma cell line sensitive to interleukin-2 activated killer (IAK) lymphocytes and K562, a human chronic myelogenous leukemia cell line sensitive to natural killer cells (but resistant to IAK) were performed. Biotinylated crude membrane digests of A549 and K562 were adsorbed respectively to IAK cells or to unstimulated human peripheral blood lymphocytes (UPBL). Adsorbed proteins were separated by SDS-PAGE, western blotted and probed with streptavidin-alkaline phosphatase. Seven to nine proteins, 28 KD to 74 KD were detected from these 4 groups. One common protein (47 KD) was detected. A549 proteins which bound to IAK compared to UPBL showed five identical bands in the range of 47 KD-72 KD and five different proteins at 31 KD-42 KD. A 37 KD protein and a 47 KD protein from A549 and K562 bound to UPBL. Additionally six different A549 and K562 proteins were detected on UPBL adsorbed blots. The reverse experiments were performed with biotinylated UPBL membrane digests adsorbed to A549 and K562. Western Blots from these preparations showed fifteen UPBL proteins that bound to K562 and seven UPBL proteins that bound to A549. These results suggest that the above approach may be useful in probing cytolytic lymphocyte-tumor interaction for binding and programming for lysis.

CE 317 TUMOUR INFILTRATING LYMPHOCYTES (TIL) FROM PATIENTS WITH BLADDER TUMOURS AND THE INFLUENCE OF TUMOUR CELL CLASS I HLA ANTIGENS EXPRESSION ON THEIR EXPANSION. AME Nouri, JP Blandy and RTD Oliver, The London Hospital Medical College, London E1 2AD.
Studies on a series of 18 snap frozen bladder tumour TURT samples have demonstrated that the majority show some degree of abnormality in class I HLA antigen expression (6 major and 8 minor changes) when normal stroma and tumour cells were compared. These tumours varied considerably in the proportion of infiltrating T lymphocytes on the basis of positive staining with CD3, CD4 and CD8 phenotyping. Single cell suspensions isolated from separate TURT chips from the same tumour have been expanded by IL-2 (100u/ml) containing RPMI 1640 plus 2.5% conditioned medium.
TIL cells were successfully expanded (continuous expansion for more than four weeks) from six of eighteen tumours (0 of 6 with major loss of class I and 6 of 12 without such loss). There was a tendancy for increased proportion of CD8 cells with increased duration of culture CD3 (80-87%), CD4 (2-40%) and CD8 (30-50%). The profiles of DR and TAC expression indicated the activated nature of these cells. These results justify further investigation of HLA antigens. Preliminary results of attempts to correct HLA defect will be presented.

CE 318 HUMAN CHORIONIC GONADOTROPHIN (βhCG) EXPRESSION AND CLASS I HLA ANTIGEN LOSS AS FACTORS IN ESCAPE FROM IMMUNE SURVEILLANCE OF BLADDER CANCER, R T D Oliver, S Popert and A Nouri, The London Hospital, Whitechapel, London E1 1BB, UK.
Recent results from this department have demonstrated that 11/23(48%) of patients with metastases have detectable levels of βhCG and that these patients are more resistant to treatment with Chemotherapy (3+7/12 (83%) of hCG negatives and 0+0/11 hCG positive patients showed complete or partial response). Because βhCG producing trophoblast lack expression of HLA class I antigen a series of 18 snap frozen bladder tumour samples (9 Ta, 3 T1 & 6 =>T2) taken from patients presenting with bladder cancer without metastases has been examined for expression of βhCG and class I HLA antigen expression using monoclonal antibodies to monomorphic and polymorphic HLA-A and B determinants. Only 4 of the 18 tumours showed equivalent staining of stroma and tumour cells and there was a trend for greater loss of class I reactions on the more invasive tumours. 0/9 non-invasive and 3/9 invasive and tumours were β hCG positive. All three βhCG positive invasive tumours had diminished class I expression compared to only 3 of 6 β hCG negative invasive tumours.

It is concluded that the most malignant form of bladder cancer mimic trophoblast to escape immune surveillance.

CE 319 REJECTION OF SARCOMA CELLS FOLLOWING TRANSFECTION OF MHC CLASS II GENES, Suzanne Ostrand-Rosenberg, Archana Thakur, and Virginia Clements, University of Maryland Baltimore County, Baltimore, MD 21228.

T helper (T_h) lymphocytes recognize antigen in the context of class II or Ia antigens of the major histocompatibility complex (MHC). The immunogenicity of tumor cells is thought to be dependent on their expression of tumor associated antigens (TAA), molecules that are restricted to, or expressed in higher quantities on malignant cells. An effective anti-tumor immune response therefore requires the presentation of TAA in the context of class II molecules to T_h cells. Since most tumor cells do not express class II antigens, TAA must be processed and presented by third party antigen presenting cells (APC). We have reasoned that a more efficient anti-tumor immune response may be generated if tumor cells are class II antigen positive and therefore able to present their own TAA to T_h lymphocytes. To test this hypothesis we have used DNA-mediated gene transfer to produce syngeneic class II antigen positive mouse sarcoma cells. The class II expressing sarcoma cells are efficiently rejected by the autologous host (A/J mice), and can protect against subsequent, simultaneous, and pre-immune challenge with the class II negative parental line. These studies suggest that induction of tumor cell class II antigen expression may be a feasible mode of specific immunotherapy.

CE 320 SUCCESSFUL *IN VITRO* IMMUNIZATION OF HUMAN PERIPHERAL BLOOD MONONUCLEAR CELLS AGAINST PROSTATE SPECIFIC ANTIGEN: AN APPROACH FOR THE ANTIGEN-SPECIFIC ADOPTIVE IMMUNOTHERAPY OF PROSTATE CANCER. J.M. Plummer, J.J. Goodwin and M.E. Osband. Boston, MA.

There is no effective therapy available for disseminated prostate carcinoma (Stage D) patients who no longer respond to hormone treatment. Renal cell carcinoma patients have been successfully treated using autolymphocyte therapy (ALT)—our form of adoptive immunotherapy that is minimally toxic, outpatient-based and antigen-specific. In order to use ALT for treating prostate carcinoma, a protocol was developed for the *in vitro* immunization of human peripheral blood mononuclear cells (MNC) against prostate specific antigen (PSA). This 33k MW serine protease is an appropriate target molecule for ALT because it is prostate-specific and is found on the surface of prostate carcinoma cells. Isolated MNC were cultured in AIM-V serum-free medium containing increasing concentrations of PSA. The maximum response occurred on day 3 to 4 at an optimal concentration of 20 µg PSA/ml. Lymphocyte activation was demonstrated by a 30–50x stimulation index and the expression of activation markers on 10 to 20% of the cultured T-cells including IL2 receptor (IL2-R), transferrin receptor and HLA-DR (Ia). Adding rIL-2 (100 to 500 U/ml) in the immunizing culture produced a 4 to 5 fold increase in stimulation but using rIL-1, TNF, IFNγ, or MDP had no effect. OKT3 and BCG increased both the background response and the response to PSA. The presence of a monoclonal antibody specific for PSA modulated proliferation against PSA. The PSA proliferative response was diminished but measureable for MNC from 4 of 4 prostatic cancer patients. Limiting dilution analysis of MNC from healthy males indicated between 1 and 5 cells per 10,000 MNC respond to PSA. These data demonstrate the ability to immunize T cells *in vitro* that are antigen-specific for PSA. We have developed the technology to identify, isolate and expand PSA-responsive lymphocytes and a clinical pilot study using these cells will be initiated shortly.

CE 321 CHARACTERIZATION OF THE CYTOLYTIC EFFECTOR TIL IN HUMAN RENAL CELL CARCINOMA, Patricia Rayman, James H. Finke, Jeannine Alexander, Mark Edinger, Raymond R. Tubbs, Robert Connelly, Edson Pontes, Siva Murthy, and Ronald Bukowski, The Cleveland Clinic Foundation, Cleveland, OH 44195.

Previously we showed that IL2 expanded TIL from human renal cell carcinoma mediated non-MHC-restricted cytotoxicity. Phenotypic analysis showed that cultured TIL were composed mostly of T lymphocytes with varying numbers of $CD4^+$, $CD8^+$ and $CD56^+$ ($Leu19^+$) populations. Here we compared the cytolytic activity of the two predominant TIL subsets, $CD3^+CD4^+$ and $CD3^+CD8^+$, to that of the $CD56^+$ populations. In some experiments $CD4^+$ and $CD8^+$ TIL were isolated by positive selection using antibody coated biomagnetic beads. In other experiments $CD5^+CD56^-$, $CD5^+CD56^+$ and $CD5^-CD56^+$ TIL were isolated by cell sorting with $FACStar^+$ and tested for cytotoxic activity. In a 4 hr ^{51}Cr release assay the highly enriched populations of $CD4^+$ and $CD8^+$ TIL showed minimal lytic activity whereas unseparated cells exhibited significant levels of cytotoxicity against autologous and allogeneic tumor targets. The lytic activity seen in the 4 hr assay with unseparated TIL appeared to be related to the presence of $CD56^+$ populations. With one exception none of the purified $CD4^+$ or $CD8^+$ TIL expressed any significant levels of CD56 while the unseparated TIL contained varying numbers of $CD3^+CD56^+$ and $CD3^-CD56^+$ populations. Cell sorting experiments confirmed that most of the lytic response of TIL culture was mediated by the $CD5^-CD56^+$ and $CD5^+CD56^+$ populations. Although CD3+CD56- TIL were minimally lytic in 4 hours, we show that both $CD3^+CD4^+$ and $CD3^+CD8^+$ subsets displayed substantial cytotoxicity in 18 hr ^{51}Cr release assay. Moreover, we show that $CD3^+CD56^-$ can mediate lysis of autologous RCC in 4 hrs. provided tumor targets are pretreated with a combination of recombinant TNFalpha and IFNgamma. Results with highly enriched TIL subsets suggest that most of the lytic response of cytokine treated targets is mediated by $CD3^+CD8^+$ and not $CD3^+CD4^+$ TIL. The lytic response observed with IFNgamma/TNFalpha treated targets was not specific since TIL will lyse allogeneic RCC that were pretreated with this cytokine combination. These results suggest that in addition to the $CD56^+$ effector cells both $CD4^+$ and $CD8^+$ TIL which lack $CD56^-$ can mediate lysis of RCC and that their mechanism of action may be different from one another and from $CD56^+$ cells.

CE 322 ANALYSIS OF CELL SURFACE PROTEINS ON MUTAGEN-TREATED MURINE TUMORS RECOGNIZED BY T CELLS. Luigina Romani, Ursula Grohmann, Paolo Puccetti, Angela M.Rossi and Cristina M.Fioretti, Dept. Experimental Medicine and Biological Sciences, University of Perugia,Italy.
Treatment of murine tumor cells with mutagens generates immunogenic variants expressing new, highly immunogenic antigens not found on the parent tumor. We have recently raised a polyclonal syngeneic antiserum to L5178Y lymphoma cells mutagenized by dacarbazine treatment (L5178Y/DTIC variant), which specifically interferes with T-cell recognition of the variant cells. Immunoprecipitation studies performed with this antiserum showed the presence of an 80KDa glycoprotein on the surface of the variant tumor, not detectable on parent cells. In addition, a 45KDa component was detected only in the lysate of L5178Y/DTIC cells. In order to explore the possible contribution of these molecules to the overall immunogenicity of the mutagenized variant, the 80 and 45KDa proteins, precipitated by the antiserum and blotted onto nitrocellulose, were injected intrasplenically into recipient mice. For comparison, the gp70 env proteins precipitaed from both parental and mutagenized cells were studied. The frequency of CTL precursors in $CD8^+$ splenocytes was evaluated. The results showed that only sensitization with the 80KDa protein resulted in a 10-fold increase in the frequency of CTL precursors to mutagenized cells. Also, sensitization with the gp70 molecules from L5178Y/DTIC but not parental cells resulted in a two-fold increase in the CTL precursor frequency to the same tumor.
Supported by PFO from Italy-CNR, contract n.88.01120.44.

CE 323 T CELL CLONES IN HUMAN METASTATIC MELANOMA POSSESSING BOTH CYTOTOXICITY AND INTERLEUKIN-2 PRODUCTION IN RESPONSE TO AUTOLOGOUS TUMOR CELLS, Marie A. Salmeron, Hidetoshi Seki, Chris D. Platsoucas and Kyogo Itoh, Departments of General Surgery and Immunology, The University of Texas, M.D. Anderson Cancer Center, Houston, TX 77030. T cell clones from human melanoma TIL were investigated for their cytotoxicity and IL-2 production in response to autologous tumor cells. Two functionally distinct types of T cell clones were identified: T cell clones possessing both cytotoxicity and the ability to produce IL-2 in response to autologous, but not allogeneic tumor cells; and those exhibiting only autologous tumor cell lysis without IL-2 production. No classic markers for T cell subsets (CD4, CD8, 4B4 or 2H4 antigens) correlated with either cytotoxicity or IL-2 production. In contrast to melanoma TIL, T cell clones from either lymph nodes with melanoma metastasis or renal cell carcinoma TIL primarily did not exhibit either cytotoxicity or IL-2 production in response to autologous tumor cells. Anti-CD3 mAb suppressed autologous tumor cell lysis by melanoma TIL clones at the effector phase, while primarily enhancing their IL-2 production in response to autologous, but not allogeneic tumor cells. Further, anti-CD3 mAb induced non-cytotoxic clones to be cytotoxic, and some non-IL-2-producing clones to produce the lymphokine. T cell clones from melanoma TIL showed the highest proliferation when incubated with autologous tumor cells and IL-2. (This research is partly supported by grant CA-47891 from the NIH).

