CELLULAR IMMUNITY AND THE IMMUNOTHERAPY OF CANCER

Organizers: Olivera J. Finn and Michael T. Lotze

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Plenary Sessions *Page*

Poster Sessions

Keynote Address (Joint)

NZ 001 IMMUNOLOGY BY VIRUSES, Rolf M. Zinkernagel, Peter Aichele, Manuel Battagay, Martin Bachmann, Daniel Brändle, Marie-Anne Bründler, Etienne Bucher, Christoph Burkhart, Giulia Freer, Urs Hoffmann Rohrer, David Kägi, Ulrich Kalinke, Thomas Kündig, Diego Kyburz, Demetrios Moskophidis, Ulrich Steinhoff, Hanspeter Pircher, Hans Hengartner; Institute of Experimental Immunology, University of Zurich (Switzerland)

Different viruses have coevolved with vertebrate hosts and their immune systems to reach evolutionarily balanced states; therefore many of these virus-host arrangements reveal limiting facets of the immune system. For example immunological unresponsiveness to viruses may be established by infection of the thymus by sequestration of antigens or presentation in an antigenic but not immunogenic form which are ignored by T cells, or by exhaustive differentiation of T cells in the periphery. Induction of antiviral T cells depends on proper antigen presentation and an environment with sufficient concentrations of cytokines; in their absence T cells are apparently neither induced nor anergized. T cell memory seems to be strictly dependent upon antigen persistence and so far no special memory T cell could be found. Mechanisms of T cell mediated antiviral protection also varies with the virus. For some either CD8+ or CD4+ T cell dependent cytokines may provide protection, dependent upon the site of viral replication. For others CD8+ T cells contact seems to be mandatory for virus elimination. The respective role of perforin, or of granzymes is still unclear. The implied cytolytic T cell effector pathway is always antivirally effective against cytopathogenic viruses. In contrast, immunopathological consequences of protective antiviral immune responses are seen whenever non- or poorly cytopathic viruses spread too widely before they can be stopped by antiviral cytotoxic T cells (CTLs), antibodies or other mechanisms.
Aggressive hepatitis by Hepatitis B virus in man or lymphocytic choriomeningitis virus (LCMV) in mice illustrate such a pathogenesis. Similarly the immunosuppression caused by LCMV, and possibly by HIV, may be caused by CTLs destroying antigen-presenting cells infected by LCMV. All these examples may easily impress as autoimmune disease if the infectious agents were not known. This conclusion is illustrated in a transgenic mouse model where the LCMV-glycoprotein (GP) is expressed as a new-self antigen in ß-islet cells of the Langerhans islets in the pancreas. Such mice do not develop diabetes sponteanously, but do so after infection with LCMV within 7-9 days. The immune system of these mice ignores apparently the new self antigen; these mice are therefore not immunologically tolerant to this new self antigen, but normaly no response is induced either. LCMV infection of antigen presenting cells causes prompt induction of CTLs and of diabetes. Again, did one not know the antigen or virus involved, this disease would be called an autoimmune disease. Accordingly, we speculate that possibly many so-called autoimmune diseases may actually reflect immunopathologies triggered by the unrecognized or unrecognizable trivial or special viruses or other infectious agents. Viruses also reveal fundamental aspects of antibody responses: B cells may be unresponsive to viral antigen because of lack of T help or because of anergy to a special form and organisation of the antigen. Also so-called affinity maturation, B cell memory, and the role of antibodies in protection against viruses reveal limitations of humoral responses. Collectively, the various balances between viruses and immune system, yield captivating and accurate data on role, efficiency and limitations of immune effector mechanisms in a biologically relevant setting.

Cell Biology of Antigen Processing and Presentation (Joint)

NZ 002 PRESENTATION OF VIRAL ANTIGENS TO CYTOTOXIC T LYMPHOCYTES, Jack R. Bennink[1], Igor Bacik[1], Cheryl Lapham[1], Daniele Arnold[2], Thomas Spies[2], Josephine Cox[1], Yuping Deng[1], Patricia Day[1], Robert Anderson[1], Nicholas Restifo[3], Laurence Eisenlohr[4], Fernando Esquivel[1], Jonathan W. Yewdell[1], [1]National Institute of Allergy and Infectious Diseases, Bethesda, MD 20892, [2]Dana Farber Cancer Institute, Boston, [3]National Cancer Institute, Bethesda, [4]Thomas Jefferson Cancer Institute, Philadelphia.

$CD8^+$ T-cells(T_{CD8+}) play an important role in controlling viral infections. T_{CD8+} recognize peptides of 8 to 10 residues derived from viral proteins located in the cytosol of infected cells. These peptides are recognized in a complex with class I molecules encoded by the major histocompatibility complex (MHC). Since the processing pathway begins in the cytosol and association with class I molecules occurs in an exocytic compartment proteolysis and peptide transport are thought to play key roles in presentation to T_{CD8+}. Two MHC encoded molecules termed Tap1 and Tap2 seem to specifically transport peptides from the cytosol into the intracellular compartment that contains class I molecules. To characterize the structure and function of the Tap genes we have inserted them into vaccinia virus. We have been studying the biochemical, immunocytochemical, and antigen specificity of the transporters in mutant cells failing to express one or both Tap genes. In addition, the endoplasmic reticulum location of peptide association with class I molecules in the exocytic pathway has been demonstrated using a vaccinia recombinant encoding an endoplasmic reticulum retained class I molecule.

NZ 003 MECHANISMS OF CLASS II MHC-RESTRICTED ANTIGEN PROCESSING, Peter Cresswell, Howard Hughes Medical Institute, Yale University School of Medicine, New Haven, CT 06510.

Class II MHC α-and β-subunits associate with invariant chain trimers in the endoplasmic reticulum. Following assembly the αβ-invariant chain complex is transported through the Golgi apparatus and then diverted from the constitutive transport pathway into the endosomal system. Here the invariant chain is proteolytically degraded and αβ dimers are released to bind peptides derived from endocytosed proteins. The precise mechanisms involved in peptide generation and binding, and the route by which αβ-peptide complexes are subsequently delivered to the plasma membrane, are unknown. A number of mutant cell lines, defective in an uncharacterized MHC-linked gene or genes, are impaired in their ability to generate functional αβ-peptide complexes. We have determined that many αβ dimers from one such cell line, T2.DR3, are associated with a nested set of invariant chain-derived peptides, and that such αβ dimers can be efficiently loaded with antigenic peptides *in vitro*. Comparative analyses of class II MHC transport, invariant chain processing, and subcellular distribution in wild-type and mutant cell lines will be presented.

NZ 004 THE USE OF CRYSTAL STRUCTURE ANALYSIS OF RECOMBINANT CLASS I MHC-PEPTIDE COMPLEXES TO ANALYZE THE MOLECULAR BASIS OF PEPTIDE/MHC/TCR INTERACTIONS. Stanley G. Nathenson, Weiguo Zhang, Aideen C.M. Young and James C. Sacchettini. Departments of Microbiology and Immunology, and Cell Biology and Biochemistry, Albert Einstein College of Medicine, Bx NY 10461.

Most of the sequence diversity among MHC class I alleles is found in the amino acid residues that line the peptide binding groove. This diversity alters the chemical composition and spatial properties of the peptide binding groove and in turn dictates the characteristics of the peptide that can be accommodated.

In order to characterize those peptides that bind to specific class I molecules in vivo, we identifed the major H2-K^b restricted peptide from VSV as a unique octamer, VSV N52-59, Arg- Gly- Tyr- Val- Tyr- Gln- Gly- Leu. Alanine substitued peptide variants were used to define the role of each amino acid residue in the octapeptide in terms of its interaction with the H2-K^b molecule and with the TCR. As a result of binding studies we postulated that Tyr3, Tyr5 and Leu8 were MHC anchor residues, while studies using a panel of T cell clones recognizing VSV/K^b complexes on cells suggested that Arg1 Val4 , Gln6 and Gly7 were important in TCR recognition.

To evaluate these hypothesis at the structural level we exploited a high yield bacterial expression system and in vitro co-complex formation (protein folding) to prepare a homogeneous MHC class I molecule containing VSV N52-59 peptide. This complex was crystallized and its structure solved using molecular replacement techniques.

The structure of mouse H-$2K^b$ revealed its similarity to three human class I HLA molecules, consistent with the high primary sequence homology and common function of these peptide-presenting molecules. Electron density was located in the peptide binding groove, to which a single peptide in a unique conformation was unambiguously fit. The peptide extended the length of the groove, parallel to the α-helices, and assumed an extended, mostly ß-strand conformation. The peptide was constrained within the groove by hydrogen bonding of its main-chain atoms and by contacts of its side-chains with the H-$2K^b$ molecule. Its amino terminal nitrogen atom formed a hydrogen bond with the hydroxyl group of Tyr171 at one end of the groove, while the carboxyl terminal oxygen formed a hydrogen bond with the hydroxyl group of Tyr84 at the other end, amino acids which are conserved among human and mouse MHC molecules. This anchoring of each end of the peptide appears to be a general feature of peptide-MHC class I molecule binding and imposes restrictions on its length. The side-chains of residues Tyr3, Tyr5, and Leu8 of the peptide fit into the interior of the K^b molecule with no appreciable surface exposure, a finding in support of previous biological studies that showed the importance of these residues for binding. Thus the basis for binding of specific peptide sequences to the MHC class I molecule is the steric restriction imposed on the peptide side-chains by the architecture of the floor and sides of the groove. The side-chains of Arg1, Val4 and Gln6 as well as the main-chain of Gly7 of the peptide are exposed on the surface of the complex, thus confirming their availability for T cell receptor contact, as previously suggested by experiments which demonstrated that a specific subset of these residues were interactive with specific TCRs. The overall picture that arises from our studies is that the TCR/MHC interaction is unique since only 3 to 4 residues of the peptide have sufficient solvent accessibility for TCR interaction, with the majority of the peptide residues being buried. T cell recognition thus depends on only a few of the residues of a peptide presented in the context of the much larger pattern of amino acid side chains of the 2α helices of the antigen presenting domain of the MHC.

NZ 005 PROCESSING AND PRESENTATION OF THE VESICULAR STOMATITIS VIRUS GLYCOPROTEIN IN ASSOCIATION WITH CLASS II MHC ANTIGENS. Carol S. Reiss[1], Lara M. Palevitz[1], Anna Wilson[1], and Stephanie Diment[2]. [1]Biology Department, New York University, New York, NY 10003, and [2]Pathology Department, New York University School of Medicine, New York, NY 10016.