CE 324 GENERATION OF MONOCLONAL ANTIBODIES FROM PATIENTS WITH PRIMARY LUNG CANCER, D. S. Schrump, K. Furukawa, F. Cacace, C. Gordon, K. O. Lloyd and L. J. Old, Sloan-Kettering Institute, NY, NY 10021. Regional lymph node lymphocytes from 22 patients with non-small cell lung cancer were immortalized with EBV and culture supernatents were analyzed for cell surface reactivity against allogeneic cancer cell lines. Of 16,378 supernatents analyzed, 1,176 (7%) demonstrated cell-surface reactivity (range 1-33% per patient). Frequency of reactivity did not correlate with histology or nodal status at time of surgery. Recurrent reactivity patterns were observed; several representative cultures were successfully expanded and fused with mouse myeloma cell lines. A stable human-mouse hybrid derived from a patient with bronchoalveolar lung cancer secretes an IgM antibody (190.653) with serologic reactivity restricted primarily to renal cancer cell lines, and to a lesser extent, with cell lines derived from normal renal epithelium. Antibody 190.653 detects a heat stable, neuraminidase-resistant antigen present in the acidic glycolipid fraction of renal carcinoma cell lines which is structurally similar to galactosylceramide sulfate based on migration in TLC plates and immunostaining data. The antigen is quantitatively increased in extracts of renal cancer specimens in comparison to corresponding normal renal tissues, but has not been detected in normal or cancerous lung tissue glycolipids including glycolipids from the patient from whom antibody 190.653 was derived.

CE 325 SUSCEPTIBILITY OF HUMAN NEUROBLASTOMA CELL LINES TO CTL-MEDIATED LYSIS. Karen Gansbacher Zier and Giuliana R. Pierson. Departments of Medicine and Microbiology, The Mount Sinai School of Medicine, New York, NY 10029.

The absence of cell surface MHC determinants may be one means by which tumor cells escape destruction by T cells. Recently, it was reported that neuroblastoma cells were resistant to lysis by CTL, even after upregulation of their class I antigens with gamma-interferon. If, however, the target determinant on the tumor cell were a complex of peptide plus class I antigen, the absence of lysis could be explained by the fact that the CTL had been generated against normal cells sharing class I with the neuroblastoma cells, not to the tumor cells themselves. To test the effect of including tumor cells in the sensitizing culture, CTL lines were generated against CHP-100 neuroblastoma cells alone or against CHP-100 plus allogeneic PBL from donor LS, who shared class I antigens with CHP-100 and who also expressed foreign class II antigens. Sensitization to CHP-100 cells alone resulted in a population with largely NK activity. Stimulation with CHP-100 and LS PBL, however, led to good lysis of CHP-100 with very low killing on K562. This suggested that NK cells were not responsible. Further studies provided evidence for a role for T cells. First, cold target inhibition studies showed the CTL recognized target antigens on CHP-100 not expressed by K562, as well as some target antigens shared between CHP-100 and LS. Blocking studies with W6/32, a monoclonal antibody directed against class I, demonstrated specific inhibition of lysis on CHP-100. Finally, a population of almost pure T cells, prepared by depletion of NK and LAK cells on the FACS, lysed CHP-100. These results show that class I positive neuroblastoma cells are susceptible to lysis by CTL and suggest the target antigen could be composed of a peptide specific for the tumor cell plus class I.

Animal Models of Adoptive Therapy

CE 400 IMMUNOADJUVANT STUDIES OF IL-2 LIPOSOMES AGAINST CANCER: MURINE PHARMACOKINETIC AND ANTI-TUMOR EFFICACY STUDIES USING INTRACAVITARY ROUTES OF ADMINISTRATION. Peter M. Anderson, Emmanuel Katsanis, Mary Rich, Arnold S. Leonard, Cynthia M. Loeffler, and Augusto C. Ochoa*., Departments of Pediatrics and Surgery, University of Minnesota, Minneapolis, MN 55455 and *National Cancer Institute, Frederick MD 21701

Liposomes containing carrier free IL-2 (Hoffmann-LaRoche) were synthesized. Over 90 percent of the cytokine was reliably incorporated as measured by the fluorescamine protein assay and CTLL-20 bioassay. Pharmacokinetics and the efficacy of intracavitary administration of IL-2 liposomes were investigated in C57BL/6 mice. Intraperitoneal IL-2 liposomes had increased serum and peritoneal elimination half-lives compared to free IL-2 (42 vs 7 and 250 min vs 18 min respectively). A sensitive IL-2 EIA using a urease detection system can detect levels of IL-2 as low as 0.5-300u/ml will be described. When ip IL-2 liposomes were used to treat hepatic metastases of the slightly immunogenic MC-38 tumor, no anti-tumor effect was seen. However, significant anti-tumor activity was seen using adoptively transferred anti-CD3 + IL-2 activated splenocytes in combination with IL-2 liposomes. In contrast, intrathoracic free IL-2 or IL-2 liposomes could effectively reduce the number of pulmonary metastases of the immunogenic MCA-106 tumor. Tumor reduction by IL-2 liposomes was significantly better than that achieved by free IL-2.

CE 401 GENERATION OF CYTOLYTIC T LYMPHOCYTES (CTL) IN PERIPHERAL BLOOD BY COMBINED ACTIVE SPECIFIC AND CYTOKINE IMMUNOTHERAPY IN A MOUSE MODEL FOR METASTATIC COLON CANCER. Pedro Arroyo, Marc K. Wallack and Jerry A. Bash, Department of Surgery and Research, Mount Sinai Medical Center, Miami Beach, Florida 33140.

A weakly immunogenic colon adenocarcinoma line (C-C36) was used to establish hepatic metastases by intrasplenic injection of syngeneic Balb/c mice. Untreated controls receiving $5x10^5$ tumor cells (day 0) die of hepatic tumors (mean survival = 33.2 days). Active specific immunotherapy with vaccinia virus-infected tumor cell oncolysates (VCO) alone (days +4, +10) failed to significantly alter mean survival (39.6 days) or tumor burden (day 14 liver colonies and weight). When low dose (25,000 U/day) interleukin-2 (human recombinant IL-2, Hoffmann La Roche) was administered on days 11-13 in VCO primed mice, significant improvement in survival($p<.05$) and decrease in tumor burden (day 14) was obtained over controls (untreated) or mice treated with VCO alone. Even further improvement in therapeutic results was achieved when interferon alpha (hybrid human recombinant A/D IFN-a, Hoffman La Roche) was administered (25,000 U/day) along with IL-2 in VCO primed mice. IFN-a alone or in combination with IL-2 contributed no significant improvement unless mice had been primed with VCO. Combination of the three modalities resulted in a synergistic therapeutic response ($p<0.002$). Evidence to suggest that the therapeutic effect of this regimen was mediated by induction of specific cell mediated immunity was obtained both by demonstration of delayed hypersensitivity to C-C36 (24 hr. foot-pad swelling) and C-C36 specific CTL (CD8+) activity(^{51}Cr release) in peripheral blood which paralleled the therapeutic effectiveness of the regimen. Achievement of these successful therapeutic results with a weakly immunogenic IFN-a resistant tumor may have important implications for combination immunotherapy in the clinical setting.

CE 402 ADOPTIVE TRANSFER OF ANTILISTERIAL IMMUNITY CORRELATES WITH IN VITRO CYTOTOXICITY IN THE IMMUNE CD8 T LYMPHOCYTE SUBPOPULATION, Jory R. Baldridge, H. G. Archie Bouwer and David J. Hinrichs, Chiles Research Institute, Providence Medical Center, Portland, OR 97213, and Immunology Research, VA Medical Center, Portland, OR 97207
Antilisterial immunity can be adoptively transferred with immune T-cells. The in vivo protective response of these immune T-cells can be significantly enhanced by stimulation in culture prior to adoptive transfer. We have found that following the culture activation step, the immune $CD8^+$ T-cell population is solely responsible for the adoptive transfer of antilisterial immunity. We have developed an in vitro cytotoxicity assay which utilizes J774 cells infected with viable Listeria, as the target cell population. We have found that culture activated immune cells (but not culture activated non-immune cells) will lyse Listeria infected J774 cells. Using negative depletion, we have found that following culture activation, the effector T-cell population which is able to lyse Listeria infected J774 cells is present in the Thy^+, $L3T4^-$, $Ly2^+$ lymphocyte subset. In addition, we have found that the immune culture activated cell population is able to lyse Listeria infected bone marrow derived macrophages. The antilisterial response appears to be class I restricted in that we have utilized Listeria infected fibroblasts in the cytotoxicity assay and found that the immune culture activated effector cells are able to mediate lysis of this target cell population. In conclusion, the in vitro cytotoxicity response of the immune culture activated effector cells correlates with in vivo antilisterial protection. These results demonstrate that immunity to a facultative intracellular parasite, which does not go through an eclipse phase during its life cycle, is mediated by the $CD8^+$ lymphocyte subset, and that the antilisterial response is MHC class I restricted, both in vivo, and in vitro. Supported by AI23455

CE 403 A P815-SPECIFIC CTL CLONE IS DEPENDENT UPON TUMOR CELL STIMULATION FOR CELL DIVISION, Harry D. Bear and Shelley K. Hoover, Massey Cancer Center and Department of Surgery, Medical College of Virginia, Richmond, VA 23298. A P815-specific CTL clone (GD11.10) was selected from spleen cells of mice bearing day 12 PHS-5 (a HAT-sensitive mutant of P815) i.d. tumors. Cell populations were enriched for T cells, depleted of CD4+ subsets, and stimulated for 6 days in MLTC culture in 24-well plates with mitomycin c-treated PHS-5 (PHS-5mc) cells, HAT, irradiated normal spleen cells, 10% secondary MLR supernatant plus rIL-2 (25 U/ml). GD11.10 was selected by two limiting dilution procedures in 96-well microtiter plates. GD11.10 cells were maintained by restimulation each 5-7 days with PHS-5mc, HAT and 20 U/ml rIL-2. GD11.10 is a CD3+, CD8+ cell specific for P815 tumor target cells (63% lysis), while not lysing P388 (1%), EL-4 (0%) or B16 (0%) with some lysis of YAC (16%) at 10:1 E:T ratios. Removal of tumor cells and rIL-2 for 7 days resulted in a decline in cell numbers and cytotoxic activity. Subsequently, cell proliferation and cytotoxicity was induced in a 6 day culture as follows:

Responders	rIL-2	PHS-5	% Cytotox.(E:T,5:1)	Fold Increase,Cell No.
GD11.10	-	+	10	0.2
GD11.10	10 U/ml	-	31	0.3
GD11.10	10 U/ml	+	42	4.8

No GD11.10 cells could be recovered after culture without antigen or rIL-2. Thus, proliferation of tumor-specific GD11.10 cells is dependent upon both antigen and IL-2, while cytotoxic activity can be induced by rIL-2 alone. Moreover, GD11.10 has in vivo anti-tumor activity.

CE 404 T cell targeting using bispecific monoclonal antibodies in a rat colon carcinoma model

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Activated non-specific T cells can lyse tumor cells in vitro if artificially linked by bispecific (monoclonal) antibodies containing anti-TCR/CD3 as well as anti-tumor specificity. Experimental animal models that reflect human non-immune syngeneic tumor-host relationships and allow the development of a bispecific treatment strategy have not yet been described.
We produced mixed hybridoma's by fusing R73 (IgG1, anti-rat-TCR framework, Hünig et al., J. Exp. Med. 1989, 169:73) and CC11, CC52 or CC101 (all IgG1, anti-surface-CC531, a weakly immunogenic Wag rat colon carcinoma) hybridoma lines. Polyclonal stimulation of Wag spleen cells with solid phase R73 +/- Con A supernatant, followed by short term (5-11 days) culture with rIL-2 (Ortho) up to 1000 U/ml gave rise to rapidly proliferating predominantly R73+OX8(CD8)+ T cells that could induce modest lysis of CC531 in the presence of mixed hybridoma supernatants (up to 25% 51Cr release at E/T ratio 25:1), while the same effectors exerted excellent cytotoxicity against P815 target cells in R73 mediated reverse ADCC (up to 80% lysis).
In view of the far greater potential in vitro of bispecific T cell targeting reported in other species (human, mouse), especially when T cell clones were used, we screen alternative stimulation protocols and target cells for comparable cytotoxic efficacy prior to in vivo studies.

CE 405 ADOPTIVE TRANSFER OF EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS AND THE RECIPIENT RESPONSE TO THE TRANSFERRED CELL POPULATIONS,

H. G. Archie Bouwer, Greg N. Dietsch and David J. Hinrichs, Chiles Research Institute, Providence Medical Center, Portland, OR 97213, and Immunology Research, VA Medical Center, Portland, OR 97207.

Experimental Allergic Encephalomyelitis (EAE) can be adoptively transferred with spleen cells from rats previously immunized with myelin basic protein (BP) emulsified in CFA only following stimulation in culture with BP or selected mitogens. BP-specific T-cell lines also require a culture activation step prior to the adoptive transfer of clinical EAE and lose their ability to transfer clinical disease when maintained only in IL-2. We have evaluated the response of recipients of BP-immune spleen cells and BP-specific cell lines subsequent to cell transfer. Following recovery from encephalitogenic T cell lines mediated adoptive disease, recipients do not develop EAE when subsequently challenged with BP-CFA. These recipients remain susceptible to adoptively transferred disease. These same T cell lines do not evoke this resistance to BP-CFA challenge in recipients receiving these lines when the cell lines are grown only in IL-2. We have found that recipients of BP-specific line cells co-stimulated with PMA + Ionomycin develop adoptive EAE, and are resistant to active EAE induction. However, an episode of cell line mediated EAE is not required to invoke the protective response, in that recipients of BP specific line cells stimulated with only Ionomycin do not develop adoptive disease, but are resistant to induction of active EAE. This is in contrast to recipients of BP-specific line cell stimulated in the presence of only PMA, in that adoptive disease does not develop, and the recipients are susceptible to active EAE. Supported by NIH NS24130

CE 406 CELL CONSTRUCTS OF MURINE FIBROBLASTS EXPRESSING MELANOMA-ASSOCIATED ANTIGENS PROTECT AGAINST MELANOMA IN MICE SYNGENEIC WITH THE TUMOR, Cohen, E.P. and Kim, Y.S., Department of Microbiology and Immunology, University of Illinois at Chicago, Chicago, IL 60612.

Current approaches toward the specific immunotherapy of melanoma involve immunizations with inactivated melanoma cells, often in combination with immune adjuvants. Viable cell constructs expressing melanoma associated antigens (MAA) in conjunction with allogeneic histocompatibility antigens may lead to augmented immunoprotective effects. We constructed murine fibroblasts expressing MAA by transfection with DNA from B16 melanoma cells. We accomplished this by co-transfecting LM(TK-) fibroblasts with unfractionated genomic DNA from B16 cells and DNA from the plasmid pTK (carries the herpes simplex gene for thymidine kinase). After initial selection in HAT medium, viable colonies of transfected fibroblasts expressing MAA were identified by erythrocyte-rosetting in situ, using polyclonal antibodies for MAA and human RBC coupled with rabbit anti mouse Ig. Confirmation of expression of MAA by the transfectants was made by flow cytometry and immunoprecipitation of radiolabeled cell extracts. C57BL/6 mice (H-2^b) are susceptible to the malignant growth of B16 melanoma (H-2^b). We immunized the mice by injecting them three times with approximately 10^6 MAA positive fibroblasts (H-2^k), without adjuvant. As a control, we injected mice with an equivalent number of x-irradiated B16 cells. Mice receiving the MAA positive constructs survived longer ($p < .01$) than mice receiving other forms of specific immunotherapy.

CE 407 POTENTIAL OF ALLOGENEIC TUMORICIDAL CYTOTOXIC T LYMPHCYTES IN BRAIN TUMOR ADOPTIVE IMMUNOTHERAPY, **Monika Fleshner*, Linda R. Watkins*, Carol A. Kruse+ & Donald Bellgrau**, Dept. of Psych., Univ. of Colorado-Boulder*, Boulder, CO 80309, Dept. of Surgery+ & Dept. of Micro/Immunol.** UCHSC, Denver, CO 80262**

Fisher rats die within 5 wks of intracranial implant of Fischer derived 9L gliosarcoma tumor cells. We tested if allogeneic lymphocytes cytolytic for the 9L tumor in vitro would kill tumor, in vivo. One wk after implantation of a cannula into the right frontal cortex of each rat, 9L tumor cells were injected via the cannula into the brain along with an exogenous source of recombinant Interleukin-2 and DA lymphocytes (DA is genetically unrelated to Fischer) either generated against Fischer 9L tumor haplotype (Group 1) or unstimulated (Group 2). Only the former population lysed chromium labelled 9L targets in vitro. Both groups were injected 5 times over a 2 wk time period with IL-2 and potential effector cells starting at the time the tumor was injected. Rats were killed when Group 2 was losing weight & showing tumor-associated morbidity. Serial sections of the brains showed rats in Group 2 displayed massive tumor whereas Group 1 rats showed little or no obvious tumor. We conclude that allogeneic cytotoxic T cells can destroy tumor in vivo. Unstimulated cells in the presence of IL-2 cannot, suggesting that tumor destruction is due to specific cytotoxicity & not to a nonspecific inflammation caused by allogeneic lymphocytes &/or exogenous sources of IL-2. Other studies indicate that this protocol should protect animals from tumor induced death & that surviving animals display no obvious neurologic dysfunction, suggesting that lymphocyte reactivity to normal brain is minimal. Ongoing studies are designed to determine the effect of this or modified protocols on already established tumors of varying sizes & whether more potent &/or specifically lytic allogeneic effector populations can be generated.

CE 408 ANTI-TUMOR EFFECT OF GENE TRANSFER MEDIATED LYMPHOKINE DELIVERY AT THE TUMOR SITE. Bernd Gansbacher, Rajat Bannerji, Karen Zier, Kathy Cronin, Ranjana Tavorah, Eli Gilboa. Department of Hematology/Lymphoma and Molecular Biology, Memorial Sloan Kettering Cancer Center and The Mount Sinai School of Medicine, New York, N.Y.
Lymphokines such as IL-2, TNF or gamma-interferon (g-INF) are important modulators of the immune response and have been used in cancer models to induce anti-tumor responses. These applications, involving the systemic administration of high doses of lymphokine, were compromised by the short half-life and toxicity of these molecules. In this study we asked whether local administration of low doses of lymphokines at the tumor site would generate an immune response leading to tumor rejection. Retroviral-based gene transfer of the human IL-2 or mouse g-INF into CMS-5, a chemically induced fibrosarcoma of Balb/C mice, was used to assess the effect of local secretion of these lymphokines on tumor growth. CMS clones secreting high, intermediate or low levels of lymphokines were injected I.D. into Balb/C mice. Tumor growth correlated inversely with lymphokine secretion. Only tumor cells which did not secrete lymphokines grew and killed the mice. Tumor cells secreting "high" levels of lymphokine did not grow and cells expressing intermediate or low levels of lymphokine grew transiently and then regressed. It is estimated that secretion of 10-100 units of IL-2 or g-INF were sufficient to block tumor growth. Our results demonstrate that local delivery of very low levels of IL-2 or g-INF can result in a potent anti-tumor effect which correlates closely with levels of IL-2 and INF produced at the tumor site. This suggests that the way in which lymphokines are delivered at the tumor site can have a decisive impact of anti-tumor responses.

CE 409 IMMUNE REGRESSION OF ROUS SARCOMA VIRUS-INDUCED TUMORS: CORRELATION WITH MHC CLASS I EXPRESSION, **Irwin H. Gelman & Hidesaburo Hanafusa, Department of Molecular Oncology, The Rockefeller University, New York, NY 10021-6399.**
Unlike all previously described Rous sarcoma virus (RSV) mutants encoding nonmyristoylated src product, those of rASV157 and rASV1702, which contain novel N-terminal domains, can transform chicken embryo fibroblasts (CEF) in culture and cause tumors in 1-week-old chicks. However, these tumors regress fully after 2 weeks. In addition, chicks preinfected with either rASV suppress tumor formation by challenge infections with RSV. This "protection" is specific for determinants encoded or induced by $p60^{v\text{-}src}$ but not for those of RSV-encoded gag, pol or env, or other oncogenes such as v-fps or polyomavirus middle T. The protective effect is elicited only by these two rASV's, and is directed by their expression of novel N-terminal src domains. Using chicken line SC, inbred for MHC Class I haplotype B^2/B^2, we show that infection with these rASV's activates a src-specific cellular immune cytotoxicity responsible for tumor rejection. Nude (athymic) mice injected with NIH3T3 cells expressing v-src or the rASVsrc's form tumors which continue to grow, further indicating that immunocytes are required for the rASV-induced regression. As in the avian system, NIH3T3 or Balb/c-3T3 cell lines expressing the rASVsrc's display characteristics of partial transformation when compared with those expressing $p60^{v\text{-}src}$. Evidence will be presented that $p60^{v\text{-}src}$ down-regulates the expression of MHC Class I at the level of transcription, and that rASV-induced tumor regression correlates with sustained expression of MHC Class I. Data will also be presented on the adaptation of this tumor rejection system to mice. We believe this to be a novel system for studying the immunogenicity of activated oncogene products and their use as targets in cancer therapy.

CE 410 PERILYMPHATIC INJECTION OF IL-4 WITH OR WITHOUT OTHER LYMPHOKINES ACTIVATES REJECTION OF POORLY IMMUNOGENIC MOUSE TUMORS, Mirella Giovarelli, Maria Carla Bosco, Andrea Modesti, Guido Forni, Dept. Exp. Med., Univ. L'Aquila, Dept. Exp. Med., Univ. Rome, and Inst. Microbiol., Univ. Turin, Italy.
IL-4 has been shown to activate B lymphocytes, MO and CTL. It was therefore decided to study the ability of perilymphatically injected IL-4 to inhibit the growth of murine tumors. Ten daily injections of 0.1-100 pg rIL-4 (Immunex) induced dose dependent inhibition of the growth of a poorly immunogenic sarcoma (CE-2) raised by methylcolanthrene in BALB/c mice. Association of rIL-1 , a synthetic nonapeptide (Sclavo), or rIL-2 created a helper system capable of recruiting an effective antitumor reactivity. This reaction is mediated by granulocytes, MO, NK cells, and T-lymphocytes: the tumor area is infiltrated by mononuclear cells and granulocytes (mostly eosinophils), which form close contacts between each other and with the neoplastic cells. The draining lymph nodes display marked expansion of the cortical follicles and massive infiltration into the medulla of histiocytes, foreign-body giant cells, plasma cells and immunoblasts, joined together by lymphocytes and by granulocytes. The local activity becomes systemic and confers a specific immune memory. The growth of a second, contralateral inoculum was inhibited in a significant number of animals. A more comprehensive assessment of the extent to this reaction was made by studying its inhibition of the growth of a spontaneous non-immunogenic and metastasizing mouse breast tumor (TS/A).

CE 411 INTERLEUKIN 2 (IL-2) INFUSION IN THE DOG INDUCES A RESPONSE IN IL-2 TARGET CELLS. Stuart C. Helfand*, Jaime F. Modiano#, Peter C. Nowell#. *University of Wisconsin, #University of Pennsylvania.

IL-2, a 15 kilodalton polypeptide hormone secreted by activated T helper cells, is a critical mediator of the cellular immune response. The ability of IL-2 to act as an antitumor agent, presumably via immune-mediated mechanisms, is currently under study in humans and animals. After interacting with its receptor, important effects of IL-2 on lymphoid cells include: 1) increased expression (upregulation) of the IL-2 receptor (IL-2R) on the plasma membrane and 2) induction of cellular proliferation. We investigated the efficacy of subcutaneous delivery of human recombinant IL-2 by continuous infusion in two dogs with mast cell tumors. Specifically, we looked for IL-2 activity in the circulation of treated and control dogs, and we analyzed the effect of the IL-2 therapy on mitogenic responses of peripheral blood lymphocytes (PBL), the presumed target cells for IL-2.

No IL-2 was measured in the serum from patients or control dogs immediately prior to the beginning of IL-2 therapy. PBL isolated from these dogs at this time proliferated in response to PHA, with a peak response on day seven, and more slowly in response to IL-2, reflecting a relatively small number of lymphocytes bearing biologically relevant IL-2R. One of the two patients showed measurable IL-2 activity in the serum on day eight of therapy. Concurrently with this, the in vitro mitogenic response of this patient's PBL to IL-2 occurred earlier reflecting a possible increase in the relative number of lymphocytes that expressed IL-2R. Neither the second patient nor the control had measurable IL-2 in the serum at any time, and there was no change in the kinetics of the IL-2 response by PBL in these dogs. These data indicate that in one of two dogs, IL-2 delivered by continuous subcutaneous infusion produced measurable amounts of IL-2 in the serum. Furthermore, there was a response in the target cells which can be explained by: 1) a clonal expansion of cells that were initially responsive to IL-2 or alternatively, 2) recruitment of new PBL into the IL-2 responsive pool possibly by upregulation of IL-2R. This could be a direct effect of the in vivo IL-2 therapy in this dog. IL-2R data is currently under analysis.

CE 412 ERADICATION OF ADENOVIRUS E1-INDUCED TUMORS BY E1A SPECIFIC CTL.
W. Martin Kast*, Rienk Offringa†, Peter J. Peters¥, Arie C. Voordouw*, Rob H. Meloen§, Alex J. van der Eb† and Cornelis J.M. Melief*. *Division of Immunology, The Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX Amsterdam, †Sylvius Laboratory, University of Leiden, ¥Center for Electron Microscopy, University of Utrecht, §Central Veterinary Institute, Lelystad, The Netherlands.

Cytotoxic T lymphocyte (CTL) clones against Adenovirus type 5 (Ad 5) early region 1 (E1)-transformed cells were generated in C57BL/6 (B6) mice. By testing these H-2D^b restricted CTL clones on target cells that had been transformed by recombinant Ad 5 / Ad 12 E1 constructs it was found that the Ad 5 E1A region encoded the target structure for the CTL clones. By generating and testing a set of 316 overlapping peptides the one peptide that was recognized by all the clones was defined. Upon intravenous injection into B6 nude mice bearing Ad 5 E1 induced tumors, these CTL clones, if combined with recombinant IL-2, destroyed subcutaneous tumor masses up to 10 cm^3. Ultrastructural analysis of tumor tissue sections taken from mice during treatment showed CTL in the blood vessels of the tumor, CTL infiltrating the tumor tissue and local tumor tissue necrosis. The *in vivo* action of CTL is highly specific because in B6 nude mice bearing both a syngeneic and an allogeneic Ad 5 induced tumor only the syngeneic tumor disappeared. Long-term tumor specific memory persisted in treated mice because no new identical tumors could be induced. DNA analysis of spleen cells taken 3 months after tumor treatment revealed a pattern of T cell receptor gene rearrangement identical to the pattern of the CTL clone used to treat the animals. Our data show an important role for CTL directed against a viral nuclear oncogene product in tumor eradication.

CE 413 COMBINATION THERAPY OF C1300 MURINE NEUROBLASTOMA WITH IL-2 LIPOSOMES, ANTI-GD2 MONOCLONAL ANTIBODY AND CYCLOPHOSPHAMIDE.
Emmanuel Katsanis, *Augusto C. Ochoa, Maria A. Bausero, Cynthia M. Loeffler, and Peter M. Anderson. Departments of Pediatrics and Surgery, University of Minnesota, Minneapolis, MN 55455 and *National Cancer Institute, Frederick MD 21701.

To better simulate human disease we have modified the C1300 murine neuroblastoma model. Intraperitoneal inoculation leads to progressive abdominal invasion with extension of the tumor into the retroperitoneal area within 14 days and a median survival of 18 days compared to 22 days with the conventional s.c. inoculation. Treatment of A/J mice with advanced intraabdominal disease (day 10-14) with IL-2 liposomes 100,000 u i.p. results in significantly increased survival. Using a 4 hr ^{51}Cr release assay the C1300 tumor cell line behaves like a NK sensitive target with relatively high lysis by IL-2 stimulated splenocytes 670 LU/10^6 effectors, compared to only moderate lysis, 22 LU/10^6 effectors, with anti-CD3 + IL-2 stimulated cells. Most human neuroblastomas express ganglioside antigen GD2. With the use of a tumor enzyme immunoassay (EIA) we demonstrated binding of anti-GD2 (Mab 126) to C1300 murine neuroblastoma cells. Antibody dependent cell mediated lysis of this tumor using Mab 126 and the immunomodulatory effect of cyclophosphamide will be presented.