Vesicular stomatitis virus (VSV) glycoprotein (G) has been shown to be a major target of both the humoral and cell mediated immune response of mice and other species to this pathogen. The cell mediated immune response is principally class II MHC restricted and includes both proliferative and cytolytic effector cells. We have studied the processing and presentation of this protein by murine B cells by both exogenous and endogenous routes. Published studies have demonstrated 1) an absolute dependence on newly synthesized Ia molecules, as emetine treatment completely prevents sensitization. 2) Acidification of the endosomal compartment is required, whether infectious virus or purified protein antigen is employed, as chloroquine, methylamine, and ammonium chloride pretreatment prevent uptake and degradation of purified protein, inactivated virions, and infectious virus. 3) Integrity of the vesicular compartments of the cell and their normal fluidity is necessary, as Brefeldin A treatment reversibly blocks sensitization of cells for CTL clone recognition and there is a reversible "cold block" in cells treated at 15°C. 4) A late post-Golgi block was observed in cells treated with acidification inhibitors, which presumably interfere with degradation of the invariant chain of Class II MHC. We have continued these studies by examining the role of the cytoskeleton in antigen processing and presentation both for effects on the processing cell and on the CTL clone recognizing Ia^d-peptide complexes on the surface of A20 cells. This has been probed with inhibitors of both microtubules (taxol, colchicine) and microfilaments (cytochalasian). As the cold treatment of cells had previously been shown to interfere with presentation, we expected microtubule-dissociation or -polymerization to substantially inhibit presentation. This was an inconsistent finding, even when the inhibitors were incorporated in assay medium to prevent reversible changes in the absence of drug; the tendency was to greater sensitivity to CTL effectors, not diminished recognition. Treatment of processing and presenting cells with microfilament-interfering drugs did not alter their recognition. However, cytochalasian treatment of T cells had profound inhibitory effects. This was not seen with either taxol or colchicine treatment of our clones, however, thus implying a central role for microfilaments (but not microtubules) in T cell cytoskeleton and lethal hit delivery. We have also been examining alternative sites (to late endosomes/early lysosomes) of protein degradation using 2 forms of the glycoprotein synthesized in the presenting cells and limited, due to either temperature sensitive mutation (tsO45 virus) or to genetic manipulation (vaccinia expressing a "poison tail" construct, generously provided by JK Rose, Yale). These protein variants are degraded within the lumen of the pre-Golgi endoplasmic reticulum, and peptides generated readily sensitize A20 cells for T cell recognition. The site of peptide acquisition and the maturation of the Ia complex are under intense study.

NZ 006 MANIPULATING THE ANTIGEN PROCESSING MACHINERY AND TUMOR IMMUNOLOGY. NP Restifo, JW Yewdell, JR Bennink, I Bacik, Y Kawakami, F Esquivel, and SA Rosenberg. NCI and NIAID, Bethesda, MD.

Some tumor cells in mouse and man clearly present peptide/MHC class I complexes recognized by $CD8^+$ CTL (T_{CD8+}). However, most cancers are not cured by T cell based immunotherapy. We have recently shown that the nonimmunogenic murine tumor, 101.WT, presented endogenously generated viral antigens in the context of MHC class I poorly, despite the presence of these antigens intracellularly in high quantities. Hypothesizing that this tumor might evade recognition by T_{CD8+} by failing to process and present tumor antigens in the context of class I molecules, we transduced 101.WT, with interferon-γ (IFN-γ) cDNA to create 101.NAT. Gene-modification increase class I expression by > 100-fold and reversed the viral antigen presentation deficit of 101.WT. Significantly, 101.NAT could be used to generate $CD8^+$ TIL that were therapeutically active *in vivo* against established pulmonary metastases from the wild-type tumor.

To examine whether or not such antigen processing deficiencies were present in *human* tumor cells, we used a recombinant vaccinia virus expressing mouse the K^d molecule. Because lysis by mouse T_{CD8+} was our read out, our assay independent of both the HLA type of the tumor, and the presence, or absence, of specific cellular proteins. Human small cell lung carcinoma (SCLC) cell lines, amongst others, processed endogenous antigens poorly. Pulse-chase experiments showed that MHC class I molecules were not transported by SCLC from the ER to the cell surface, suggesting that peptides were not available for binding to nascent MHC and β_2-microglobulin molecules. Consistent with this interpretation, northern blot analysis revealed low to non-detectable levels of mRNAs for MHC encoded proteasome components *LMP-7* and *LMP-2*, as well as the putative peptide transporters TAP-1 and TAP-2. Treatment of cells with IFN-γ enhanced expression of these mRNAs, and completely reversed the observed functional and biochemical deficits. In order to bypass the need for the processing of intracellular antigens, we constructed a vaccinia virus capable of endogenously synthesizing a 9 amino acid long "minimal determinant" from the nucleoprotein gene of influenza A/PR/8/34 preceded by an ER insertion signal sequence, (Anderson *et al*, J Exp Med 174:489, 1991) thus eliminating the need for both protease activity and transporter activity. The NP 9-mer preceeded by a signal sequence was very efficiently presented by the SCLC lines to T_{CD8+}. Thus, antigens, of precisely the right size, targeted to the endoplasmic reticulum, can bypass much of the antigen processing machinery.

Effector Cell Activation, Trafficking and Endothelial Cell Interactions

NZ 007 GENERATION AND FUNCTIONS OF HELPER T CELL SUBSETS. Susan L. Swain, Linda M. Bradley, Michael Croft, David D. Duncan, Girija Muralidhar, Susanne Koch and Filippa Shub, University of California, San Diego, La Jolla, CA 92093-0063.

Only in the past few years has the extensive heterogeneity of helper T cells (Th) become clear. We have characterized and studied CD4 Th at different stages of differentiation and with different programs for patterns of cytokine secretion within those stages. Naive Th are short lived and secrete predominantly IL-2. They proliferate vigorously in response to Ag presented by dendritic cells or activated B cells and they differentiate into primary effector cells and memory cells. Primary effector cells are large, activated cells that are probably transient. They can be Th0-, Th1-, or Th2-like in their secretion of cytokines. The Th2 effectors are excellent helpers for the response of B cells generating a large Ab response of a variety of isotypes. The Th2-like effectors can drive both cognate and bystander B cell responses. The generation of Th2-like effectors is dependent on and promoted by IL-4 and is inhibited by IFN-γ and TGFβ. Production of IL-4 by effector cells may be influenced by the APC type on which they encounter Ag. Memory helper cells are also derived from naive cells. We have studied the features of memory Th subsets by generating Th2 and Th2 effectors in vitro and then transferring each of the effector populations or naive CD4 T to irradiated, thymectomized recipients. We find that memory is generated by both effector types and that their unique patterns of cytokine secretion are maintained indefinitely (>40 weeks). The memory cells are persistent, while naive transferred cells decline in numbers and function with time after transfer. Even effectors generated in response to peptide antigen give rise to long-term memory in vivo in the absence of any further antigen stimulation. Thus the memory persistence of these populations is apparently independent of Ag. The most potent population of helpers is a population we term 'memory effectors'. These effectors come from memory cells, are generated rapidly and give the highest levels of lymphokine secretion and help. In vitro we can regulate the generation of effectors with IL-4, IFN-γ, and TGFβ, promoting Th2 and Th1 patterns of cytokine production. We have also studied the induction of anergy in vivo to both bacteria and viral Superantigens. We are investigating the conditions which will favor induction of effectors and memory versus those which favor generation of anergy. The ability to generate particular Th effector populations which have potent Ag-specific activities and which can persist in a completely functional state for long times after transfer, should provide an opportunity for therapeutic intervention and in vivo augmentation of specific immune response.

Tumor Antigens Defined by T Cells

NZ 008 GENETIC ANALYSIS OF HUMAN TUMOR REJECTION ANTIGENS. Pierre G. Coulie, Pierre van der Bruggen, Benoît Van den Eynde, Etienne De Plaen, Patrick Chomez, Christophe Lurquin and Thierry Boon, Ludwig Institute for Cancer Research, Brussels unit and Cellular Genetics unit, Catholic University of Louvain, Brussels, Belgium

We isolated the gene coding for antigen MZ2-E recognized by autologous cytolytic T cells (CTL) on a human melanoma (1). This gene, named MAGE-1, is expressed by 20% of melanoma tumors as well as by a significant proportion of tumors of other histological types (20% of lung tumors, 17% of breast tumors). No expression of MAGE-1 is observed in a large panel of normal tissues. The only tissue in which we detect the expression of gene MAGE-1 is testis.

MAGE-1 belongs to a family of closely related genes, localized on chromosome X. Fourteen members of this family have been identified, sharing 65 to 85% homology with MAGE-1. Of these genes, only MAGE-1, -2,-3 and -4 are significantly expressed by some human tumors. None of them has so far been shown to be expressed in normal adult tissues.

The CTL clone directed against antigen MZ2-E recognizes a nonapeptide that is encoded by the third exon of gene MAGE-1 and is presented by the HLA-A1 molecule (2). The CTL also recognizes this peptide when it is presented by mouse cells transfected with HLA-A1 gene, confirming the association of antigen MZ2-E with the HLA-A1 molecule. Other members of the MAGE gene family do not code for the same peptide, suggesting that only MAGE-1 produces the antigen recognized by the anti-MZ2-E CTL. Our results open the possibility of immunizing HLA-A1 patients whose tumor expresses MAGE-1 either with the antigenic peptide or with autologous antigen-presenting cells pulsed with the peptide. Another possibility is to immunize these patients with cells engeneered to express high levels of HLA-A1 and MAGE-1, together with the appropriate adhesion molecules and interleukins to stimulate a CTL response in vivo.

In order to follow these immunizations and to document any increase in the frequency of the MAGE-1 specific CTL's, we set up a protocol of limiting dilution analysis tailored to this type of responses (3). We use cold-target inhibition with an excess of NK target K562 to inhibit the NK-like activity. Using autologous tumor cells to stimulate the proliferation of the anti-tumor CTL-P, we measured the anti-tumoral CTL-P frequencies in the blood of 15 patients and obtained frequencies ranging from 1/1000 to less than 1/100,000. We are modifying this assay in order to avoid the use of autologous tumor cells as stimulator cells.

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NZ 009 CELLULAR, MOLECULAR AND STRUCTURAL ANALYSIS OF HUMAN T CELL - TUMOR MUCIN INTERACTIONS. Olivera J. Finn, Ph.D., Department of Molecular Genetics and Biochemistry, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261

One of the primary challenges to tumor immunologists in recent years has been to define antigens on tumor cells capable of stimulating the immune system, primarily the effector T cells. One of these molecules, first identified by monoclonal tumor specific antibodies, is breast mucin. Mucin consists of multiple tandem repeats of a 20 amino acid sequence which is highly glycosylated by O-linked sugars. Since both normal and malignant epithelial cells produce mucin, the tumor specificity of anti-mucin antibodies has been postulated to result from either 1) genetic changes of the mucin gene in tumors; or 2) incomplete glycosylation of mucin by malignant cells. The importance of mucins as tumor-associated antigens has recently been increased by our finding that cytotoxic T lymphocytes (CTL) can recognize mucin. The epitope recognized by the CTL is present on mucin produced by malignant cells, yet not on mucin produced by normal ductal epithelial cells. This CTL epitope is closely related to the tumor-specific mucin epitope defined by the mAb SM-3.

The above information becomes more valuable now that the gene for mucin has been cloned and immune responses to its product can be manipulated by various gene transfer and biochemical techniques. We constructed several expression vectors which allow mucin production in different cell types, including autologous antigen presenting cells. Each transfected line expresses a number of tumor-specific mucin epitopes, recognized by such tumor-specific antibodies as SM-3, DF-3, and BC-1, suggesting that the defects in mucin synthesis seen in breast and pancreatic tumors are present in other transformed cells as well. Furthermore, although the cell lines were transfected with the same expression construct, each expressed a different subset of tumor-specific epitopes, indicating that the specificity of these epitopes for tumors is not due to genetic alterations in the mucin gene. Finally, the expression of tumor-specific epitopes was increased by incubating transfected cells with phenyl-N-acetyl-α-galactosaminide, an inhibitor of O-linked glycosylation, confirming the role of incomplete glycosylation in the tumor specificity of these epitopes. Glycosylation-inhibited transfectants were capable of maintaining proliferation of mucin specific T cells and serving as targets for CTL killing.