CE 414 IMMUNE CONTROL OF TUMOR APPEARANCE IN SV40 ANTIGEN TRANSGENIC MICE Barbara B. Knowles and James McCarrick, The Wistar Institute, 3601 Spruce St., Philadelphia, PA 19104.

Mice transgenic for the simian virus 40 early region gene (SV40 Tag) were investigated to determine the role of the immune response in controlling the development of SV40 T antigen-initiated tumors. The CTL response to SV40 Tag immunization was determined in mice from several lineages bearing different SV40 Tag hybrid genes which target SV40 Tag expression to distinct tissues. In some mice, tumor appearance and immune tolerance to SV40 Tag were correlated in contrast, SV40 Tag-responsive animals remain tumor free. However, mice of the RIP Tag 4 lineage (Hanahan D. Nature, 1985, 315, 115) mount a CTL response following SV40 Tag immunization yet succumb with SV40 Tag induced insulinomas. Immunization of these animals delays insulinoma onset by an average of 6-7 months. The role of MHC class I antigen expression in the β-islet cell on the induction and/or maintenance of SV40-specific CTL is under investigation.

CE 415 MURINE MELANOMA CELLS TRANSFECTED WITH A HUMAN MELANOMA-DERIVED GENE: AN EXPERIMENTAL MODEL FOR ACTIVE IMMUNOTHERAPY OF HUMAN MELANOMA. Shaohong Liang, Alban Linnenbach, Traci Zell, Hans Bender, Stanislaw Szala, Hilary Koprowski, and Dorothee Herlyn, The Wistar Institute of Anatomy and Biology, Philadelphia, PA 19104.

Active immunotherapy against a human melanoma-associated antigen can now be studied in an animal model. In this model, murine melanoma cells were transfected with a human gene encoding the 30/60 kDa human melanoma-associated antigen ME491. Several transfectant clones were obtained with the human ME491 gene integrated into the mouse genome. The transfectants were positive for human mRNA, and stably expressed the antigen on their surfaces. The density of the ME491 antigen on the surfaces of transfectants was approximately 10 times higher than the antigen density on human melanoma cells. The antigen was shed by the transfectants into the culture medium similar to shedding of this antigen by human melanoma cells. Each of the clones tested was tumorigenic in syngeneic mice. Mice with progressively growing transfectants did not develop humoral immune responses to the ME491 antigen. However, a high titer of serum antibodies against the antigen was induced in naive mice by immunization with immunoaffinity-purified ME491 antigen in adjuvant. This model will be useful to study protective effects of active immunotherapy against human melanoma experimentally before the clinical application of this approach. (Supported by NIH grant CA25874).

CE 416 ADOPTIVE IMMUNOTHERAPY OF A CLASS I NEGATIVE MURINE TUMOR WITH SPECIFIC CYTOLYTIC T CELL CLONES, Edith M. Lord, Timothy M. Blieden and John G. Frelinger, Cancer Center and Department of Microbiology & Immunology, University of Rochester Medical Center, Rochester, NY 14642.

A series of cytolytic T cell clones which recognize line 1, a low class I antigen expressing BALB/c lung carcinoma, were generated by immunization with inactivated tumor cells. These are $CD3^{+}, 4^{-}, 8^{+}$ T cells which specifically lyse line 1 tumor cells which have been induced to express class I antigens but not uninduced line 1 cells or a variety of other murine tumor cell lines. Line 1 tumor cells grown in spinner flasks form clusters called multicellular spheroids which contain about 5×10^4 tumor cells and have many of the properties of solid tumors. When these spheroids are implanted into the peritoneal cavity of syngeneic mice, they grow progressively but injection of the T cell clones at a ratio of 3 T cells: 1 tumor cell resulted in complete destruction of the tumor cells. The effectiveness of the T cell clones was also tested in a metastases model. Uninduced class I negative line 1 tumor cells (10^5) were injected i.v. and the number of lung metastases evaluated 14 days later. Injection of the specific T cell clones (10^6) at day 0 reduced the number of lung metastases by 95% and a significant reduction was observed if the T cells were given 4 days after the tumor cells. Injection of an irrelevant T cell clone (BALB anti-$H2^b$) had no effect on the number of metastases. Interestingly, the clones are effective against uninduced tumor cells as well as class 1 expressing tumor cells, which implies that induction of class 1 antigens may be occurring in vivo.

CE 417 ADOPTIVE IMMUNOTHERAPY OF UV-INDUCED TUMORS, David H. Lynch and Robert E. Miller, Immunex Corp., 51 University St., Seattle, WA 98101.

The fact that tumor cells express a number of neoantigens to which immune reponses can be elicited has provided the basis for the development of a variety of immunotherapeutic strategies, including the use of tumor-reactive T cells. We have demonstrated that the draining lymph nodes (DLN) of tumor-immunized mice contain populations of lymphoid cells which are capable of differentiating into functional cytotoxic T lymphocytes (CTL) during an in vitro culture period. The data demonstrate that intravenous injection of the tumor reactive CTL generated using this protocol effectively eliminates both intradermal and subcutaneous challenges of tumor cells from the tumor used for immunization. The tumor reactive effector cells were found to traffic appropriately in the recipient mice since the tumor challenge is implanted at a distal site. Negative selection studies demonstrated that the cells that mediate elimination of the tumor challenge are radiosensitive, Thy 1^+, $CD4^-$, $CD8^+$. Further, positive selection studies have demonstrated that adoptive transfer of as few as 1×10^6 $CD8^+$ anti-tumor CTL were capable of eliminating a tumor challenge at a distal site. These data indicate that generation of tumor-reactive CTL from lymphoid cells contained in the DLN may provide a valuble source of effector cells which can be used in the effective immunotherapeutic treatment of cancer.

CE 418 ERADICATION OF A LARGE MOPC-315 TUMOR IN ATHYMIC NUDE MICE BY CHEMOIMMUNOTHERAPY WITH LYT 2^+ SPLENIC T-CELLS FROM MELPHALAN TREATED BALB/C MICE BEARING A LARGE MOPC-315 TUMOR[1], Margalit B. Mokyr, Larry M. Weiskirch and Edward Barker, Department of Micro/Immuno., University of Illinois College of Medicine at Chicago, Chicago, IL 60612.

Spleen cells from BALB/c mice which are in the process of eradicating a large MOPC-315 tumor following low-dose (2.5 mg/kg) melphalan therapy can, in conjunction with a subcurative dose of drug (adoptive chemoimmunotherapy; ACIT), cause the complete regression of a large (15-20 mm) s.c. MOPC-315 tumor in a large percentage of T-cell-deficient (athymic nude) tumor bearing mice. Spleen cells which were effective in ACIT of athymic nude mice displayed in vitro a substantial direct lytic activity against MOPC-315 tumor cells. The cells responsible for the therapeutic effectiveness of the spleen cells in ACIT of athymic nude mice, as well as the cells responsible for the direct in vitro anti-MOPC-315 lytic activity of the spleen cells, were of the Lyt 2 and not the L3T4 phenotype. Most of the athymic nude mice that completely eradicated a large MOPC-315 tumor as a consequence of ACIT were capable of rejecting a challenge with 30 to 100 fold the minimal lethal tumor dose administered more than one month after the ACIT. The ability of these athymic nude mice to resist the tumor challenge was associated with the presence of a greatly elevated percentage of cells expressing T-cell surface markers in their spleens. Thus, it is conceivable that splenic Lyt 2^+ T-cells from L-PAM treated BALB/c mice bearing a large MOPC-315 tumor mediate their therapeutic effectiveness in ACIT of athymic nude mice bearing a large MOPC-315 tumor, at least in part, through direct cytotoxicity for MOPC-315 tumor cells.

CE 419 ROLE OF IL-2 ACTIVATED KILLER CELLS IN REJECTION OF ALLOGRAFTS, Allen J. Norin, Mary O. Mondragon and Stephan L. Kamholz, Department of Medicine and Anatomy & Cell Biology, SUNY Health Science Center at Brooklyn, NY 11203

Invivo cell mediated effector mechanisms of rejection were investigated in a canine single lung transplantation model. This large animal model permits direct longitudinal studies of immune effector cells from the allografts of individual recipients by bronchoalveolar lavage (BAL). In addition to the detection of typical allospecific cytolytic T lymphocytes (CTL) the activity of putative IL-2 activated killer (IAK) cells, ie LAK cells, was observed during the course of rejection (due to drug dose tapering) in cyclosporine (CyA) treated recipients. Putative IAK cell activity was detected in BAL fluid of lung allografts by lectin dependent cell mediated cytotoxicity in the absence of cytolytic activity against target cells from the organ donor. Such cytolytic activity was also detected in the initial stage of lung rejection in non immunosuppressed recipients and in the early period (3 days) of mixed lymphocyte culture. Current interpretation of these data is that these latter effector cells have the characteristics of IAK cells. The appearance of increased IAK activity in BAL fluid is associated with evidence of graft rejection as assesed by physiologic (reduced blood flow to the graft) and morphologic (histopathologic changes) criteria. Increased dosage of CyA diminished the physiologic and morphologic signs of rejection with concommittant reduction in cytolytic lymphocyte activity (IAK and CTL). Substantial delays in the detection of intragraft donor specific CTL relative to IAK activity were observed in recipients undergoing CyA dose tapering compared to nonimmunosuppressed recipients suggesting that appropriate CyA treatment may lead to prolonged inhibition of the generation of donor specific CTL compared to reactions that result in production of intragraft IAK activity.

CE 420 THERAPY OF MURINE HEPATIC METASTASES USING CD3-LAK CELLS AND IL2-LIPOSOMES, *Augusto C. Ochoa, +Cynthia Loeffler, +Arnold Leonard and +Peter M. Anderson, *Program Resources, Inc., NCI-Frederick Cancer Research Facility, Frederick, MD 21701 and +University of Minnesota, Minneapolis, MN.

The difficulty in obtaining sufficient cells for adoptive immunotherapy is a well-documented problem in adoptive immunotherapy. Stimulation of human or murine lymphocytes with anti-CD3 + IL2 generates large numbers of cells with anti-tumor activity (CD3-LAK cells). Data obtained using isolated subpopulations have demonstrated that the cells which develop lytic activity in the CD3-LAK cultures are Lyt2+ lymphocytes. The in vivo therapeutic efficacy of these non-specific cytotoxic T cells was tested in mice bearing hepatic metastases of colon carcinoma C-38. Mice were treated with three doses of intraperitoneal CD3-LAK cells and 50,000 units qd of IL2 (Hoffmann-LaRoche) encapsulated in liposomes. The data obtained demonstrated that the only combination which induced a significant reduction in the number of hepatic metastases was CD3-LAK cells and IL2-liposomes. CD3-LAK cells with free IL2 or IL2-liposomes alone did not result in any tumor reduction even when given at much higher doses. Immunohistological stains of liver sections demonstrate that Lyt2+ cells infiltrate the tumor sites of mice receiving CD3-LAK cells and IL2-liposomes, while mice receiving other therapies do not show lymphocyte infiltrates. The origin and possible role of these cells will be discussed.

CE 421 GENERATION OF AN ANTI-TUMOR RESPONSE BY TUMOR CELLS ENGINEERED TO SECRETE INTERLEUKIN-2, Drew Pardoll, Paul Golumbek, Hy Levitsky, Eric Fearon, Jonathan Simons, Phil Frost, Bert Vogelstein and Hajime Karasuyama, Departments of Medicine, Oncology, and Molecular Biology, Johns Hopkins University School of Medicine, Baltimore, MD 21205.
A poorly immunogenic murine colon cancer was used to investigate mechanisms of anti-tumor immunity. The injection of tumor cells engineered by gene transfection to secrete the helper lymphokine, interleukin-2, stimulated an MHC class I-restricted cytolytic T lymphocyte (CTL) response against the parental tumor. The tumor cells secreting IL-2 produced an anti-tumor response in vivo, even in the absence of $CD4^+$ T cells. In contrast, in vivo depletion of $CD8^+$ T cells abrogated the anti-tumor response. Animals immunized with the engineered cells were protected against subsequent challenge with the parental tumor cell line. Thus, the provision of a helper lymphokine in a paracrine fashion induced a tumor-specific immune response involving activation of endogenous CTL and other immune effector cells. These findings demonstrate that in certain cases, the failure of an effective anti-tumor immune response may be primarily due to a deficiency in the helper arm of the immune system rather than a paucity of tumor-specific cytotoxic effector cells. Furthermore, they outline a novel strategy for augmenting tumor immunity.

CE 422 THE CELLULAR IMMUNE RESPONSE TO FRIEND VIRUS: GENETIC FACTORS AND IMMUNOLOGICAL MECHANISMS. David Polsky, Sally Ishizaka and Frank Lilly. Department of Molecular Genetics, Albert Einstein College of Medicine, Bronx, NY 10461. Friend virus (FV), a murine retrovirus complex, induces erythroleukemia characterized by massive splenomegaly as early as 12 days post-infection. Mice of the congenic BALB.B strain ($H-2^b$) are more resistant to the disease than BALB/c mice ($H-2^d$). Studies in congenic BALB mice bearing recombinant H-2 haplotypes have shown that the resistance of BALB.B mice is tightly linked to the class I molecule, D^b, and this molecule is also the exclusive restriction element recognized by BALB.B anti-FV cytotoxic T lymphocytes (CTL). However, in F_1 crosses between BALB.B and D^b-negative strains, resistance is a recessive trait. Resistance is not due to fewer target cells for viral infection as all strains tested by spleen focus assay show equal foci at 9 days post-infection. In addition, these strains show no difference in NK activity. The incidence of splenomegaly in these strains is directly associated with CTL activity. In crosses between BALB.B and other strains, the resistant strains show greater CTL activity than the susceptible strains. The generation of IL-2 in vitro and the level of IL-2R expression by these strains will also be discussed. In an effort to determine which elements in the mouse response are important in resistance to the virus we have utilized an adoptive immunotherapy model. Mice receive 500R, a transfer of immune, in vitro boosted spleen cells, and viral challenge. Our data demonstrate that $Thy1^+$ cells are necessary for long-lived protection against viral challenge. $I\text{-}A^+$ cells are not required for protection, and immune serum will only generate temporary protection. The importance of T cell subsets in this model will also be discussed.