Using mucin transfected B cells as APC we generated specific T cell lines from breast cancer patients and analyzed over 200 T cell clones. T cell clones could be divided into the major group which requires inhibition of mucin glycosylation for target cell recognition, a smaller group which is not affected by it, and rare clones which loose activity when mucin is not fully glycosylated. In agreement with our earlier observations derived from T cell lines generated against allogeneic mucin producing tumors, mucin on both syngeneic and allogeneic APC could stimulate mucin specific CTL and serve as their target. Inasmuch as we had postulated that the MHC-unrestricted recognition is due to the identity of multiple, repeated T cell epitopes along the mucin polypeptide core, which allow simultaneous binding, cross-linking and aggregation of multiple T cell receptors, we performed structural analysis of the mucin tandem repeat region to confirm the structural, and not only sequence identity of the T cell epitopes. We prepared synthetic peptides corresponding to one, two and three mucin tandem repeats and analyzed their three dimensional structure by one and two dimensional ^{1}H-NMR correlation spectroscopy (COSY). The proof of the equivalence of particular residues within each copy of the tandem repeat was obtained from the analysis of the side chain protons C2 and C4 of histidine when dissolved in D_2O. These two are always resolved from each other by distinct magnetic resonances which are usually separated by at least 1ppm. The two histidines in the two amino acid peptide which had the potential of resolving into four distinct resonances were resolved into only two resonance peaks. The three repeat peptide which can potentially yield six peaks, also yields only two peaks. These data indicate that each histidine is present in an identical three dimensional environment in each tandem repeat.

Our cellular, molecular and structural data suggest that altered post-translational modification of normal gene products can result in the expression of novel, tumor specific epitopes, which can be utilized to induce an effective anti-tumor response, when presented on an immunizing cell of choice. These observations are now being utilized to construct a mucin - based cellular vaccine in the form of an antigen presenting cell transfected with an appropriate mucin expression vector and cytokines or costimulatory molecules of choice.

NZ 010 T CELL RECOGNITION OF HUMAN MELANOMA ANTIGENS, Yutaka Kawakami, Bert H.O'Neil, Michael I.Nishimura, Nicholas P.Restifo, Suzanne L. Topalian, Sophia S.Hom, John R.Yannelli, Joel Shilyansky, Peter Shamamian, Cynthia H.Delgado, Siona Eliyahu, Patrick Charmley[1], Jonathan W.Yewdell[2], Jack R.Bennink[2], Leroy E.Hood[3], and Steven A.Rosenberg. Surgery Branch, National Cancer Institute and [2]Laboratory of Viral Diseases, National Institute of Allergy and Infectious Disease, National Institutes of Health, Bethesda, MD 20892, [1]Virginia Mason Research Center, Seattle, WA 98101, and [3]Department of Molecular Biotechnology, University of Washington, Seattle, WA 98195.

Adoptive transfer of tumor infiltrating lymphocytes (TIL) along with IL2 has antitumor activity in mice and humans. Traffic studies using Indium-111 labeled TIL or TIL marked with the neomycin resistance gene showed accumulation of transferred TIL in melanomas. Immunohistochemical studies showed massive infiltrates of T-cells and macrophages in biopsies from melanomas that regressed after IL2-related therapy. Therefore, T-cells are likely to be important immune cells in in vivo tumor regression.

We were able to establish T-cell lines that specifically lyse autologous and some allogeneic melanomas sharing particular class I MHC molecules by culturing TIL in IL2 containing media. HLA-A2 is the most frequently expressed class I HLA molecule and possibly a dominant restriction element for the induction of melanoma-specific T-cells. Several TIL lines or clones were established from HLA-A2 patients. We have analyzed 3 components important in the T-cell recognition of melanoma, MHC, antigen and T-cell receptor (TCR). Depending on TIL lines tested, these TIL lyse 8/16(50%)- 14/15(93%) HLA-A2 melanoma cell lines derived from different patients. In addition, these TIL lyse 11/17(65%) of naturally HLA-A2 negative melanoma cell lines after HLA-A2 was expressed on these tumors by introducing the HLA-A2.1 gene with recombinant vaccinia virus. None of the non-melanoma cell lines tested were lysed by these TIL. These results suggest the existence of shared melanoma-specific antigenic peptides that are recognized by T-cells in the context of HLA-A2 molecules and also suggest that precursor proteins for shared melanoma antigenic peptides exist in HLA-A2 negative melanomas. Different peptides derived from antigenic proteins may bind to different MHC molecules and be presented to T cells which are restricted at MHC loci other than HLA-A2. If we can isolate such antigenic proteins, we may be able to develop vaccines to induce T-cell responses in patients expressing a variety of HLA types. Isolation of melanoma antigens at both peptide and gene level are in progress. T-cell component of anti-melanoma responses was also analyzed by determining gene structure of TCR in melanoma specific TIL using PCR and cDNA cloning.

Defects in the expression of MHC may lead to tumor escape from T-cell immunesurveillance. We found 4 melanoma cell lines which express little class I MHC on their surface. It is likely that these melanoma cell lines have defects in ß2-microglobulin expression, because introduction of the gene coding for normal ß2-microglobulin by vaccinia virus vectors to these cell lines increased class I MHC expression. Two of these cells expressed little mRNA for ß2-microglobulin. This may be one of the mechanisms by which some melanomas escape from T-cell recognition.

References

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Oncogene Products as Tumor-Specific Immunostimulatory Antigens

NZ 011 T CELL IMMUNITY TO ONCOGENIC PROTEINS INCLUDING MUTATED p21 *RAS* AND CHIMERIC p210 *BCR-ABL*.
Martin A. Cheever, David J. Peace, Mary L. Disis, and Wei Chen. Department of Medicine, Division of Oncology (Mailstop: RM-17), University of Washington, Seattle, WA 98195.

Ras oncogenes are activated by point mutation in approximately 20% of human malignancies. The mutations occur primarily at codons 12 or 61 which results in expression of a $p21^{ras}$ protein with a single substituted amino acid at residues 12 or 61. Only a limited number of amino acid substitutions occur. Murine studies will be presented demonstrating that both class II restricted $CD4^+$ T cell responses and class I restricted $CD8^+$ T cell responses specific for mutated $p21^{ras}$ protein can be elicited and can affect tumor viability. Thus, activated $p21^{ras}$ can serve as a cancer-specific antigen.

To elicit $CD4^+$ T cell responses, mice were immunized with synthetic peptides corresponding to the mutated region of $p21^{ras}$ protein. Each mouse strain was capable of responding to a distinct spectrum of mutated peptides. In some but not all circumstances, the peptide-specific T cells could respond to $p21^{ras}$ protein. In these circumstances, T cells specific for the mutated segment of $p21^{ras}$ could also be elicited by immunization with whole protein. In two *in vivo* models, $CD4^+$ $p21^{ras}$-specific T cells could be shown prevent growth of *ras*-positive tumors.

Class I MHC restricted cytotoxic T cells which recognize the mutated segment of activated $p21^{ras}$ protein have been generated in one prototype model. To elicit $CD8^+$ CTL responses, *ras* peptides with an amino acid motif potentially appropriate for binding to murine class I MHC molecules ($H\text{-}2^b$) were synthesized and analyzed for the ability to bind to class I MHC molecules. RMA-S, a mutant B6 leukemia line which expresses unstable "empty" class I molecules ($H\text{-}2^b$) was used as a binding assay system. Peptides corresponding to the mutated segment of the $p21^{ras}$ protein enhanced the surface expression of class I MHC on RMA-S. Incubation of B6 spleen cells with some but not all of the binding *ras* peptides under primary *in vitro* immunization conditions induced CTL specific for the immunizing *ras* peptides. Long-term cultured $CD8^+$ T cell lines retained specific cytolytic activity and were able to lyse tumor cells transformed by the corresponding $p21^{ras}$ protein. Thus, activated $p21^{ras}$, an intracellular protein, is available for processing and presentation via the class I MHC pathway.

Data from experiments to similarly evaluate the generation of T cell immunity to the joining region segment of $p210^{bcr\text{-}abl}$ protein will be presented. The hallmark of chronic myelogenous leukemia is the translocation of the human *c-abl* proto-oncogene from chromosome 9 to the specific breakpoint cluster (*bcr*) region on chromosome 22. The t(9;22) translocation results in the formation of a *bcr-abl* fusion gene that encodes at 210-kDa chimeric protein with abnormal tyrosine kinase activity. The joining region segment of chimeric *bcr-abl* protein is composed of a unique combination of *c-abl* and *bcr* amino acids and is expressed only by malignant cells. Immunization of mice with synthetic peptides corresponding to the joining region segment elicited peptide-specific $CD4^+$ class II MHC-restricted T cells. The peptide-specific T cells recognized only the chimeric joining region sequence and could recognize $p210^{bcr\text{-}abl}$ proteins. The response to protein demonstrates that $p210^{bcr\text{-}abl}$ protein can be processed by APC so that the joining region segment is bound to class II MHC molecules in a configuration similar to that of the immunizing peptide, and in a concentration high enough to stimulate peptide-specific T cells. Thus, $p210^{bcr\text{-}abl}$ protein, as well as $p21^{ras}$ represent potential tumor-specific antigens related to the transforming event and shared by multiple individuals with malignancies. Preliminary experiments to elicit human *bcr-abl*-specific T cells by primary *in vitro* immunization will be presented.

NZ 012 PEPTIDE VACCINATION WITH A CYTOTOXIC T CELL EPITOPE DERIVED FROM THE HUMAN PAPILLOMA VIRUS TYPE 16 ONCOGENE E7 CONFERS PROTECTION AGAINST HPV16 INDUCED TUMORS, Mariet C. W. Feltkamp[1,2], Henk L. Smits[2], Michel P. M. Vierboom[1], René P. Minnaar[2], Barteld M. de Jongh[2], Jan Wouter Drijfhout[1], Jan ter Schegget[2], Cornelis J. M. Melief[1], and W. Martin Kast[1], [1]Department of Immunohematology and Bloodbank, University Hospital Leiden, P. O. Box 9600, 2300 RC Leiden, The Netherlands, [2]Department of Virology, Academic Medical Centre, Meibergdreef 15, 1105 AZ Amsterdam, The Netherlands.