CE 423 EFFECTS OF INTERFERON-α (IFN-α) AND INTERLEUKIN-2 (IL-2) ON *IN-VIVO* GENERATION OF LYMPHOKINE ACTIVATED KILLER (LAK) CELLS AND NATURAL KILLER (NK) CELLS IN THE ORGANS OF MICE, Raj K. Puri and Pamela Leland, Laboratory of Cellular Immunology, Division of Cytokine Biology, Center for Biologics Evaluation of Research, FDA, Bethesda, MD 20892
IFN-α has been shown to synergize with IL-2 in the regression of a variety of established murine tumors and some human tumors. To understand the mechanism of synergy we have studied NK and LAK cell activity in the organs of mice in response to IFN-α and IL-2 administration. C57BL/6 mice were injected intraperitoneally with HBSS, 250,000 IU IL-2 (Cetus) alone, 50,000 U IFN-α (Hoffman La Roche) alone or both two times a day for 7 days. On day 4 and 8, LAK and NK activity were tested in the cells obtained from lungs, spleen and liver by 4 hour chromium release assay *in-vitro* utilizing fresh MCA-102 tumor cells and Yac-1 line as targets. The cells from the HBSS treated mice failed to lyse the MCA-102 target. IL-2 caused the generation of LAK activity in all the organs at 4 days of injection. IFN-α failed to generate LAK activity but when administered along with IL-2, caused synergistic enhancement of LAK lysis of MCA-102 target when compared to cells from animals treated with IL-2 alone. After 7 days of IL-2 therapy LAK activity significantly decreased compared to that observed after 3 days of therapy while after IFN-α and IL-2 administration activity remained at high level as observed after 3 days of therapy. Pre-treatment irradiation (500R) of mice completely abrogated the capability of IL-2 or IL-2 plus IFN-α to generate LAK activity. IL-2 and IFN-α when injected alone caused the enhancement of NK mediated lysis. The animals treated with both IL-2 and IFN-α had greater NK activity in hepatocytes than did animals treated with IL-2 alone: however there was no significant difference in splenic NK activity. These studies indicate that induction of 'super LAK' and NK actvity may be partly responsbile in the mediation of synergistic anti-tumor effects of IFN-α and IL-2.

CE 424 ANTITUMOR T LYMPHOCYTES BUT NOT LAK CELLS SYNERGIZE WITH rIL-2 IN ADJUVANT ADOPTIVE IMMUNOTHERAPY OF SPONTANEOUS LUNG METASTASIS OF A MOUSE CARCINOMA. Monica Rodolfo, Carolina Salvi, Cinzia Bassi, Giovanni Rovetta and Giorgio Parmiani, Division of Experimental Oncology D, Istituto Nazionale Tumori, 20133 Milan, Italy.
Animal studies have established the antitumor activity of rIL-2 administration with or without LAK cells but few data are available on the effectiveness of such approach in an adjuvant setting. To see whether rIL-2 could be effective as adjuvant treatment in combination with lymphocytes and to assess whether LAKs or tumor specific T cells are more effective in such system, BALB/c mice were operated of a s.c. colon carcinoma 26 (C-26) and treated, 7 days later, with a $3x10^4$ U/day of Glaxo rIL-2 i.p. for 5 days, while a total of 10^8 lymphocytes (LAKs or T cells) were given i.v. on alternate days during rIL-2 administration. Antitumor immune effectors resulted in the highest percentage of cures (63%) compared with LAKs (33%), rIL-2 alone (31%) or surgery alone (21%). In vitro conditions were then studied in order to obtain "immune" T lymphocytes from tumor-bearing or tumor-excised mice. Cultivation of splenic lymphocytes with irradiated C-26 cells and rIL-2 (5-10 U/ml) for 7 + 5 days (MLTC lymphocytes) resulted in the activation of cytotoxic lymphocytes lytic on the C-26 cells and not on B16 target, while the same lymphocytes cultured with rIL-2 only (100-250 U/ml) displayed LAK activity. When used in the adjuvant therapy setting in combination with rIL-2 treatment, MLTC lymphocytes again showed a higher therapeutic activity than LAKs obtained from the same donors. (Supported by the Italy-USA Project on "New Therapies in cancer").

CE 425 TRANSFER AND EXPRESSION OF THE HUMAN IL2 GENE IN A TRANSPLANTABLE RAT SARCOMA, Russell SJ, *Eccles SA, *Johnson C, Collins MKL, Institute of Cancer Research, Royal Cancer Hospital, Chester Beatty Laboratories, Fulham Road, London, SW3 6JB and *Section of Medicine, Clifton Avenue, Sutton, Surrey.
We have generated recombinant retroviral vectors for transfer and expression of the human IL2 gene. High-titre, helper-free recombninant retroviral stocks were used to infect a population of highly metastatic transplantable HSNLV rat sarcoma cells which were subsequently shown to secrete biologically active IL2. The efficiency of gene transfer was estimated at close to 100 percent by Southern blot analysis and this was confirmed by analysis of subclones derived from the bulk-innfected cell population. High doses (10^6-10^7) of IL2-secreting HSNLV were completely rejected by syngeneic immunocompetent rats following subcutaneous inoculation. In contrast, low doses (10^2-10^3) of IL2-secreting HSNLV were tumorigenic. Explanted tumours continued to secrete IL2 and were serially transplantable even at high cell doses. IL2-secreting HSNLV were tumorigenic at all cell doses in nude rats and the rate of tumour growth did not differ from that of parental HSNLV. Inoculation of admixed parental and IL2-secreting HSNLV significantly delayed and sometimes abrogated the tumorigenicity of parental cells in a dose dependent manner.

Further studies will focus on the mechanism of rejection at high cell doses and of tumorigenicity at low cell doses. HSNLV clones secreting different quantities of IL2 will be analysed for ability to stimulate a rejection response and other lymphokines will be tested in the same system.

CE 426 IMMUNOTHERAPY OF BOVINE OCULAR SQUAMOUS CELL CARCINOMA (BOSCC): THE POTENTIAL ROLE OF TUMOR INFILTRATING LYMPHOCYTES (TIL).

Victor P.M.G. Rutten[1], Wim R. Klein[2], Mieke A. de Jong[1], P.A Steerenberg[4], Wim den Otter[3], E. Joost Ruitenberg[1,4]. Departments of 1) Immunology and 2) Surgery, Fac. of Vet. Med., Univ. of Utrecht 3) Pathol. Inst., Fac. of Med., Univ. of Utrecht, 4) RIVM, Bilthoven, The Netherlands.

BOSCC is sensitive to intralesional immunotherapy with BCG and recombinant human IL2 (rhIL2)(tumor regression in 50% of the cases, respectively 3/5 animals treated). Alterations in concentration of IL2 (and possibly other factors), by way of regional injection or production, may induce killer cell activity and tumor regression. TIL were isolated by mechanical fractionation of tumor biopsies (8 different cows), and were incubated in vitro with various doses of rhIL2 (500, 100, 10 U/ml). Cell numbers increased 50-150 fold during 2-6 weeks of culture. Growth characteristics of all TIL cultures were comparable. In ^{51}Cr release assays TIL from 4/8 animals tested so far showed cytotoxicity for BOSCC derived tumor cell lines in general. A transient increase of cytotoxicity during culture was noticed. Killing of YAC1, P815 and K562 was less efficient. TIL from one animal showed preferential killing of an autologous cell line. Phenotypes of cells in culture were analysed. So far no conclusions can be drawn with respect to the cells responsible for cytotoxicity.

CE 427 EFFECT OF TREATMENT WITH INTERLEUKIN-1 ALPHA (IL-1a) AND INTERFERON BETA (IFN-b) ON ESTABLISHED P 815 MASTOCYTOMAS. Deepak M. Sahasrabudhe, M. D. and Craig S. McCune, M. D. The Cancer Center and the Department of Medicine, University of Rochester School of Medicine and Dentistry, Rochester, N.Y. 14642.

The immune response to progressive P 815 mastocytoma is characterized by concomitant antitumor immunity which is down regulated by tumor induced suppressor T lymphocytes (Ts). Inhibition of Ts-function is a prerequisite to successful adoptive immunotherapy of established tumors. Since the concomitant immunity is sufficient to reject a **secondary** tumor initiated 6 to 9 days after the primary tumor, we reasoned that augmenting concomitant immunity while simultaneously inhibiting Ts-function may result in regression of established **primary** P 815 tumors. IL-1a was used to augment the concomitant immunity and IFN-b was given to inhibit Ts-function. We report that such treatments inhibit Ts-activity, prolong the duration of concomitant antitumor immunity, and (in preliminary experiments) can result in regression of the primary tumors.

CE 428 SUPPRESSION OF ORGAN-COLONIZATION, CELLULAR ADHESION AND INVASION OF METASTATIC TUMOR CELLS BY VIRUS INFECTION,

Volker Schirrmacher, Georg Brunner and Paul von Hoegen, Institut für Immunologie und Genetik, Deutsches Krebsforschungszentrum, D-6900 Heidelberg, FRG

Infection of high metastatic B16-F10 melanoma cells or of murine ESb lymphoma cells by a non-lytic strain of Newcastle Disease Virus (NDV) has a strong suppressive effect on organ colonization following intravenous cell inoculation. This anti-metastatic effect was seen in normal unimmunized mice and also in animals which had been sublethally irradiated by 5 Gy. The effect was independent of specific immune reactions. NDV modification of tumor cells also significantly reduced the adhesion to various extracellular matrices and tumor cell invasion into fibrin gels. In addition to these anti-tumor effects NDV infection also leads to an augmentation of tumor cell immunogenicity and to augmentation of protective tumor-specific immune responses.

CE 429 ACTIVE IMMUNIZATION WITH TUMOR-ASSOCIATED TN (GALNAC-O-SER/THR) ANTIGEN IN MOUSE MAMMARY ADENOCARCINOMA, Anil Singhal, Melinda Fohn and Sen-itiroh Hakomori, The Biomembrane Institute and University of Washington, Seattle, WA 98119.
A block in carbohydrate chain elongation of O-glycosylated mucins results in expression of Tn antigen in more than 60% of human adenocarcinoma. Mice (CAF-1) immunized with purified desialylated ovine or bovine submaxillary mucins (A-OSM or A-BSM), containing large quantities of Tn antigen, coated on Ribi adjuvant showed significantly increased survival compared to the adjuvant controls upon challenge with a highly invasive Tn expressing syngeneic mammary carcinoma cell line, TA3-Ha. High titers of anti-Tn antibodies (both IgG and IgM), which bound strongly to tumor cells, were detected in sera of mucin-immunized animals. Lymphocytes prepared from lymph nodes of mucin immunized mice exhibited strong Tn-specific proliferation *in vitro*, and this proliferation was abolished by pre-treating lymphocytes with anti-thy1.2 antibody and complement. Mice immunized with A-OSM displayed delayed type hypersensitivity when they were injected in the footpad with A-OSM or irradiated TA3-Ha cells. These studies demonstrate the protective effect of Tn antigen and will be useful in designing a vaccine against human adenocarcinoma. Investigations are currently underway to determine the effect of immunization with synthetic Tn antigen.

CE 430 ANTITUMOR EFFECT OF EHRLICH'S ASCITES FLUID ADSORBED OVER NON-VIABLE AND FORMALINE FIXED PROTEIN A CONTAINING STAPHYLOCOCCUS AUREUS COWAN I., Ashish S. Verma, Arun K. Prasad, Premendra D. Dwivedi, and Prasant K. Ray, Director's Lab., Industrial Toxicology Research Centre, Lucknow, India -226001.
Ray et. al. (Cancer Res. 42,4970,1982;J.Biol.Resp.Modif.8,39,1984;Ray,P.K. In:Contemporary Topics In Immunobiology,Vol.15, 147,1985) reported the antitumor effect of *ex vivo* adsorbed serum and plasma over heat attenuated and formaline fixed Protein A containing *Staphylococcus aureus* Cowan I (SAC), when reinfused into syngeneic animals. This was thought to be due to the removal of blocking factors responsible for immunosuppressor in tumor bearing animals as well as human patients. Here we report for the first time the antitumor effect of ascites fluid drawn from Ehrlich's ascites tumor bearing host adsorbed *ex vivo* over heat attenuated SAC and then inoculated to the same host. The administration of SAC adsorbed ascites fluid (alternate day right from the day of tumor transplantation) resulted in significant ($p<0.001$) decrease in the body weight (a parameter used to asses tumor regression in ascites tumor) reduction in the viable tumor cell count alongwith subsequent immunopotentiation as judged by increased DTH, PFC, haemagglutination and an increase in the weight of lymphoid organs compared to untreated control. Our results provide a new lead to cause ascites tumor destruction by the administration of *ex vivo* SAC adsorbed ascites fluid to tumor bearers. The significance of this procedure appears to be in the fact that the withdrawl of ascites fluid on the one hand physically reduces the tumor burden and also the inoculation of adsorbed fluid immunopotentiare the host to cause tumor regression. Thus it might produce a novel approach in the experimental therapy of cancer.

CE 431 TREATMENT OF MURINE RENAL CANCER BY COMBINED MODALITIES: ROLE OF CYTOKINES. R.H. Wiltrout, H. Futami, T. Back, H.A. Young, T.J. Sayers, and R.L. Hornung, NCI-FCRF and BCDP, PRI, Frederick, MD 21701. Murine renal cancer (Renca) can be successfully treated (80% disease-free survival) using lymphokine-activated killer (LAK) cells and rIL2 in combination with doxorubicin hydrochloride (DOX). Similar therapeutic effects (40-80% disease free survival) can be achieved against Renca by using the investigational drug flavone acetic acid (FAA) in combination with rIL2. Mice cured of Renca by high-dose Dox or by Dox + LAK + rIL2 are not immune to rechallenge while those cured by FAA + rIL2 are specifically immune to rechallenge. Northern blot analysis of LAK cells has shown that the LAK effector cells exhibit upregulated levels of mRNA for TNFα and IFNγ, while FAA administration induces gene expression in splenic or hepatic leukocytes for TNFα, IFNγ, as well as IFNα and IL1. Subsequent studies have demonstrated that alkalinization of Renca-bearing mice prior to FAA administration, in a manner analogous to that used in FAA clinical trials, virtually abrogates therapeutic activity of FAA + rIL2 (3% vs. 42% long-term survivors). Northern blot analysis of spleen cells from FAA-induced mice performed in parallel with these studies demonstrated a dramatic reduction in mRNA for TNFα, IFNγ, and IFNα in alkalinized mice that coincided with reduced production of the relevant biologically active proteins. These results demonstrate that LAK cells and leukocytes from FAA-treated mice produce a variety of immunoregulatory cytokines which may contribute to the therapeutic efficacy of both approaches, and may be part of the mechanism of action of adoptive cellular therapy for some tumors.