Human papilloma viruses (HPV) are considered to be involved in the pathogenesis of congenital cancers. In up to 100% of the cervical carcinomas, HPV-DNA can be detected, of which HPV16 accounts for over 50%. Apart from *in vitro* immortalizing human keratinocytes and transformation of human fibroblasts, HPV16 also immortalizes mouse cells. This allows a detailed study of the immune responses against HPV16 induced tumors in mice and of possible vaccination strategies against HPV16 induced tumors. The possibility of conferring protection against an HPV16 induced tumor was investigated by vaccination with virus-derived peptides able to induce tumor-specific cytotoxic T lymphocytes (CTL). Potential peptide epitopes derived from the viral oncoges HPV16 E6 and E7 were identified by testing a complete set of 240 overlapping synthetic peptides for binding to major histocompatibility complex class I molecules. Peptides of 9 amino acids (aa) length and 8 aa overlap were analyzed for binding to "empty" MHC class I K^b and D^b molecules present on the antigen-processing defective cell line RMA-S. In a set of 30 binding peptides, 5 putative CTL epitopes were identified, one of which is binding to the H-2 D^b molecule with the highest affinity, is located in E7, and is partially overlapping a previously described T helper cell epitope. Immunization of B6 mice ($H\text{-}2^b$) with a peptide comprising these two epitopes or with a peptide comprising the CTL epitope alone, both suspended in an adjuvant, induced CTL memory and resulted in protection against a subsequent challenge (3 weeks later) with HPV16-transformed tumor cells. Furthermore, the CTL response against this CTL epitope measured *in vitro* cross-reacted with the HPV16 induced tumor cell line, indicating that this peptide is naturally processed in HPV16-transformed cells of B6 mice.

NZ 013 *Abstract Withdrawn*

NZ 014 EVIDENCE FOR PEPTIDE - CHAPERONING BY THE ENDOPLASMIC RETICULAR HEAT SHOCK PROTEIN GP96 : IMPLICATIONS FOR VACCINATION AGAINST CANCER AND INFECTIOUS DISEASES, Pramod K Srivastava, Heiichiro Udono, Nathalie E Blachere, Zihai Li, Anna M Feldweg, Robert G Maki and Daniel L Levey, Dept of Pharmacology, Box 1215, Mount Sinai School of Medicine, New York, NY 10029.

Gp96 heat shock proteins (HSPs) elicit immunity specific to the tumors from which they are isolated, but not to antigenically distinct tumors (1). Gp96 isolated from normal tissues does not elicit immunity to tumors tested (2). However, no differences are seen in gp96 cDNA sequences between tumors and normal tissues or among tumors. Specificity of immunogenicity also does not arise from differential glycosylation. In view of the lack of tumor-specific differences inspite of antigenic specificity and in view of the observations that a number of HSPs bind to a wide array of molecules, including peptides, we proposed (3) that *gp96 molecules are not immunogenic per se, but are carriers of antigenic peptides, which associate with them. In view of the predominant localization of gp96 in the endoplasmic reticulum (ER), we further suggested that gp96 acts as peptide-acceptor for peptides transported to ER and may be accessory to peptide-loading of MHC class I molecules.* Our suggestion is supported by the following observations :

- ***Peptides can be eluted from gp96 by acid-stripping.*** Two 7-mer peptides have been characterized by spectroscopy;
- Gp96 contains ATP-binding casettes, *binds ATP in vitro and in vivo and is a Ca++, Mg++ dependent ATPase (4);*
- Apart from eliciting protective tumor immunity, immunization with gp96 elicits class I -restricted CTLs, specifically against the tumor from which it is prepared. This phenomenon is not restricted to tumors. Consistent with our prediction (3), *gp96 isolated from influenza virus-infected cells, SV40 transformed cells and a mouse leukemia can immunize and elicit specific CTLs in each system (5;*
- Addition of gp96 to RMA-S extracts facilitates assembly of MHC class I - β2 microglobulin complexes;
- Similar to other elements involved in presentation by MHC class I, *expression of gp96 is up-regulated by γ-interferon.*

1. Srivastava et al. 1986 Proc Natl Acad Sci USA 83, 3407. **2.**Udono & Srivastava 1992 See abstract this meeting.**3.** Srivastava & Maki 1990 Curr Top Micro and Immun 167, 109. **4.** Li & Srivastava 1992 See abstract this mtg **5.** Blachere 1992 See abstract this meeting.

NZ 015 CTL RECOGNITION OF DNA VIRUS ONCOGENE, Satvir S. Tevethia, Nancy L.Lill, Alison M. Deckhut, Mary J. Tevethia, John Lippolis and Tong- Ming Fu, Department of Microbiology and Immunology, The Pennsylvania State University College of Medicine, Hershey, PA 17033,

Simian virus 40 (SV40), a DNA virus, codes for a 94 kD oncogene, known as large tumor or T antigen. The large T antigen contains all the necessary information to immortalize and transform nonpermissive cells *in vitro* and *in vivo*. The T antigen also induces the generation of MHC class I restricted cytotoxic T lymphocytes (CTL). The recognition of T antigen by CTL clones established from C57BL/6 mice immunized with T antigen is limited to five epitopes in T antigen. The CTL recognition epitopes have been localized to amino acid residues 207-215 (site I), 223-231 (sites II/III), 404-411 (site IV) and 489-497 (site V) in T antigen and were detected by CTL clones Y-1, Y-2, Y-3, Y-4 and Y-5 respectively. CTL clones Y1, Y-2, Y-3 and Y-5 are H-2D^b restricted and the Y-4 CTL clone is H-2K^b restricted. In order to correlate structure-function relationship, epitope loss variants were selected *in vitro* which were resistant to lysis by the site specific CTL clones. However, these variants maintained the expression of MHC class I antigen. Variants resistant to lysis by the CTL clone Y-1 had lost the expression of epitopes I and II/III and variants selected by either CTL clone Y-2 or Y-3 lost the expression of epitopes II/III. Variants generated after selection of SV40 transformed cells with CTL clone Y-4 or Y-5 had lost the expression of epitopes IV or V respectively. Molecular analysis of Y-1 resistant variants identified a deletion in the region of SV40 T antigen which codes for epitopes I, II/III. Point mutations were identified in varaints selected by CTL clones Y-2 and Y-3 at residues 228 and 230 respectively. Point mutations at residues 491 and 496 were identified in variants selected by the CTL clone Y-5. In order to determine the role of residues in the recognition of epitopes by CTL clones, point mutations identified in the epitope loss variants, were reconstructed into wild type T antigen coding sequences by site-directed mutagenesis and the mutant T antigens were expressed in B6 embryo fibroblasts. These cells were used as target cells in the cytotoxicity assay against CTL clones Y-2, Y-3 and Y-5. The results indicated that each amino acid substitution caused the loss of the relevant CTL recognition epitope. The identical amino acid substitutions in the synthetic peptides representing the CTL recognition epitopes II, III and V also resulted in the loss of peptide recognition by site-specific CTL clones. Since variants selected by the CTL clone Y-1 were shown to contain deletions in the region of CTL epitopes I, II/III, point mutants were generated in the coding sequence for site I and residues critical to the recognition by the CTL clone Y-1 have been identified. We conclude that the spontaneous mutations and deletions in the coding region of CTL recognition epitopes in the DNA virus oncogene leads to the escape of transformed cells from immnunosurveillance as the variant cells retain their ability to form tumors in athymic nude mice.

Immune Suppression and Anergy (Joint)

NZ 016 THE ROLE OF CELL DIVISION IN THE INDUCTION OF T CELL ANERGY, Marc K. Jenkins, Julia Johnson, Besty Kearney, Dimuthu R. DeSilva, University of Minnesota, Minneapolis.

Secretion of maximal amounts of IL-2 by CD4+ Th1 clones depends not only on T cell antigen receptor (TCR) signaling but also on non-specific costimulatory signals from antigen-presenting cells (APC). When both signals are provided, for example when dendritic cells present antigen, the T cells undergo multiple rounds of cell division in response to the IL-2 that they produce, returning eventually to a resting state in which they are ready to respond to antigenic stimulation once again. We and others have shown that the T cell surface molecule CD28 transduces a costimulatory signal either when occupied by monoclonal antibodies or by its ligand, the APC surface molecule B7. CD28 crosslinking greatly enhances the amount of IL-2 and other lymphokines produced by T cells stimulated through their TCRs. CD28 signal transduction involves a cyclosporin A-resistant biochemical pathway distinct from the hydrolysis of inositol phospholipids.

If Th1 cells do not proliferate following TCR signaling they become unable to produce IL-2 in response to subsequent antigenic stimulation. Unresponsiveness (also called anergy) results when the TCR is occupied and CD28 signaling does not occur, for example when B7-negative APCs present antigen or when the CD28/B7 interaction is blocked by an uncrosslinked anti-CD28 antibody. Anergy can also be induced when the TCR is occupied and subsequent cell division is inhibited. This occurs when Th1 cells are stimulated with peptide antigen and APC in the presence of anti-IL-2 and anti-IL-2 receptor antibody or agents that inhibit IL-2 responsiveness such as rapamycin, genistein, or PGE_2. Chronic stimulation of Th1 cells with anti-CD3 antibody when APC are present results in IL-2 production, however the capacity of the T cells to proliferate is inhibited by an unknown mechanism. Anergy is also induced under these conditions. Therefore, based on these results the optimal situation for lymphokine production, clonal expansion, and retention of subsequent responsiveness by Th1 cells is transient antigen-presentation by a B7-expressing APC.

Using a differential screening approach we have recently shown that although T cells stimulated with anti-CD3 antibody in the absence of APC produce very little IL-2 mRNA, they do produce large numbers of transcripts that encode macrophage inflammatory protein-1α and another unknown member of the small cytokine family. The accumulation of neither of these transcripts is enhanced by CD28 costimulation. Although the functional significance of these findings is presently unclear it is possible that these cytokines may be involved in the unresponsive state either by preventing T cell responsiveness to IL-2 or by modifying the costimulatory properties of APC.

NZ 017 ROLE OF T CELL TOLERANCE IN THE PERSISTENCE OF HEPATITIS B VIRUS INFECTION, David R. Milich, Toshiyuki Maruyama, Joyce Jones, and Janice Hughes, The Scripps Research Institute, La Jolla.

Infants born to HBeAg-positive HBV carrier mothers invariably become persistently infected. To investigate the role of immunologic tolerance mechanisms in chronic infection of the newborn, we have generated HBeAg-expressing transgenic mice (B10.S/e31). These mice were tolerant to both HBeAg and the nonsecreted HBcAg at the T-cell level. Furthermore, nontransgenic littermates born to HBeAg-expressing mothers showed reduced T-cell responses to HBc/HBe antigens, suggesting that tolerogenic HBeAg may transverse the placenta. Tg mice did not produce antibody to HBeAg but did produce IgM antibodies to HBcAg via a T cell-independent pathway. The coexistence of tolerance to HBc/HBe T cell determinants and production of antibody to HBcAg *in vivo* parallel the immunologic status of neonates born to carrier mothers. The maintenance of T cell tolerance to HBc/HBe antigens required the continued presence of the tolerogen and in the absence of HBeAg persisted for less than 16 weeks. The reversibility of T cell tolerance to HBc/HBe antigens may explain the inverse correlation between age of infection and rates of viral persistence. These observations suggest that a function of the HBeAg may be to induce immunologic tolerance *in utero*. Expression of HBeAg may represent a viral strategy to guarantee persistence subsequent to perinatal infection. Further studies in F_1 hybrid Tg mice (B10 x B10.S/e31) illustrated that "self" tolerance to HBeAg is variable depending on the MHC genotype. A dominant T cell site on HBeAg (p120-131 and I-A^s-restricted) is tolerogenic, whereas a proportion of T cells recognizing p129-140 in the context of I-A^b evade induction of tolerance, persist in the periphery, and can be activated *in vivo* by a single injection of the 12 residue T cell self-peptide. Furthermore, the self-reactive T cells can cooperate with self-reactive, HBeAg-specific B cells to mediate *in vivo* production of autoantibody sufficient to neutralize detection of the autoantigen in serum. This model illustrates that T cells specific for an immunnogenic T-cell site on a nonsequestered autoantigen can escape induction of tolerance (i.e., are not deleted or anergic) and, more importantly, can mediate autoreactivity *in vivo*. These murine studies suggest that chronic HBV (CH-B) carriers may also possess "quiescent" T cells that have evaded tolerance induction. In order to examine the relevance of this murine model, 200 HBeAg-positive CH-B carriers with varying degrees of liver disease (ie. CAH, CPH, ASC) were analyzed with novel and sensitive serological assays capable of detecting serum anti-HBe and anti-HBs antibodies regardless of the simultaneous presence of their respective antigens. All 200 patients were seronegative for anti-HBs and anti-HBe by commercial assay. This analysis revealed: (1) virtually all CH-B patients with liver disease and approximately 50% of CH-B patients without liver disease demonstrated ongoing humoral immune responses specific for HBeAg, HBsAg, and pre-SAg in addition to variable responses to HBcAg; (2) three serologic profiles of CH-B infection were identified; (3) these "silent" immune responses may occur for a number of years prior to liver disease or viral clearance; (4) the quantitative or qualitative characteristics of the immune responses correlate with clinical status; and (5) this array of humoral immune responses appears to be mediated exclusively by HBeAg-specific T helper cells. These results redefine the serology of CH-B infection. The serological data is also consistent with the hypothesis that T cell tolerance to HBeAg plays a role in chronicity, and that HBeAg-specific T cells emerging from the tolerant state mediate liver injury and HBV clearance in CH-B infection.