Clinical and Preclinical Studies of T Cell-Mediated Rejection (Tumor and Related Models)

CE 500 TUMOR INFILTRATING LYMPHOCYTES (TIL) FROM RENAL CELL CARCINOMA (RCC) AND THEIR STATE OF ACTIVATION: PHENOTYPIC AND FUNCTIONAL ANALYSIS, Jeannine Alexander, Robert Connelly, Mark Edinger, Raymond R. Tubbs, James Boyett, Edson Pontes, Ronald Bukowski, and James H. Finke, The Cleveland Clinic Foundation, Cleveland, OH 44195.

Here we defined the state of activation of TIL in human renal cell carcinoma. Freshly isolated TIL and PBL were examined for the expression of transient activation markers such as IL2R (CD25) and HLADr using 3 color flow cytometry. In addition we compared PBL and TIL for their expression of stable markers known to distinquish antigen activated T cells from naive T cells. Antigen activated cells express high densities of CDw29 and CD45RO (UCHL1) and express no or low levels of CD45RA (CD45R), a marker found on naive cells. Our results showed that the majority of TIL and PBL did not express CD25 whereas a significantly higher percentage of TIL expressed HLADr than PBL (28 vs 15%). When compared to PBL TIL *contained fewer cells expressing CD45RA (12.7% in TIL vs 50.6% in PBL). In contrast TIL contained more cells that expressed* CDw29 (78.7%) than PBL (47.4%). Whereas PBLs contained similar percentages of activated ($CD45RO^+$) and nonactivated ($CD45RA^+$) T cells, in TILs there was an 8 fold increase in the percentage of activated (42.2%) cells over the nonactivated (5.3%) subset. An evaluation of T cell subsets revealed that approximately equal numbers of $CD4^+$ and $CD8^+$ TIL were expressing CD45RO. When compared to PBLs, $CD4^+CD45RO^+$ cells were not increased in the TILs, however, there was a 4 fold increase in number of $CD8^+$ TILs expressing CD45RO (5.0% PBL vs 20.3% TIL). Functional studies on TIL populations enriched for cells expressing CD45RO indicated that the activated cells contained most of the proliferative response to rIL-2 or autologous tumor plus rIL-2. Although the nonactivated $CD45RA^+$ subset of TIL proliferated in response to PHA, these cells did not proliferate well in response to rIL-2 with or without autologous tumor when compared to the activated $CD45RO^+$ enriched subset. The results of this study suggest that the T cells infiltrating RCC are indeed activated as judged by the expression of CDw29 and CD45RO and that the presence of these cells in the tumor support the hypothesis that there is a T cell immune response to RCC.

CE 501 IFNγ ADMINISTRATION IN OVARIAN CARCINOMA PATIENTS WITH MINIMAL RESIDUAL DISEASE AFTER CHEMOTHERAPY: MODULATION OF IMMUNE PARAMETERS, Paola Allavena, Fedro Peccatori, Cristina Bonazzi°, Nicoletta Colombo°, Maude Brandely‡, Costantino Mangioni° and Alberto Mantovani, Laboratory of Immunology, Mario Negri Institute, Milan, Italy, °San Gerardo Hospital, Monza, Milan, Italy, ‡Roussel Uclaf, Paris, France.

In direct prosecution with previous studies on local administration of BRM in advanced ascitic ovarian carcinoma patients, we have now investigated the efficacy of Interferon γ (IFNγ) in patients with minimal residual disease. Eligible patients had previously been treated with conventional chemotherapy and had a residual tumor <2 cm. Patients received 20 MU/m^2 of IFNγ (Roussel Uclaf, France) twice a week for 16 weeks. After the first week of intraperitoneal (i.p.) treatment, patients were randomized for i.p. versus subcutaneous (s.c.) administration. Up to Sept. '89, 13 patients entered this protocol. Side effects included: fever up to 38.5°C in all the patients, transient increase of hepatic enzymes in 4 patients, abdominal pain in 1 and septic peritonitis in 1 patient. Modulation of the cytotoxic activity of natural killer cells and monocyte-macrophages have been performed throughout the study both in the periphery and in the peritoneal cavity. An increase of cytotoxic activity was observed in the peritoneal compartment in all patients treated i.p. and in 2/4 patients treated s.c.; on the other hand increase in the peripheral blood was only occasionally found. These results indicate that i.p. and s.c. IFNγ administration is well tolerated and induces an increase in lymphocyte and macrophage cytotoxicity, more frequently in the peritoneal compartment.

CE 502 SUMMARY OF PHASE II TRIALS OF LOW AND HIGH DOSE INTERLEUKIN-2 (IL-2) PLUS LYMPHOKINE ACTIVATED KILLER (LAK) CELLS IN TREATMENT OF ADVANCED CANCER. Jerry A. Bash, Marc K. Wallack and Enrique Davila, Department of Surgery and Medicine, Mount Sinai Medical Center, Miami Beach, Florida 33140.

Two Phase II trials of IL-2 plus LAK cells in treatment of advanced cancer were sponsored by Hoffman La Roche, Inc. Both trials were performed on a schedule of 5 days of priming with IL-2, a 48 hour rest period, followed by 3 sequential leukaphereses (with CS3000 cell separator) to obtain cells for LAK cell generation. Five days after culture with IL-2 in Fenwall bags, LAK cells were harvested and administered on days 8, 9 and 10 along with IL-2 which continued through day 17. The first trial was performed with low dose IL-2 (500,000 U/M^2day) and the second was performed with high dose IL-2 ($6x10^6$ U/M^2day). Variables studied also included comparison of bolus and continuous i.v. infusion, Ficoll separation vs. whole cell LAK culture, culture bag size and orientation and use of RPMI 1640 culture medium containing 2% human serum or serum-free AIM-V medium. Six patients (5 melanomas, 1 colon cancer) were treated by the low dose bolus regimen and one patient (melanoma) was retreated by continuous infusion. No clinical responses were noted in this group and absence of rebound lymphocytosis resulted in low LAK cell yields. A total of 8 patients (6 melanomas, 2 colon cancer) were treated with high dose IL-2 by continuous infusion. LAK cell yields were markedly higher as a result of increased rebounds and unseparated cell culture. Two partial responses, (melanomas) 1 stable disease (colon) and 1 complete response (melanoma) was obtained in this study. The patient with a complete response who remains recurrence-free 18 months later was remarkable in view of having extensive liver metastases, being HIV+ (low CD4+) and having an exceptionally high rebound lymphocytosis which generated CD8+CD16 - T/LAK cells in culture.

CE 503 SYSTEMIC ADMINISTRATION OF RECOMBINANT INTERLEUKINE 2 (IL2) AFTER AUTOLOGOUS BONE MARROW TRANSPLANTATION (BMT) : A pilot study in 19 patients, D. Blaise 1, A.M. Stoppa 1, M. Attal 2, C. Pourreau 3, D. Boule 4, S. Negrier 5, P. Viens 1, D. Olive 1, M.H. Gaspard 2, T. Philip 5, C. Jasmin 4, P. Palmer 3, C. Francks 3 and D. Maraninchi 1. 1 : IPC Marseille France ; 2 : CHU Toulouse ; 3 : Eurocetus Amsterdam; 4 : Hopital Paul Brousse Paris ; 5 : CLB Lyon.

PTS with Leukemias treated by auto BMT, have more frequent relapses than after allo BMT, mainly by a lack of "graft vs tumor" reaction. IL2 can stimulate cytotoxic cells and induce regression of metastases. Systemic IL2 after auto BMT may enhance immune stimulation leading to this "graft vs disease" effect. We submitted 19 pts (median age 30 (range 7-56) M/F = 9/10), treated for poor risk malignancies (leukemias : 6; lymphomas : 7; ST : 7) (status pre IL2 : RC : 11; evol. : 8) to a constant infusion of IL2, 77 d +/- 13 after auto BMT. In this phase 1 study, the first 8 pts were scheduled to receive 6 days (completion : 87% of scheduled doses) and the following 11 pts a second 6 days IL2 course after 1 day rest (completion of cycle 1 : 100% cycle 2 : 58% of scheduled doses). All pts had usual toxicities but no toxic death occured. Hematological toxicity was moderate with no graft failure. Increase in eosinophilia and lymphocytes counts were significant. Immune stimulation was intense and prolonged specially for CD3+ lymphocytes ($p<0.05$), NK cells ($p<0.01$) and NK and LAK activity ($p<0.01$ and $p<0.05$). With a follow up of 9 mths (6-12), 16 pts are alive, 8 of them in CCR. This trial demonstrate the feasability of IL2 administration post BMT leading to a high immune stimulation. In this setting, 6 days fo treatment represent the regular reproducible schedule.

CE 504 THE DTH RESPONSE TO MELANOMA VACCINE IMMUNIZATION. Jean-Claude Bystryn, Ruth Oratz, Matthew N Harris, Daniel F Roses, Milagros Henn. New York University School of Medicine, New York, NY 10016

We examined the relation between delayed type hypersensitivity (DTH) responses to immunization to a melanoma vaccine and tumor progression. Ninety five patients with surgically resected stage II (regional metastases) malignant melanoma were repeatedly immunized to a soluble, polyvalent, melanoma antigen vaccine. DTH responses to melanoma was measured, by skin test to 10 ug of vaccine, at baseline prior to immunization and following the 4th immunization. The vaccine induced or augmented DTH responses in 53 (56%) of the patients. There was a relation between the magnitude of the DTH response (as measured by the size of induration at 24 hrs) and delay in tumor progression. Median disease free survival in the 39 patients with the strongest response (>10 mm increase in induration over baseline) was >57 months vs 14 months in pts who did not respond. The difference in median disease free survival between responding and non-responding patients was statistically significant ($p=0.03$). Overall immune status as evaluated by frequency and size of response to recall antigens and sensitization to DNCB was similar in both groups. These results suggest that the DTH response to melanoma vaccine immunization provides an immunological correlate of vaccine efficacy.

CE 505 ADOPTIVE IMMUNOTHERAPY OF ADVANCED MELANOMA WITH SENSITIZED T LYMPHOCYTES, A. E. Chang and S. Shu, Dept. of Surgery, University of Michigan, Ann Arbor, MI 48109.

Based on principles and techniques we have established in animal studies, a phase I clinical trial was initiated where patients with advanced melanoma are treated with autologous sensitized lymphocytes. Patients were first vaccinated with a mixture of tumor cells and BCG. Ten-day draining lymph nodes (LN) were harvested to provide tumor-primed lymphocytes. Secondary in vitro sensitization (IVS) of the LN cells was carried out by culture with irradiated tumor cells in the presence of IL-2 (100 u/ml) for 10-12 days. Resulting cells were subsequently infused to patients with the concomitant administration of IL-2 (30,000 u/kg, tid) for 5 days. Our experience with 3 patients indicates that up to 14-fold expansion of IVS cells could be obtained and up to 6×10^9 of such activated cells were infused i.v. without apparent toxic side effects. Phenotype analysis of freshly harvested tumor-primed LN cells revealed 58% T lymphocytes ($CD3^+$) comprised of 49% $CD4^+$ and 9% $CD8^+$ cells. These cells did not express detectable IL-2R (CD25). Following IVS, recovered cells consisted of >90% T cells. The majority (>80%) of these IVS cells were of the CD4 phenotype. In addition they expressed HLA-DR antigens and IL-2R, indicative of activation. Specific cytotoxicity toward autologous tumor cells was evident in 1 patient by the 4-hr ^{51}Cr release assay. Considerable amount of cross-reactive cytotoxicity was found in the IVS cells of the other 2 patients. Furthermore, similarly activated PBL were characteristically different from that of primed LN cells. Thus, our results indicate that IVS cells can be safely administered. The therapeutic efficacy of this procedure has yet to be established.

CE 506 IMMUNOHISTOCHEMICAL ANALYSIS OF THICK-NEEDLE BIOPSIES FROM METASTATIC LESIONS FROM PATIENTS TREATED WITH Mab AGAINST THE HUMAN TUMOR ASSOCIATED ANTIGEN (TAA) Co17-1A FOR COLORECTAL CANCER, Christensson B., Shetye J., Venkateswaran S., Frödin J-E., Rubio C., Skoog L., Mellstedt H. Departments of Pathology and Oncology, Karolinska Hospital, S-10401, Stockholm, SWEDEN.
Patients with primarily surgically resected, metastatic colorectal carcinoma, were given repeated infusions of a Mab directed against the TAA Co17-1A (a glycoprotein with a m.w. of 37kD). To monitor the effect of the treatment, a method was deviced for immunohistochemical detection of the infused Mab as well as identification of leucocyte populations potentially involved in immune mediated tumor destruction. Sequential thick-needle biopsies were taken guided by ultrasound and fine-needle aspiration cytology. The biopsies were analysed morphologically and immunohistochemically using conventional immunoenzyme techniques. The identification of mouse Ig associated to the tumor cells and the immunophenotype of various infiltrating leucocyte populations were analyzed semiquantitatively. The current results show that appreciable amounts of mouse Ig is located in the tumor in a seemingly dose related manner. Also an accumulation of components of complement was found in tumors indicating a possible complement mediated lysis of the tumor cells. A variation in the infiltration of T-cell subpopulation and monocyte-macrophages associated with Mab infusion was also seen. These results indicate that the effect of MAb therapy for solid cancers can be directly monitored within the tumor lesions by immunohistochemical analysis in repeated thick-needle biopsies.

CE 507 **CELLULAR EVENTS INVOLVED IN THE ACQUISITION OF LYMPHOKINE ACTIVATED KILLER (LAK) CELL FUNCTIONS BY CD56+ CELL SUBSETS,** Thomas M. Ellis, Barbara A. Helfrich, Patricia E. Simms, and Richard I. Fisher. Section of Hematology/ Oncology, Loyola University School of Medicine, 2160 South First Avenue, Maywood, IL. 60153. The molecular events required for the acquisition of LAK cell function by FACS-purified CD56 "bright" and CD56 "dim" subsets were evaluated following activation by IL-2 or IFNs. Whereas both CD56 "bright" and CD56 "dim" subsets rapidly developed COLO 205 target cell binding activity (4 hrs post stimulation), CD56 "bright" cells exhibited significantly more rapid acquisition of this function (52 hours) than CD56 "dim" cells. Despite the early appearance of COLO binding capabilities, anti-COLO cytolysis appeared later (> 4 hours). The acquisition of COLO binding activity was completely abrogated by the protein synthesis inhibitors emetine and cycloheximide. The induction of COLO binding activity in CD56 "bright" and "dims" by IL-2, IFN-γ , and IFN-α was all abrogated by the protein kinase C inhibitor, H-7. These studies indicate that 1) recognition of NK resistant targets by CD56+ cells is rapidly induced by IL-2 and requires protein synthesis, 2) IL-2 induced target cell binding and lytic activities by LAK cells are dissociable events, and 3) activation of CD56+ cells by both IL-2 and IFNs involves a protein kinase C signal transducing pathway. (Supported by CA48069)

CE 508 LONG-TERM CULTURE OF CYTOTOXIC CELLS FROM PATIENTS WITH ACUTE LEUKEMIA. Hans W. Grunwald and Fred P. Siegal, Div. of Hematology, Queens Hosp. Ctr. Affil. of L.I. Jewish Med. Ctr., Jamaica, NY 11432
Peripheral blood mononuclear cells (Ficoll-Hypaque separated) from patients with acute leukemia at diagnosis were cultured in AIM-V medium with 1,000 U of recombinant Interleukin 2 (rIL-2) per ml added, at an initial concentration of 10^6 cells/ml, and replenished with fresh medium at least once a week. Most of the cells initially have myeloid (if AML) or CALLA (if ALL) markers, with <20% having T cell markers (T11). Within 10 days confluent "balls" of cells are prominent, and absolute cell counts rise geometrically. After 4 weeks in culture the predominant cell population has T markers (T11), with nearly even proportion of T4 and T8 positive cells; a smaller proportion of cells have B markers (sIg) or NK markers (NKH-1), and the original leukemic cells are no longer detectable either by morphology or by surface markers. After 8 weeks in culture the cell growth rate decreases, and T4 positive cells begin to predominate; after 12 to 16 weeks the cultures still remain highly viable, but consist virtually only of T4 positive cells. The cultured cells are cytotoxic (by Cr^{51} release assay) not only against NK-sensitive K562 cells, but also against NK-resistant Raji cells and against the promyelocytic leukemia HL-60 cells. Cytotoxicity against frozen-thawed autologous leukemic (bone marrow) cells is also demonstrable, but with low statistical confidence due to low Cr^{51} uptake of such cells. Maximal yield of cytotoxic cells is observed between 4 and 8 weeks of culture, and correlates well with the proportion of T8 positive cells present. These studies confirm that rIL-2 stimulates the growth of cytotoxic cells in acute leukemia, and support the use of rIL-2 in-vivo for this purpose. A CALGB study to test rIL-2 in patients with AML in second remission is currently underway.

CE 509 PHASE I TRIAL OF INTRACAVITARY CETUS rIL-2 + LAK CELLS FOR THE TREATMENT OF PRIMARY ADULT MALIGNANT GLIOMA: A PRELIMINARY REPORT, Roberta L. Hayes, Maxim Koslow, Emile M. Hiesiger, Ellery Moore, DonnaMarie Pierz, Doris Chen, Kenneth Hymes, Helen Feiner, Gleb Budzilovitch, Douglas Miller, Arlene Wise & Joseph Ransohoff, Dept. Neurosurgery, NYU Med Ctr, NY, NY 10016. Despite surgery, radiation and chemotherapy, the prognosis for patients with malignant glioma is poor, with a median survival of 52 wks. We report the completion of a Phase I trial of intracavitary IL-2 and LAK cells for patients with recurrent glioblastoma (8 pts) or malignant glioma (3 pts) that have failed conventional therapy. LAK cells ($1x10^8$-10^{10}) and IL-2 ($1.2x10^6$ IU or $2.4x10^6$ IU/bolus) were infused into the tumor bed cavity through an Ommaya reservoir which was implanted at the time of second surgery. Therapy consisted of two, 2 week cycles, with LAK/IL-2 given on M followed by IL-2 alone WF, MWF, with 2 wks between cycles. Patients with stable disease were retreated at 3 month intervals. A maximum of $2x10^{10}$ LAK and $3x10^7$ IU IL-2 were administered in up to 25 doses. The cumulative MTD was considered to be $3x10^7$ IU IL-2. Both acute and cumulative CNS toxicities were manifested, although no systemic toxicity was noted. Grade 3 toxicity was seen in 3/3 pts receiving multiple bolus doses of $2.4x10^6$ IU, and in 1 pt at $1.2x10^6$ IU/bolus who had received prior interstitial therapy. Immunologic changes noted included lymphocytic infiltration and marked eosinophilia in Ommaya fluid aspirates, and increased circulating OKT9+ and IL-2R+ PBL. On re-operation and/or autopsy, marked areas of tumor necrosis and lymphocytic infiltrations were identified. Of 6 evaluable pts thus far, mean survival or time to re-operation has been 41.2 wks $\pm$ 4.2 SEM (versus 22 wks). Four others have stable disease, and one partial response was noted on CT. Supported by CA46788.

CE 510 *IMMUNOLOGIC AND CLINICAL PARAMETERS OF ANTI-LYMPHOMA RESPONSES IN PATIENTS TREATED WITH AUTOLOGOUS PHOTOINACTIVATED LYMPHOCYTES. Peter W. Heald, Maritza Perez ,Inger Christiensen, Richard L. Edelson. Department of Dermatology, Yale School of Medicine New Haven, CT 06510. Nineteen patients with erythrodermic, leukemic cutaneous T-cell lymphoma (CTCL) under went extracorporeal photochemotherapy as their first systemic therapy. A portion of the peripheral lymphocytes are treated with 8 methoxypsoralen and UVA light then returned to the patients. Treatments were conducted at 4 week intervals for a minimum of 9 months. Five patients showed >90% clearing of their skin involvement, four patients showed no response and the remaining 10 had 25-75% clearing. The CD4/CD8 ratios in responders (7.5) were less than the CD4/CD8 ratio in nonresponders (39.6). This was largely due to a greater level of CD8 cells in responders (8%) than nonresponders (3%). Therapy was also more successful when started earlier in the course of the disease with responders. This group had an average disease duration of 1.2 years vs. an average > 3 years. These results demonstrated that extracorporeal immunotherapy can be successful in leukemic CTCL and that CD8 cells may be an important component of therapy.*

CE 511 SELECTIVE EXPANSION OF A FEW $CD4^+$ AND $CD8^+$ T CELL CLONES IN THE BULK CULTURE OF HUMAN MELANOMA TIL WITH INTERLEUKIN 2, Jotereau F., Gervois N., Heuze F. and Diez E., U211 INSERM, Centre de Biologie et Physico-Chimie cellulaires, 2 rue de la Houssinière, 44072 NANTES CEDEX.
A few T lymphocyte clones, identified by their γ and β gene configuration, were shown to preferentially and successively develop in the bulk culture of human melanoma TIL established from melanoma fragments with rIL2. $CD4^+$ clones developed during the 1st month of culture. A $CD8^+$ clone, specific and cytotoxic for the autologous melanoma cell line, selectively expanded later and overgrowed the culture. This clone proliferated during one month and expanded to more than 10^8 cells, in the bulk culture, without any restimulation by autologous tumor cells. The specific cytotoxic activity of this clone was inhibited by monoclonal antibodies directed against TCR or TCR associated molecules and MHC classI antigens, showing that lytic activity was mediated through the TCR and MHC restricted . Frozen cells from this clone could be additionally expanded by a factor $4x10^8$ in limiting dilution culture with autologous melanoma cells and EBV B cells. Immunotherapeutic assays with a such clone would therefore be possible. Specificity of the $CD4^+$ clones is under study.

CE 512 IMMUNE RESPONSES OF HUMAN CANCER PATIENTS TO MOUSE MONOCLONAL ANTIBODIES INJECTED FOR DIAGNOSTIC AND THERAPEUTIC PURPOSES. R. J. Kinders, G.M. Hass, D. Johnson, J. Jennings, S. Stevens, S. Drukman and T. Griffin. Diganostics Division, Abbott Laboratories, North Chicago, IL, and Univ. Mass. Medical Center, Worcester Mass. Under FDA INDs patients with colon or ovarian cancers at the University of Massachusetts Medical Center are being injected with imaging and therapeutic doses of mouse monoclonal antibodies 110 (anti-CEA) and OC 125, respectively. As would be predicted, many of these patients have mounted immune responses to the MAbs (HAMA). Characterization of HAMA antibodies has revealed that both anti-isotype and anti-idiotype responses are present. The time courses of the responses show that the anti-idiotypic antibodies appear later, but remain in circulation longer. HAMA from selected patients were purified and partially characterized, in an attempt to correlate HAMA titres to IgG concentrations. Both high and low affinity HAMA were identified. Putative anti-idiotypic antibodies of the Ab2beta type were identified from one patient that received the OC125 MAb. The HAMA are of the IgG class, principally IgG1, but IgG2 and 3 are present.

Presence of HAMA has profound effects on immunoassays for circulating tumor antigens (markers). This is of concern because marker elevation is a key element in monitoring patient status under the INDs. The nature of HAMA interference has been studied, revealing that both positive and negative interferences occur, depending on the specificity of the HAMA response and the configuration and spcificity of the immunoassay being performed.

CE 513 A PHASE I CLINICAL TRIAL WITH CHIMERIC ANTI- CD4 MONOCLONAL ANTIBODY IN PATIENTS WITH MYCOSIS FUNGOIDES S. J. Knox, S. Hodgkinson, B. Bell, S. Brown, G. Wood, R. Hoppe, E. A. Abel, L. Steinman, A. Hanham*, G. Young*, J. Bindl*, C. Gaiser*, T. Reichert* and R. Levy. Stanford University School of Medicine, Stanford, CA and *Becton-Dickinson, Mountain View, CA.
A phase I clinical trial was recently completed using a chimeric anti-CD4 monoclonal antibody in 7 patients with mycosis fungoides. Successive patients received doses of 10, 20, 40 and 80 mg of antibody twice a week for 3 consecutive weeks. During the treatment course and for a 12 week follow-up period, a variety of parameters were followed in order to monitor toxicity, pharmacokinetics, immunogenicity, tolerogenicity, and T cell function. In addition, immunophenotyping of peripheral blood lymphocytes, and biopsies of skin lesions for immunophenotyping and determination of antibody penetration were periodically performed.

All patients had some clinical improvement with a reduction in one or more of the following parameters: erythema and induration of lesions, eythroderma and adenopathy, but the responses were of relatively short duration -- ranging from 9 days to 12 wk following the completion of therapy. Serum levels varied as a function of dose. At the 80 mg dose level, antibody was readily observed in biopsied skin lesions. Although there was coating by antibody of most CD4 + cells in the blood, there was no significant depletion of CD4+ cells. All but two patients made antibody and T cell proliferative responses to KLH. In 3/7 patients there was depression of the T cell proliferation response to tetanus toxoid. In conclusion at the dose levels studied the chimeric anti-CD4 monoclonal antibody 1. was well tolerated in general, 2. had some clinical efficacy against mycosis fungoides, 3. had some immunosuppressive activity but, 4. did not prevent the immune response to a coinjected antigen. 5. the immunogenicity of the chimeric antibody is being determined and will be presented.

CE 514 NK AND LAK ACTIVITIES OF CRYOPRESERVED LYMPHOCYTES FROM PATIENTS RECEIVING ADOPTIVE IMMUNOTHERAPY IN VIVO: IL-2 ACTIVATED LYMPHOCYTES ARE NOT DEPENDENT ON IL-2 FOR THEIR IN VITRO CYTOTOXIC FUNCTION. C.H.J. Lamers, G. Stoter and R.L.H. Bolhuis, Dept. of Immunology, Dr Daniel den Hoed Cancer Center, Groene Hilledijk 301, 3075 EA Rotterdam, The Netherlands. Patients with advanced renal cell cancer received adoptive immunotherapy with their in vitro IL-2 activated PBL and were monitored for cytolytic activity of their in vivo and in vitro IL-2 activated lymphocytes. Moreover, the manifestation of LAK activity following reinfusion of in vitro activated cells was assessed. Assays were done in series using cryopreserved cells. Cryopreservation only slightly decreased NK and LAK activities of in vitro IL2-activated PBL derived from in vivo IL2-primed cancer patients or from healthy donors. However, NK and LAK activities of in vivo (IL-2) activated PBL were decreased following cryopreservation when tested without further IL-2-activation. Lytic activity was reconstituted after "resting" the cells for 18 hrs. IL-2,when added to the medium during the cytolytic assay, only increased in vivo induced but not in vitro induced LAK activity. From the kinetics of the IL-2 enhancement of cytolytic capacity, i.e. with and without IL-2 present prior to, and/or during the cytolytic assays for various time periods, it became clear that the enhancement level of LAK activity resulted from recruitment of LAK activity and not from an intrinsic dependency of the lymphocytes on IL-2 in order to exert LAK activity. Our findings have two important implications: 1) from the point of view of immune parameter monitoring assays i.e. addition of IL-2 to the cytolytic assay may lead to an "overestimate" of actual lytic activity; 2) our data suggest that prolonged in vivo administration of low dose IL-2 appears warranted in order to maintain maximum levels of in vivo LAK activity.