NZ 018 IMMUNE REGULATION BY T CELL CYTOKINES, Tim Mosmann, Department of Immunology, University of Alberta, Edmonton, Alberta T6G 2H7, Canada.

The immune system can respond to infectious agents by a variety of effector mechanisms, each of which is appropriate for different types of pathogen. The decision between these responses is under stringent regulation, and part of this control is mediated by the cross-regulation of T cell subsets secreting different cytokine patterns. Two major T helper subsets are TH1 and TH2 cells, which differ markedly in the patterns of cytokines that they secrete after antigen stimulation. These patterns are mainly responsible for the different functions of the two subtypes. TH1 cells induce an inflammatory response, including activation of granulocytes and macrophages. In contrast, TH2 cells are excellent helpers for B cell antibody production, and in the absence of a significant Interferon γ response, TH2 cells induce a strong allergic response due to secretion of IL4, IL5 and IL10. Other T cell subtypes also exist, but the functions of these cells, and the interrelationships between them, are less well understood. The functions of TH1 and TH2 cells are often reciprocal, especially in strong immune responses such as occur during many parasite infections. Subset-specific cytokines are involved in this regulation: IL10 inhibits TH1 responses at the level of macrophage activation and indirectly, by inhibiting TH1 cytokine production; IL4 enhances differentiation of TH2 but not TH1 cells; and IFNγ inhibits the proliferation of TH2 cells. We have recently been studying the functions of another TH2-specific cytokine, P600, which was initially characterized as an induction-specific cDNA clone isolated from TH2 cells. We have now expressed recombinant P600 protein and characterized the functions of this cytokine. P600 does not have activity in many of the assays in which TH2-specific cytokines are active. However, P600 enhances the production of an adherent cell population from bone marrow precursors. The resulting cells have high expression of the macrophage markers MAC1 and F4/80. Although these cells are not phagocytic for antibody-coated erythrocytes, they are effective antigen-presenting cells for T cell clones specific for particulate, soluble or alloantigens.

Related Systems: Alloimmunity, Autoimmunity, GVH

NZ 019THE ROLE OF INTERLEUKIN 1 (IL-1) AND IL-1 RECEPTOR ANTAGONIST IN GRAFT-VERSUS-HOST DISEASE

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Graft versus host disease (GVHD) is the major complication of allogeneic bone marrow transplantation. The inflammatory cytokine tumor necrosis factor has been shown to be an important mediator of GVHD in both clinical and experimental transplantation. We have investigated the role of the inflammatory cytokine IL-1 in GVHD using a mouse bone marrow transplantation model. In this model (B10.BR --> CBA) donor and recipient mice are identical at the H-2 locus but they differ at multiple minor histocompatibility loci. We have used this model to analyze cytokine dysregulation in GVHD target organs with a quantitative cytokine MAPPing technique that used competitive PCR. To perform the competitive PCR we constructed a competitive template which could be used to quantitate mRNA transcripts for five different cytokines as well as actin. Using this technique we found that IL-1 mRNA was significantly induced in splenocytes at this time. Surprisingly, IL-2 was not signficiantly increased. The critical role of IL-1 in GVHD was confirmed by the administration of an IL-1 receptor antagonist (IL-1ra) *in vivo*. Injection of IL-1ra either as prophylaxis (day 0 - 10) or as treatment (day 10 - 20) significantly reduced the mortality of GVHD. In addition, the immunosuppression associated with GVHD was also significantly reduced in mice receiving IL-1ra. We conclude that (1) quantitiative cytokine MAPPing is an important technique for the analysis of cytokine dysregulation during allogeneic reactions such as GVHD; (2) GVHD is a systemic inflammatory process in which the monokine IL-1 is a critical effector molecule; and (3) inhibition of IL-1 by IL-1ra offers a novel approach to both the understanding and control of GVHD. We have tested this last conclusion in a Phase I/II dose escalation trial of a seven day constant IV infusion of IL-1ra for patients with steroid-resistant GVHD. All patients had received bone marrow transplants from HLA identical siblings or matched (5 or 6 HLA antigens) unrelated donors at the Children's Hospital or the Brigham and Women's Hospital in Boston. Initial evaluation of IL-1ra in twelve patients shows that it has minimal toxicity, with only one patient experiencng a transient rise in SGOT which resolved completely upon cessation of the drug. No deleterious effects of IL-1ra were observed on peripheral blood counts or peripheral blood progenitors. Approximately fifty percent of patients showed stabilization or improvement of their GVHD, and two patients had complete responses. Phramacokinetic studies showed that steady-state serum concentrations were all within predicted range of values. IL-1ra appears to be both safe and effective at the doses tested and further evaluations of this novel agent are justified in treatment and prophylaxis of GVHD.

Gene Therapy: Transduction of Cytokine Genes into Tumor or Effector Cells

NZ 020 CYTOKINE GENE-MODIFIED TUMOR VACCINES; EFFICACY AND MODE OF ACTION, Eli Gilboa[1], John Connor[2], Rajat Bannerji[1], Shiro Saito[2], Johannes Vieweg[2], Warren Heston[2], William Fair[2], [1]Program in Molecular Biology and [2]Department of Urology, Memorial Sloan-Kettering Cancer Center, New York, New York.

We have previously shown that injection of IL-2 or IFN-gamma gene-modified fibrosarcoma (CMS-5) cells into BALB/c mice prevented the growth of parental tumor cells. In the current study we have explored the utility of cytokine gene-modified tumor vaccines using another mouse tumor model, MBT-2, which constitutes an excellent model for human bladder cancer. To approximate the animal model as closely as possible to its human counterpart, irradiated tumor cells were used for immunization, and a surgical procedure was used to implant orthotopically the tumor challenge into the bladder wall of the mouse. The main findings from this study were that IL-2, and to a lesser extent, IFN-gamma gene-modified MBT-2 cells were capable of curing mice from a significant burden of a preexisting primary tumor and its metastasis, and that mice cured from their existing tumor were resistant to a subsequent challenge with parental tumor cells. Preliminary results from studies using a rat model for prostate cancer will also be discussed.

In-vitro cytotoxicity assays, in-vivo depletion experiments, and histological analysis at the site of tumor challenge have shown that cytotoxic T cells (CTL) are the main effector arm responsible for the eradication of tumor cells. Immunohistological analysis has shown, unexpectedly, that NK cells or macrophages are the predominant cell type infiltrating in response to secretion of IL-2 or IFN-gamma, respectively, and that T cells are absent from the site of immunization with the cytokine-secreting tumor cells. A model for the induction of antitumor CTL responses consistent with the absence of T cells at the site of immunization will be presented and its implications for the establishment of peripheral tolerance, as well as the controversial issue of immunogenicity of tumors, will be discussed.

NZ 021 DISSECTION OF THE IMMUNE RESPONSE INDUCED BY TUMORS ENGINEERED TO SECRETE LYMPHOKINES

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There is mounting evidence that many if not all tumors possess antigens capable of being recognized by T cells. A number of molecular strategies aimed at enhancing the immunogenicity of tumors have strengthened the hypothesis that the failure of an adequate anti-tumor immune response may be due to defects in immune regulation rather than an absence of tumor specific antigens and therefore an absence of tumor specific T cells. Over the past few years, our group has been analyzing the immune response induced by immunization with tumors engineered to secrete cytokines locally. Immunization of animals bearing small amounts of established renal cell carcinoma were in fact able to be cured *of their disease by* the systemic immune response induced by syngeneic tumor cells transduced with a murine Interleukin-4 gene. More recently, in collaboration with Richard Mulligan's laboratory, we have evaluated the immune responses generated by tumors transduced with retroviral vectors carrying the GM-CSF gene. In this model of paracrine cytokine production, GM-CSF stood out as the most effective lymphokine in inducing systemic immune responses against the poorly immunogenic F10 variant of B16 melanoma. Hystologic analysis of the inflammatory infiltrate within the GM-CSF transduced tumor cells revealed a large number of activated macrophage-like cells together with a moderate number of granulocytes. The systemic immune response generated by immunization with GM-CSF transduced tumor cells was found to be dependent both on CD4+ T cells and CD8+ T cells. Furthermore, PCR analysis revealed a burst of endogenous IL-2 and IL-4 synthesis by T cells in draining lymph nodes at day seven after immunization. Furthermore, despite the fact that CD8+ tumor specific T cells were generated by this immunization scheme, the rejection of MHC class I+ challenge tumors did not require that the immunizing tumor express MHC class 1 molecules on its surface. Taken together, these findings suggest that immunization with GM-CSF transduced tumor cells alters the presentation of tumor specific antigens such that powerful antigen presenting cells are brought to the tumor site which process and present both MHC class I and MHC class II-restricted antigens. Generation of CD8+ tumor specific T cells by this immunization strategy has allowed us to analyze the complexity of tumor specific peptides recognized by the CTL population. In one colon tumor there was a single predominant tumor specific peptide identified. In the B16 melanoma system, three peaks of peptide activity were identified. Upon dilution, one immunodominant peptide could be discerned among the three specific tumor peptides. Implications of these analyses for the development of strategies for human cancer immunotherapy will be discussed.