CE 515 BACTERIAL ENTEROTOXIN AS A CANCER TARGETING ANTIGEN.
Atsuo Ochi and Fujio Wake, Division of the department of Immunology Mt. Sinai Hospital Research Institute and Department of Immunology and Medical Genetics, University of Toronto 600 University Avenue Toronto Ontario Canada M5G 1X5.
Immunotargeting of T cells is becoming an important approach for cancer therapy . Although the devices and therapeutic protocols are heterogeneous, those are mostly based on the bifunctional construct of cancer cell-specific antibody and activation reagents directed to T cells. The development of such a molecular complex, possessing evident target cell specificity and strong T cell activation, is a prerequisite for successful therapy. To this end we have been working on the targeting of $CD4^+$ cytotoxic T cells to B lymphoma using either specific antigen-conjugated anti-idiotype (Id) antibody or anti-CD3 - anti-Id antibody heterodimer (Eur.J.Immunol.17:1645 (1987),J.Immunol.142:4079 (1989)). Recently in developing an unique and more advanced device for T cell targeting we have constructed a complex of an anti-tumor antibody and bacterial enterotoxin. The enterotoxin conjugated to anti-Id antibody was specifically recognized by T cells and induced cytotoxicity. Thus reagents were found to target T cells against the tumor in vitro and efficiently inhibit the growth of the tumor in vivo. The results implicate that the bacterial enterotoxin modified tumor specific antibody will be a highly useful tool in cancer therapy. In vitro and in vivo results of tumor targeting by this newly designed antibody reagents will be discussed.

CE 516 TREATMENT OF STAGE IV RENAL CELL CARCINOMA WITH AUTOLYMPHOCYTE THERAPY: AN OUTPATIENT, LOW TOXICITY APPROACH TO ADOPTIVE IMMUNOTHERAPY. Michael E. Osband, Robert J. Krane and Susan D. Ross, Joint Clinical Immunotherapy Program, Boston University School of Medicine and New England Baptist Hospital, and Cellcor Therapies, Inc., Boston, MA, 02135.
We report the interim results from a randomized, controlled clinical trial to study the effect of autolymphocyte therapy (ALT) in Stage IV renal cell carcinoma. ALT is a form of adoptive immunotherapy based upon the infusion of relatively small numbers of autologous lymphocytes that are depleted of suppressor cells and immunized *in vitro* with an autologous lymphokine mixture using a method designed for antigen-specific activation. Patients receive 6 monthly infusions of cells, all on an outpatient basis, and also receive oral cimetidine to reduce *in vivo* suppressor cell function. Two previous single-arm studies with ALT showed an apparent survival advantage in this disease. The majority of patients showed no toxicity with ALT, and only 10% of cell infusions were accompanied by mild, self-limited fever and chills. Consequently, we initiated this multi-site, controlled trial in which patients are randomized to receive either ALT and cimetidine, or cimetidine alone. 82 patients have been entered at the 5 participating sites. Median follow-up time is currently at 12 months. Interim analysis, using the Cox proportional hazards model, reveals that Treatment A has an estimated 2.45x survival advantage over Treatment B for all entered patients, and an even greater survival advantage (>4x) for those patients who survive to receive at least 2 months of treatment. The increase in survival in Treatment A patients is accompanied by a stable quality of life. Kaplan-Meier life table analysis shows an actual survival advantage with Treatment A that is consistent with the Cox model prediction in that 25% of patients receiving Treatment B have already died by day 138, but that survival milestone is not reached until day 274 in Treatment A patients. Moreover, there is a significant difference in response to therapy between the sexes, in that males show a very significant response to Treatment A, while females do not. The response of pre- and post-menopausal females is the same. Survival is also associated with good performance status at study entry, and negatively correlated with the presence of large tumor at the primary tumor site. Final analysis of the on-going randomized trial will be necessary to confirm these findings.

CE 517 HUMAN TUMOR-INFILTRATING LYMPHOCYTES (TIL): IMPROVED LARGE SCALE PROCESSING FOR CLINICAL USE. P. Simon, A. Nahapetian and S. Toy. Medical Products Department, DuPont Co., Glasgow, Delaware, 19714.
TIL cells obtained from tumor masses can be activated and expanded in the presence of IL-2 to clinically useable quantities. Such cells have been used successfully in the adoptive immunotherapy of patients with melanoma and renal cell carcinoma. The large scale culture of TIL cells is facilitated by the use of gas-permeable plastic bags capable of supporting cell densities of up to $2x10^6$/mL. In addition, the multiple sterile transfers of cell suspensions is more easily accomplished with the aid of a Sterile Connecting Device (SCD™). The gradient separation, washing and harvesting is performed using a flow-through centrifuge (SteriCell™ Processor). It is now routinely possible to expand TIL over 100-fold to produce in excess of 10^{11} cells in 4 weeks. Careful manipulation of the primary culture environment can improve average expansion to 1,000 - 10,000-fold at full scale. The mature TIL cells predominate in CD3+ CD8+ HLA-DR+ T-cells, and are generally low in CD4+ and CD56+ lymphocytes. TIL cells from melanoma patients often exhibit preferential cytotoxicity for the original tumor cells in vitro. This approach to T-cell immunotherapy of cancer patients continues to hold significant promise.

CE 518 **IMMUNE MODULATORY EFFECT OF INTERLEUKIN-2 AND INTERFERON ALFA-2A GIVEN ON ALTERNATE WEEKS IN PATIENTS WITH RENAL CELL CARCINOMA AND MELANOMA.** Rolf A. Stahel, Lorenz M. Jost, Walther Fierz, and Gaby Pichert. Division of Oncology and Division of Clinical Immunology, Department of Medicine, University Hospital, CH-8091 Zurich, Switzerland.

Patients with previously untreated metastatic renal cell carcinoma or melanoma were given interleukin-2 (Ro 23-6919) 3 MU/m2 continuous infusion day 1-4 and interferon alfa-2a (Ro 22-8181) 6 MU/m2 subcutaneously day 1 and 4. Treatment was given on alternate weeks. This report describes the immune modulatory effects in 8 patients who received three or more cycles of treatment. Functional LAK and NK activity of peripheral blood mononuclear cells was determined on day 1 (baseline) and day 8 of the first and the third cycle of treatment. Immune phenotyping, including NK markers CD16, CD56, and CD57 was performed at the same time. Baseline LAK activity was 6.7 LU/ml in cycle 1 and 67.5 LU/ml in cycle ($p<0.05$). Under treatment LAK activity increased 14- fold in cycle 1 ($p<0.01$) and 1.4-fold in cycle 3 (n.s.). Baseline NK activity was 117 LU/ml in cycle 1 and 754 LU/ml in cycle 3 (n.s.). Under treatment NK activity increased 2 fold in cycle 1 (n.s.), but decreased 2 fold in cycle 3 ($p<0.05$). Baseline CD16 positive cells were 0.25 G/l in cycle 1 and 0.85 G/L in cycle 3 ($p<0.01$). CD16 positive cells increased 2.8 fold in cycle 1 ($p<0.05$) and 1.2 fold in cycle 3 (n.s.). CD56 and CD578 positive cells showed a similar pattern. Our findings a demonstrate an impairment of functional response of peripheral blood mononuclear cells with continued treatment with interleukin-2 and interferon alfa-2 given on alternate weeks, despite an increase in the overall number cells positive for NK markers. The discrepancy between functional status and phenotype of peripheral mononuclear cells and the possibility of exhaustion of the lytic NK response with continued treatment have implications for the design of future studies.

CE 519 STABILITY AND SPECIFICITY OF PURIFIED ANTI-TUMOUR*ANTI-CD3 BISPECIFIC ANTIBODIES, THEIR LYTIC POTENTIAL AND USEFULLNESS FOR TREATMENT OF CANCER R.J. van de Griend, H.H. van Ravenswaay Claasen, J.van Dijk G.J. Fleuren and S.O. Warnaar.Department of Pathology State University Leiden, P.O.Box 9603, 2300 RC Leiden, The Netherlands.

Bispecific antibodies (bspcfc Ab) were generated by fusion of hybridoma cells producing tumour specific antibodies with anti-CD3 producing cells (quadroma's). Such antibodies, have potential advantages as compared to for instance chemically coupled antibodies. We have characterized several bspcfc Ab against various ovarian tumour or renal tumour antigens and analyzed their binding capacity by immunohistochemistry and flowcytometry (FACSCAN), as well as their capacity to induce cytolysis. A nice correlation between the different parameters was found: in those cases where cytotoxicity was lost, parental antibodies were produced but few or no bspcfc Ab whereas "cytotoxic" quadroma's continued to produce up to 40% bspcfc Ab. Both TCRαβ and TCRγδ cells show enhanced cytolytic activity with bspcfc Ab. Next we have studied the in vivo localization of i.v. administered 125I labelled IgG or (Fab)2 MOV18*CD3 bspcfc Ab using a human ovarian cancer xenograft nude mice model. Best results were obtained with (Fab)2: up to 40%! of all radioactivity was localized in the tumour after 48 hr Our data demonstrate that functionally active anti-tumour*anti-CD3 bspecfc Ab can specifically immage the tumour site.

CE 520 Interleukin 6 is produced by Ovarian Cancer Cell lines and Primary Ovarian Tumor Cultures. J.M. Watson, J.L. Sensintaffar, M. Vander Meyden, J.S. Berek, O. Martinez-Maza. Depts. of Microbiology and Immunology and Obstetics and Gynecology, UCLA School of Medicine, Los Angeles, CA 90024.

To determine the role of various growth factors in the progression and development of epithelial ovarian carcinoma, we examined the production and utilization of interleukin 6 (IL-6), a multifunctional cytokine with diverse biological effects, by both ovarian cancer cell lines and primary ovarian tumor cultures. We have found that epithelial ovarian cancer cell lines (CAOV-3, OVCAR-3, SKOV-3) constituitively produce varying amounts of IL-6. This molecule is biologically active as determined by the proliferation of an IL-6-dependant hybridoma cell line, MH60.BSF-2, and shares antigenic epitopes with human IL-6 detectable by an IL-6 ELISA. By cytoplasmic immunoperoxidase staining it appears that all cells produce at least some IL-6 as there is variation in the staining intensity between individual cells. The ovarian cancer cell-produced protein has a molecular weight of approximately 22-25kDa, and exhibits little heterogeniety often associated with epithelial cell produced IL-6. Our data suggest that IL-6 is not an autocrine growth factor for the established ovarian tumor cell lines as the addition of either exogenous IL-6 or antibodies to IL-6 did not effect the cellular proliferation of the cell lines. We also found significant levels (>3ng/ml) of IL-6 in ovarian cancer patient ascitic fluids and in the supernatants of primary cultures from freshly excised ovarian tumors. The production of IL-6 by epithelial ovarian cancer cells may prove to be a useful diagnostic tool, and aid in investigation of the host immune response to ovarian cancer.

CE 521 CHARACTERIZATION OF THE SPONTANEOUS HUMAN ANTI-TUMOR RESPONSE

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We have developed an original system in order to investigate the putative presence of a spontaneous, tumor specific cellular immune response in cancer patients. We have established Tumor Infiltrating Lymphocytes (TIL) clones as well as autologous tumor cell lines and Antigen Presenting Cells (EBV-transformed PBL) from colon carcinoma bearing patients. 6 TIL lines were obtained. Their phenotypical and functional characterization was done using respectively FACS analysis and a retargeting assay based on the fact that T cells can be activated to perform their effector function through the cross-linking of their T cell receptor complex (TcR and CD3 complex). TIL lines of both $CD4^+$ and $CD8^+$ phenotypes were obtained.

We have also established autologous tumor cell lines. The 6 tumor cell lines were characterized with specific anti-colorectal carcinoma monoclonal antibodies. Eventhough TIL can be maintained in culture by stimulation with allogeneic oxidized PBL, the availability of nominal antigen greatly facilitates the culture and cloning of TIL lines. We are currently studying the specificity of TIL lines and clones for the autologous tumor by testing the direct cytotoxicity of $CD8^+$ TIL and proliferation and secretion of lymphokines of $CD4^+$ cells in presence of tumor cells and APC.

The establishment of TIL, tumor cells and APC for each patient allow us to analyse the functional phenotype of the cells involved in an ongoing, presumably weak anti-tumor response.

CE 522 IMMUNOMODULATORY EFFECTS OF COMBINATION IMMUNOTHERAPY WITH LOW-DOSE INTERLEUKIN-2 (IL-2) AND TUMOR NECROSIS FACTOR-ALPHA (TNF) IN PATIENTS WITH NON-SMALL CELL LUNG CANCER (NSCLC), Stephen C. Yang, Elizabeth A. Grimm, and Jack A. Roth, Departments of Thoracic Surgery and Tumor Biology, M. D. Anderson Cancer Center, Houston, TX 77030

The purpose of this study was to assess the antitumor and immunomodulatory effects *in vivo* of a combination of low dose IL-2 and TNF in patients with NSCLC. Sixteen patients with stage IIIb or IV NSCLC received a continuous daily IV infusion of $6x10^6$ IU/m^2 of IL-2 and a simultaneous IM dose of TNF (25-100 $\mu g/m^2$) for 5 days. Treatment was given every 3 weeks. Thrombocytopenia (<50 K/µL) was the dose limiting toxicity, and established 50 $\mu g/m^2$ of TNF with IL-2 as the maximum tolerated dose. In 12 evaluable patients, there was one partial and three minor responses, and radiographic stabilization of disease in 8 patients before progression (median 12 wks). All patients showed increased lymphokine-activated killer (LAK) and natural killer activities (range 11-250 and 6-242 LU, respectively) compared to pretreatment levels. Increased lysis over pretreatment levels of available autologous tumor targets (range 70-4150 LU) was demonstrated for 4 patients. Pretreatment IL-2 serum levels of succeeding cycles (obtained 2 weeks after cessation of therapy) ranged from 10-3600 IU/ml; posttreatment levels measured 2 days after stopping therapy ranged from 0-30,000 IU/ml. This serum IL-2 was functional and generated LAK activity when added to allogeneic lymphocytes. Serum TNF levels were measured in a similar fashion, with concentrations up to 2000 and 6000 pg/ml measured pre- and posttreatment, respectively. Phenotypic analysis of circulating lymphocytes revealed transient increases in $CD3^+$, $CD16^+$, and Leu 19^+ cell populations following therapy, with no consistent changes in $CD4^+$ or $CD8^+$ subpopulations. These observations demonstrate that combined low doses of IL-2 and TNF produce significant biologic activity with acceptable toxicities.