Activation and Function of MHC-Unrestricted Effector Cells

NZ 022 CD28/B7 CO-STIMULATION OF HUMAN T CELLS. L.L. Lanier, J.H. Phillips, M. Cayabyab. and M. Azuma. DNAX Research Institute for Molecular and Cellular Biology, Inc., Department of Immunology, 901 California Avenue, Palo Alto, California 94304

Interaction between CD28, a membrane antigen expressed on T lymphocytes, and B7, an activation antigen present on activated B cells, macrophages, and dendritic cells results in augmentation of T cell activation initiated via the CD3/TcR complex. CD28/B7 co-stimulation enables small, resting human peripheral blood T cells to mediate anti-CD3 re-directed lysis against murine target cells transfected with human B7. We now demonstrate that cytotoxicity is mediated by pre-existing cytotoxic effectors within the small, resting "memory" T cell population and by the de novo generation of additional CTL within both the "memory" and "virgin" T subsets. This conclusion is based on analysis of the kinetics of the response and the effects of metabolic inhibitors on the generation of CTL function. "Memory" CD45RO+ T cells demonstrated cytotoxicity within 4 hr of co-culture with anti-CD3 mAb and B7+ P815 cells and cytolysis was only partially prevented by inhibitors of protein synthesis. By contrast, "virgin" CD45RO- T cells demonstrated anti-CD3 induced lysis against B7+ P815 targets only after 6 hr or 8 hr of co-culture and cytotoxicity was completely prevented by inhibiting protein synthesis. Induction of cytotoxicity was B7-dependent in that parental P815 cells and P815 cells transfected with CD72 and VCAM-1, ligands for T cell-associated membrane receptors CD5 and VLA-4 respectively, did not initiate cytotoxicity. Our studies further revealed cooperation between the CD28 / B7 and LFA-1 / ICAM pathways in the generation of CTL from small, resting T cells. However, after CTL generation, the CD28 - B7 interaction was not required for cytotoxic effector cell function. These observations may have important physiological implications since this would permit activated CTL to lyse targets in vivo that do not express B7, after the CTL were generated by antigen-presenting cells that do express B7 or possibly other co-stimulatory molecules.

Vaccine Design and Strategies (Joint)

NZ 023 SYNTHETIC PEPTIDE STRATEGIES IN THE INDUCTION AND ANALYSIS OF T-CELL RESPONSES TO HIV AND TUMORS, Jay A. Berzofsky, Molecular Immunogenetics and Vaccine Research Section, Metabolism Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892 USA.

Recent advances in the understanding of antigen processing and presentation facilitate the identification of antigenic sites recognized by $CD4^+$ helper T cells and by $CD8^+$ cytotoxic T lymphocytes (CTL), and suggest methods to optimize the activity of these for construction of synthetic peptide vaccines aimed at eliciting T-cell immunity. We have characterized the functional role of each residue in an HIV envelope peptide recognized by helper T cells from mice and humans. Only a few of the amino acid residues were necessary for binding to class II major histocompatibility complex (MHC) molecules and immunogenicity, as shown using a peptide consisting of only four of the original residues, with the rest replace by Ala. The other residues were more remarkable for the negative impact they could have. Several of the non-critical residues could play a deleterious role if replaced with the wrong substitution, and removal of one negative charge in the natural sequence led to a peptide 100-fold more potent for stimulating specific T cells and for binding to MHC molecules, emphasizing the importance of adverse interactions in peptide-MHC binding. Similarly for a CTL site presented by multiple murine and human class I MHC molecules, CTL specificity was shown to focus on the distinction between aromatic and aliphatic side chains at one position. This result, in turn, allowed the construction of chimeric peptides that induced broadly crossreactive CTL responses for multiple strains of HIV-1, and that may reduce the risk of outgrowth of escape mutants. Thus, understanding the molecular basis for MHC binding and T-cell recognition can facilitate improvements in synthetic vaccines beyond the use of just natural peptide sequences from the pathogen.

Another problem in the design of a synthetic vaccine is the polymorphism of the MHC, leading to differential recognition of different antigenic determinants of the same protein by individuals of different MHC types. One approach to overcome this problem that we have used for helper T-cells specific for the HIV-1 envelope protein was to identify multideterminant regions encompassing several overlapping determinants presented by different MHC molecules. Synthetic peptides spanning such multideterminant regions elicited T cell responses in multiple strains of mice and in humans of multiple HLA types. An alternative approach is to identify truly promiscuously recognized antigenic determinants. We have found one HIV CTL determinant to be presented by four different class I MHC molecules in mice and by at least two different class I molecules in humans. At least in mice, the same minimal core 10-residue sequence is presented by all four class I molecules. These antigenic sequences can then be coupled to produce a synthetic vaccine eliciting neutralizing antibodies and CTL in animals (or humans) of multiple MHC types. Thus, analysis of the T-cell responses to viral proteins can contribute to the rational design of new vaccines. Similar approaches can be applied to tumor antigens recognized by T cells, including mutant oncogene products and viral oncogene products as potential targets for vaccine prophylaxis and immunotherapy of cancer.

NZ 024 POOL SEQUENCING FOR DETERMINING MHC PEPTIDE MOTIFS

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MHC molecules are peptide receptors of particular specificity. Many MHC class I molecules, for example, require nonapeptides with two positions conserved, whereas the other positions can be occupied by various amino acid residues. This information can relatively easily be obtained by immunoprecipitation of the MHC molecule of interest, elution of the associated peptides, and sequencing of those peptides all together as a pool. This laboratory has used that approach for several MHC molecules, including H-2 K^d, L^d, D^d, K^b, D^b, K^k, K^{kml}; Qa-2; HLA-A2 (*0201 and *0205), A11, A31, B35, B7, B37, B27 (*2705; *2702), Cw7, Cw4, Cw6, DR 1, and DR 5. The results indicate the use of pool sequencing for the purpose of obtaining information on MHC-peptide motifs, including the analysis of class II ligands. Several interesting aspects have been noted and will be discussed; for example, Qa-2 molecules are peptide receptors of higher stringency than ordinary class I molecules, and A11 molecules obviously can accommodate peptides from 8 to 11 residues, always with a charged C-terminus. For determining class II peptide motifs, a useful way appears to be to use both pool sequences as well as a few individual peptides. Participation in this work of the following persons is acknowledged: M. Takiguchi, D. Schendel, J. Strominger, M. Soloski, E. Weiss (contribution of cell lines); S. Stevanovic, G. Jung (peptide sequencing).

Cell Mediated Immunotherapy: Animal Models

NZ 025 ANTIGEN-PULSED DENDRITIC CELLS ARE POTENT STIMULATORS OF TUMOR-SPECIFIC $CD4^+$ T CELLS,
Peter A. Cohen, Steven A. Rosenberg, and James J. Mulé, Surgery Branch, National Cancer Institute, Bethesda, MD.

Fresh immune CD4+ T cells can mediate potent antitumor effects in mice upon adoptive transfer. It was previously impossible, however, to grow murine anti-tumor $CD4^+$ T cells in culture except when putative antigens such as tumor-associated viruses were available to stimulate cultures. Such antigens are unavailable for the great majority of murine as well as human tumors. We have overcome this obstacle using syngeneic spleen dendritic cells (DC) to present crude tumor antigen to $CD4^+$ T cells. Snap freeze-thawed tumor cells were used to pulse fresh DC overnight; then the latter are cocultured with immunoaffinity column-purified $CD4^+$ T cells freshly harvested from either tumor-hyperimmunized or tumor-bearing mice.

Splenocytes from C57BL/6 mice hyperimmunized to weakly immunogenic, syngeneic sarcomas such as MC-203 and 207 demonstrated highly specific $CD4^+$ T cell proliferation when tested in five day ^3H-thymidine proliferation assays. Crossreactive proliferation was seen to fresh digests of other syngeneic tumors, but this proved to be a result of immunization to the bacterial collagenase added during tumor digestion. When tumor digests were cultured in vitro to remove the collagenase, then harvested as a source of tumor antigen, there was complete elimination of crossreactive $CD4^+$ proliferation both to non-sarcomatous syngeneic tumors such as 3LL and B16, and to other syngeneic sarcomata.

Although capable of initiating highly specific proliferation of anti-tumor $CD4^+$ T cells in vitro, tumor-pulsed DC were by themselves inadequate to sustain longterm $CD4^+$ T cell growth. Addition of a single dose of rhIL-7 (100 ng/ml) 36 hours after culture initiation resulted in sustained $CD4^+$ T cell proliferation over 2-3 weeks (3-8 fold expansion) without addition of exogenous IL-2. $CD4^+$ cultures could be restimulated repeatedly or rested for weeks without irradiated feeder cells. Restimulated $CD4^+$ T cells themselves secreted IL-2 and γ-INF (30-50 units/ml/72 hrs and 120-200 units/ml/24 hrs, respectively), and could be grown in the presence of exogenous γ-INF, properties suggestive of TH1 $CD4^+$ cells.

Anti-tumor $CD4^+$ T cells could also be raised from the draining lymph nodes of sarcoma-bearing mice. IL-7 was essential to demonstrate initial proliferation in this instance. The added use of TGFβ-1 conferred markedly greater specificity of proliferation

We conclude that the use of dendritic cells and IL-7 enables the successful propagation of murine anti-tumor $CD4^+$ T cells in vitro. Adoptive therapy experiments utilizing these cultured cells are in progress. We are applying similar techniques to grow $CD4^+$ cells T from patients with cancer.

NZ 026 CYTOKINE-INDUCED TUMOR IMMUNOGENICITY: FROM EXOGENOUS CYTOKINES TO GENE THERAPY. Guido Forni[1], Federica Cavallo[2], Federica Pericle[2], Francesco Di Pierro[2], Mirella Giovarelli[2], Alberto Gulino[3], Alessandra Vacca[3], Mario P. Colombo[4], Giuliana Ferrari[5], Andrea Modesti[6], Piero Musiani[6], Antonella Stoppacciaro[7], Pier Luigi Lollini[8], Patrizia Nanni[8], Giordano Nicoletti[8], Carla De Giovanni[8], Maria Carla Bosco[9], Howard Young[9], [1]CNR Center of Immunogenetics and Histocompatibility, 10126-Turin, [2]University of Turin, [3]University of L'Aquila, [4]Istituto Nazionale Tumori, Milan, [5]Istituto Scientifico S. Raffaele, Milan, [6]University of Chieti, [7]University of Rome, [8]University of Bologna, Italy, and [9]NCI-Frederick, MD.

The majority of spontaneous tumors are not able to induce a significant immune response. This inability rests mainly on poor presentation of tumor associated antigens (TAA) by the very few MHC molecules expressed by tumor cells, absence of adhesion molecules, TAA inefficient indirect presentation by professional antigen presenting cells, absence of the appropriate cytokines, and the rise of suppressor activities.

The use of cytokines is now becoming a simple way to try to increase tumor immunogenicity. Repeated local administration of low amount of exogenous cytokines around the tumor growth area is a procedure that can be easily undertaken with both mice and tumor patients. The goals are to first activate a reaction strong enough to affect tumor progression, and eventually elicit a systemic, TAA-specific immune memory. In syngeneic mice challenged shortly before with the minimal 100% tumor inducing dose of an apparently non-immunogenic spontaneous adenocarcinoma (TS/A) of the BALB/c strain, as little as 10 U IL-2, 12-300 IU IFN-γ, 10 pg of IL-4 or 1-10 pg of IL-1β injected daily for 10 days lead to tumor rejection in a significant number of mice. In clinical practice, infiltration of IL-2 around lymph nodes draining small head and neck squamous cell carcinomas or in the tumor mass induces a substantial number of complete or partial responses. By contrast, the insertion of an cytokine gene in tumors requires the *in vitro* growth of tumor cells, insertion of the gene, selection of transduced cells, and evaluation of their tumorigenicity. Non-tumorigenic cells releasing appropriate amounts of cytokines can then be used for the treatment of mice and eventually of tumor patients. The primary goal is to elicit a systemic immune memory. Its eventual quick establishment could also affect the growth of already existing tumors. Several data show that cytokine secreting neoplastic cells are no longer tumorigenic, but induce a protective immune response against the parental tumor. These data confirm those we have observed after injecting exogenous cytokines, and emphasize the concept that the local presence of cytokines transforms a tumor unable to elicit an immune response into a signal able to activate a response that is prompt enough to overcome tumor ability to grow.

To evaluate the immunogenic potential of cytokine releasing neoplastic cells, we are currently using expression vectors to introduce the cDNA coding for murine cytokines (IL-1β, IL-2, IFN-γ, IL-4, IL-6, IL-7, and IL-10) into the cells of the apparently non-immunogenic TS/A adenocarcinoma. Rejection of IL-2-releasing TS/A cells depends on neutrophils infiltration, the intensity of which is directly proportional to the amount of IL-2 released. $CD4^+$ T-lymphocytes are ininfluential, while $CD8^+$ lymphocytes play only a minor role. This neutrophil-dominated rejection leaves a long-lasting, tumor-specific, T-lymphocyte mediated immune memory. For its induction, $CD4^+$ lymphocytes are required. Their specific activation appears to depend on both the amount of IL-2 released and the granulocyte mediated-reaction that may lead to a more efficient presentation of tumor antigens. The rejection of IL-4 releasing cells depends on eosinophils and CD8+ lymphocytes, while CD4+ lymphocytes are ininfluential. It elicits a very efficient tumor-specific immune memory that is more powerful than that induced by IL-2-releasing cells. With both IL-2 and IL-4 the efficient induction of a tumor-specific immune memory rests on T-lymphocytes, the number of transfected tumor cells used, and the amount and the combination of cytokine released.

NZ 027 IL-1 ENHANCEMENT OF CHEMOTHERAPEUTIC EFFICACY IN MURINE TUMOR MODEL SYSTEMS, Candace S. Johnson[1], Ming-Jei Chang[1], Wei-Dong Yu[1], Ruth A. Modzelewski[1], Daniel R. Vlock[2], Merrill J. Egorin[3], Leonard Reyno[3], and Philip Furmanski[4], [1]University of Pittsburgh, Pittsburgh, PA 15213, [2]Brigham and Women's Hospital, Boston, MA 02115, [3]University of Maryland, Baltimore, MD 21201 and [4]New York University, New York, NY 10003.

Biologic response modifiers (BRMs) participate in a wide variety of regulatory functions including effects on immune function, stimulation of hematopoiesis, direct tumor cell cytostatic or cytotoxic activities, protection of hematopoietic stem cells, effects on vascular function and enhancement of cytotoxic drug-mediated anti-tumor responses. Interleukin-1 (IL-1), a multifunctional BRM, may offer the potential to exploit these attributes towards therapeutic design. We have demonstrated that IL-1 has acute anti-tumor activity in vivo, characterized by hemorrhagic necrosis, microvascular injury, enhanced clonogenic tumor cell kill and a decrease in tumor blood flow. IL-1's acute anti-tumor activities can be significantly suppressed by glucocorticoids, are not the result of a direct tumor effect, appear to be T cell independent, and are not mediated through TNF. While significant tumor cell kill is observed in vivo, IL-1 hemorrhage does not result in sustained inhibition of tumor cell growth. However, when IL-1 is combined with bioreductive agents such as mitomycin C and porfiromycin, enhanced anti-tumor activity is observed in vivo when compared to that observed with either agent alone. We focus our discussion here on IL-1's ability to enhance the efficacy of the platinum agents, cisplatin (cDDP) and carboplatin (CBDCA) in the RIF-1 tumor system in C3H/HeJ mice. In vitro, IL-1 had no effect on either cDDP or CBDCA mediated tumor cell kill. To determine IL-1's in vivo effect, excision clonogenic tumor cell assays were performed using tumor-bearing animals that were treated and tumor removed 24 hr later. A significant increase in dose-dependent clonogenic tumor cell kill was observed with IL-1/cDDP as well as IL-1/CBDCA when compared to drug alone and at the lowest doses tested. Using median-dose effect analysis, the interaction between IL-1 and cDDP was found to be strongly synergistic. To examine whether the mechanism of this enhancement could be explained by IL-1's profound effect on the vasculature, platinum levels were determined in tumor, plasma and normal tissue. When drugs were given concurrently, IL-1 had no effect on platinum levels in both normal and tumor tissue from animals treated with cDDP whereas a significant increase was observed in tumor platinum levels from animals treated with IL-1/CBDCA when compared to CBDCA alone. These differences in cDDP versus CBDCA may be due to differences in the pharmacokinetic behavior of the two drugs. In addition, tumor cell kill appeared time-dependent with maximum activity observed when IL-1 was administered 4-6 hr before CBDCA. Studies are in progress to examine the potential mechanisms of these effects and form the basis for further investigation on the use of IL-1 in combination with cytotoxic drugs for the therapy of solid malignancies. Supported by NIH grant CA48077.

NZ 028 SPECIFIC IMMUNE T CELLS GENERATED FROM TUMOR-DRAINING LYMPH NODES BY ACTIVATION WITH BACTERIAL SUPERANTIGENIC TOXINS, Suyu Shu[1], Robert Krnock[1], Takafumi Matsumura[1], Jeffrey Sussman[1], Bernard Fox[1], Scott Strome[1], John Krauss[2], Alfred Chang[1], and David Terman[3], [1]Departments of Surgery and [2]Internal Medicine, University of Michigan, Ann Arbor, MI 48109, and [3]Iatros Corporation, Carmel, CA 93921.

An essential requirement for developing successful clinical adoptive immunotherapy of cancer is the identification of potent immune effector cells in cancer patients. In animal studies, we have found that lymph nodes (LN) draining a progressively growing tumor contain tumor-sensitized but functionally deficient T lymphocytes. These cells, termed "pre-effector", can be stimulated in vitro with irradiated tumor cells in the presence of interleukin-2 (IL-2) to differentiate into mature immune effector cells which upon adoptive transfer, mediate the regression of established metastases. Recently, we have identified another in vitro culture system with which draining LN cells are activated with anti-CD3 monoclonal antibodies (mAb) followed by culture in IL-2. Although anti-CD3 interaction with T cells is polyclonal, the activated cells mediate immunologically specific effects and the specificity is determined by the tumor which stimulates the draining LN. Since normal LN cells proliferate equally well in response to the anti-CD3/IL-2 activation, albeit therapeutically ineffective, the functional immune cells in the activated draining LN cells must represent a small fraction of the entire population and most cells are probably not contributing to the antitumor effects.

In an attempt to selectively activate tumor-sensitized pre-effector cells, we have examined the effects of stimulating draining LN cells with microbial superantigenic toxins. This group of toxins is a family of small proteins (20-30KDa) which bind the MHC class II molecules on the antigen presenting cells and provide a triggering signal to T cells. One striking feature of the superantigens is their interactions with T cell receptors of distinct Vβ regions regardless of other variable components. The use of these bacterial toxins for stimulation of LN cells may selectively activate and expand distinct Vβ T cells that may otherwise represent small fractions of the total T cells. Three toxins including staphylococcal enterotoxins A (SEA), B(SEB) and streptococcal pyrogenic exotoxin type A (SPE A) were examined. LN cells draining the MCA 205 sarcoma were stimulated with the toxins followed by culture in low dose (4 U/ml) of IL-2. Proliferation assays revealed that LN T cells were optimally stimulated by 0.002, 2 and 1 μg/ml of SEA, SEB and SPE A, respectively. Flow cytometric analyses with fifteen available Vβ specific mAb indicated that each toxin expanded distinct Vβ bearing T cells with Vβ3 and 11 for SEA, Vβ3, 8.1, 8.2 and 8.3 for SEB, and Vβ8.2 and 11 for SPE A.

The antitumor reactivity of toxin-stimulated tumor-draining LN cells was tested for in vivo therapeutic efficacy. Pulmonary metastases were initiated in syngeneic mice by i.v. inoculation of MCA 205 tumor cells. Three days later, mice were treated by i.v. infusion of activated cells. The therapeutic efficacy was evident by the reduction of numbers of metastases evaluated 3 weeks after tumor inoculation. The adoptive immunotherapy experiments revealed that SEB and SPE A-stimulated cells mediated antitumor effects. On a per cell basis, SPE A-activated cells appeared to be more potent than SEB-activated cells. In spite of being the strongest T cell activator, SEA-stimulated cells demonstrated minimal antitumor reactivity. With the use of another antigenically distinct sarcoma, MCA 207, we found that tumor regression mediated by either SEB or SPE A activated cells was immunologically specific toward the tumor that stimulated the draining LN. Although effective in vivo, the SEB and SPE A stimulated cells failed to mediate in vitro cytotoxicity in a 4-h ^{51}Cr-release assay. These results demonstrate that the interactions of bacterial superantigens with tumor-sensitized pre-effector cells could result in differentiation and generation of specific immune effector cells. Our results also suggest that T cells bearing Vβ8 might be more preferentially responding to a progressively growing tumor than T cells bearing Vβ3 or 11.

Cell Mediated Immunotherapy: Clinical Trials

NZ 029 RECONSTITUTION OF VIRAL IMMUNITY IN IMMUNOCOMPROMISED HUMANS BY THE ADOPTIVE TRANSFER OF T CELL CLONES. Phil Greenberg, Käthe Watanabe, Mark Gilbert, Brad Nelson, and Stan Riddell, University of Washington and Fred Hutchinson Cancer Research Center, Seattle, WA 98195.

Restoring or augmenting immunity by the adoptive transfer of *in vitro* cultured antigen-specific T cells has proven therapeutically effective for viral infections and tumors in animal models. Our laboratory has applied insights from studies in these animal models to the treatment of human viral disease in immunocompromised hosts. This clinical setting was selected since the disease occurs as a consequence of an inadequate T cell response, the antigenic target for the T cell response can be identified, T cell clones specific for the antigen can be generated, and the persistence and efficacy of transferred T cell clones can be monitored. Thus, principles defined in this experimental context should promote the development of analogous approaches for the treatment of malignant diseases. Our initial studies have focused on reconstitution of CMV-specific host CD8+ cytotoxic T cell responses in CMV-seropositive patients immunosuppressed by the preparative regimen given prior to bone marrow transplantation (BMT) from an HLA-matched sibling. The essential role of CD8+ Tc for protection from CMV disease is supported by murine studies and correlative human studies. Five patients have been entered on our initial adoptive T cell therapy protocol. CD8+ CMV-specific T cell clones were derived from HLA-matched seropositive sibling bone marrow donors and infused in escalating cell doses over 4 consecutive weeks. Infusion of >2.2 x 10^9 CD8+ CMV-specific T cell clones was non-toxic and performed safely in an out-patient setting. Transfer of immunity was detectable following infusion of even the lowest cell dose (3.3 x 10^7 T cells/m^2) and responses greater than those present in healthy donors with protective immunity were detectable following transfer of higher cell doses. Transferred CD8+ Tc immunity was detectable for >6 weeks, and long-term persistence of transferred clones is being analyzed by PCR using amplimers specific for the TCR of the transferred clones. None of the patients who received adoptive T cell therapy developed subsequent evidence of CMV disease.

Our laboratory is now evaluating methods to improve the efficacy and safety of adoptive T cell transfer by the introduction of genes into T cell clones. To improve safety, a retroviral vector containing an inducible suicide gene has been constructed by Steve Lupton and Bob Overell (Targeted Genetics Corporation). The herpes virus thymidine kinase (TK) gene has been fused in frame with the *hph* gene, resulting in a gene encoding a single bifunctional protein conferring hygromycin resistance and sensitivity to ganciclovir. Preclinical studies have demonstrated that T cell clones expressing this gene can be readily killed *in vitro* and *in vivo* by ganciclovir. A clinical trial including adoptive T cell therapy is now underway in HIV seropositive patients undergoing allogeneic BMT for the treatment of HIV-related lymphomas. These patients are receiving high-dose chemoradiotherapy pretransplant to eliminate the lymphoma cells and ablate their lymphohematopoietic system, which represents the major reservoir of HIV-infected cells, and are receiving AZT drug therapy and adoptively-transferred TK-modified CD8+ T cell clones post-transplant in an effort to prevent or minimize infection of donor bone marrow derived cells. If patients develop toxicity at sites of infection potentially related to transfer of the T cell clones, such as encephalitis or pulmonary alveolitis, ganciclovir will be administered. The persistence and/or elimination of the transferred T cells will be monitored by PCR.

The therapeutic efficacy of transferred CD8+ T cell clones in murine models is limited by the inability of the clones to proliferate and survive long-term *in vivo* in the absence of either exogenous IL-2 or a concurrent CD4+ helper T cell response. Therefore, we are attempting to modify CD8+ T cells to render them independent of exogenous growth factors and capable of proliferating in response to TCR ligation. Several types of gene constructs are being evaluated. The first is designed to provide the additional signals necessary to result in endogenous IL-2 production following T cell activation. Based on previous studies, CD8+ T cell clones were transduced with a vector containing the gene for the IL-1 receptor. Such T cells proliferate in response to binding of ligands to both the TCR and IL-1R, but fail to proliferate in response to either signal alone. A second type of construct involves the construction of a hybrid gene, in which an inserted IL-2 gene is under control of a promoter normally activated by TCR ligation. Preliminary studies are being performed with a vector containing the IFN-γ promoter driving an IL-2 cDNA. Finally, a third type of construct containing chimeric cytokine receptors potentially capable of providing an autocrine loop and delivering the signal normally provided by the binding of IL-2 to its receptor is being evaluated.

NZ 030 LIPOSOME-ENCAPSULATED MTP-PE: A NOVEL BIOLOGIC FOR CANCER THERAPY, Eugenie S. Kleinerman, Takeshi Asano, Jacalyn B. Gano, Dennis Johnston, Robert S. Benjamin, and Norman Jaffe, The University of Texas, M. D. Anderson Cancer Center, Houston, Texas, 77030

MTP-PE is a synthetic lipophilic analog of muramyl dipeptide, the minimal component of mycobacterium capable of stimulating immune function. Liposome-encapsulated MTP-PE (L-MTP-PE) was designed to target the immune modulator to monocytes and macrophages. Human monocytes/macrophages phagocytize L-MTP-PE with subsequent upregulation of IL-1α, IL-1β, IL-6, and TNF genes and with the production and secretion of these cytokines. L-MTP-PE-activated macrophages kill tumor but not normal cells *in vitro*. Following the i.v. infusion of L-MTP-PE to cancer patients in a Phase I study, uptake was demonstrated in liver, spleen, lung, and in and around lung metastases. Extensive preclinical data demonstrated that L-MTP-PE is only effective against microscopic but not bulk disease. We therefore sought to design a phase II clinical trial in which L-MTP-PE could be administered to patients with minimal residual disease in an area where drug uptake could be achieved.

Relapsed osteosarcoma in the lung presented such a disease setting. These patients have a short disease-free interval (DFI) following the surgical excision of all visible and palpable lung metastases. Eighty percent of patients will relapse within one year. Post-operative chemotherapy has done little to alter the disease course. We investigated if L-MTP-PE therapy could improve the DFI in this high risk group of patients.

All patients that entered this study had histologically proven osteosarcoma and pulmonary metastases that developed during adjuvant chemotherapy or that were present at diagnosis and did not respond to chemotherapy. Patients received either a 12 or 24 week course of L-MTP-PE after the surgical removal of all metastases. Induction of circulating TNF, IL-6, neopterin, and C-reactive protein was demonstrated following L-MTP-PE infusion. Disease-free intervals were calculated from the day of surgery to the day of relapse in each group and compared to the disease-free interval of a historical control group. There was a significant prolongation in time to relapse in those patients receiving 24 weeks ($p < 0.03$) but not 12 weeks of L-MTP-PE. The median time to relapse for the 24 week treatment group was 9.0 months compared to 4.5 months for the control group. Histologic changes in the pulmonary tumors were demonstrated in 6 patients in the 12 week treatment group. Peripheral fibrosis with inflammatory macrophage infiltration into the lesions were the key changes observed. These data indicate that L-MTP-PE is an active agent in osteosarcoma which deserves further investigation in a more appropriate adjuvant setting. The data also suggest that while 24 weeks of L-MTP-PE was superior to 12 weeks, a more prolonged administration of the drug (*i.e.* 1 year) may prove to be even more effective.

Late Abstracts

ENDOTHELIAL CELL SURFACE ADHESION MOLECULES IN TUMOR METASTASIS,
Michael P. Bevilacqua, M.D., Ph.D., Howard Hughes Medical Institute, University of California, San Diego

Vascular endothelium can express several cell surface molecules that support the adhesion and extravasation of blood leukocytes. These molecules play critical roles in the development of inflammatory and immunological processes. Unfortunately, blood-borne tumor cells may also be able to utilize these adhesion molecules. My colleagues and I have demonstrated that human melanoma and osteosarcoma cells can bind to activated vascular endothelium through an interaction of tumor cell surface $\alpha 4\beta 1$ integrins and endothelial cell surface VCAM-1/INCAM-110. In addition, colon cancers can bind endothelial E-selectin, an interaction that appears to depend on tumor cell expression of specific carbohydrates, some of which are related to the tetrasaccharide sialyl-Lewis x (sLe^x). *In vivo* models demonstrate that infusion of cytokines can dramatically increase the development of metastatic tumor nodules. Reagents which block the specific cell adhesion mechanisms diminish these experimental events.

Modulation of murine reactivity to tumor and transplantation antigens by cytokine gene therapy. Michael T. Lotze, Hideaki Tahara, Barbara Pippin, Quan Cai, Elaine Elder, William Jacob*, Yawen Chen*, Jill Siegfried, Paul Strumpf, Sally Carty, Paul Robbins and Walter Storkus. From the Departments of Surgery, Molecular Genetics and Biochemistry, and Pharmacology, University of Pittsburgh, Pittsburgh, PA. 15261 and *Genetic Therapy Incorporated Gaithersburg, MD. 20878.

We, like others, have developed retroviral vectors to introduce cytokine genes to elicit a local, and nominally a systemic, immune response to tumor by incorporating them into vaccines (IL-2, IL-4 and IL-12). We have also created retroviral vectors to introduce immunosuppressive cytokines (IL1Rα, TGF-β, vIL-10) into cellular transplants such as islet cells so that local immunosuppression may be attained without the debilitating effects of systemic immunosuppression.

Local production of IL-2 delivered by transfected Bl6 melanoma cells leads to reduction of tumor size at 21d from 2200 +/- 106 to 34 +/-10mm^2. Similar reduction to 226 +\- 94mm^2 can be attained by delivering PEG-IL-2 at the site of tumor. This approach can totally eliminate the local growth of tumor by providing 3×10^5 IU of PEG IL-2 with a 10^6 tumor cell inoculum. Delivery of IL-2 by transfection cells decreases pulmonary metastases from a mean of 200 to <10 on day 23 following injection of 10^5 tumor cells. Local production of either IL-4 or IL-12 by retroviral vectors that we have created leads to delayed tumor growth in murine models. We can routinely introduce the IL-4 gene using these vectors into cultured human fibroblasts as well as cultured human tumors (melanoma, lung carcinoma) and shown production of as much as 10^4-10^5units/10^6cells/24hrs, even after irradiation with 5000 rads. We have recently received approval from the Recombinant DNA Advisory Committee to deliver IL-4 using irradiated transfected autologous cultured fibroblasts mixed with noncultured autologous tumor as part of a vaccine. We plan to biopsy these sites of vaccination to 1) demonstrate continuous production of the cytokine, 2) evaluate endothelial activation mediated by IL-4, 3) determine presence of T cell infiltrate and specific TCR usage and 4) demonstrate specific T cell response to tumor.

Interleukin-12 (IL-12), a potential candidate for cancer therapy, is a disulfide-linked heterodimeric cytokine composed of light (p35) and heavy (p40) chains and requires the expression of both subunits to obtain biological activity. We (Nastala C) and others (M. Brunda/M. Gately) have shown that IL-12 systemic delivery leads to markedly delayed growth of various murine tumors and increase in survival. Similar findings have been observed with delivery of IL-12 by transfected fibroblasts *in vivo* to test tumor establishment of BL6, a poorly immunogenic murine melanoma cell line. These tumors were significantly delayed from growing in experiments using 1×10^6 tumor inoculum with 5×10^5 fibroblasts (NIH3T3 cells) which expressed 150 U/24 hr/5×10^5 cells of IL-12. We have constructed retroviral vectors that can express either of the two chains of IL-12 (MFG-p35 and MFG-p40) or both genes with an internal ribosome entry site sequence of the encephalomyocarditis virus (DFG-MIL-12). We have recently demonstrated high level of expression of IL-12 in NIH3T3 cells transfected with the DFG-IL-12 provirus (3100 U/24hr/10^6 cells).

Viral IL-10 and IL-1Rα retroviral vectors (MFG-vIL-10, and MFG-IL-1Rα) have been constructed to deliver these gene products at the site of an allogeneic or xenogeneic pancreatic islet transplant. Islet cells themselves have no capacity to proliferate. However, we have successfully infected the other components of human islet cell preparations grown in culture with our retroviral vectors, and have gotten good expression of IL-1Rα (14ng/ml/islet) using these supernatants. Using this system, we are currently evaluating the possibility of altering the rejection of human islets transplanted under the renal capsule in a chemically induced diabetic mouse model. Direct cytokine incubation of these cytokines in islet cell culture did not decrease islet function (production of insulin induced by glucose). Human trials will require the evidence of such decreased rejection and lack of effects on normal islet production of insulin *in vivo*. Use of these constructs to transfect murine tumors (MCA-105 sarcoma) appears to possibly delay the time to rejection in an allogeneic host.

IDENTIFICATION OF HUMAN MELANOMA DERIVED EPITOPES RECOGNIZED BY HLA-A2 RESTRICTED, CD8+ TUMOR INFILTRATING LYMPHOCYTES, *Walter J. Storkus, Mark J. Maeurer, Herbert J. Zeh III, and Michael T. Lotze. From the Departments of Surgery and Molecular Genetics and Biochemistry, Pittsburgh School of Medicine, Pittsburgh, PA 15261.*

Using a novel pH 3.3 acid elution technique, peptides were extracted by denaturation of class I molecules on the surface of normal human melanocytes, primary melanomas, and metastatic melanomas. This material was subsequently fractionated on reverse-phase high performance liquid chromatography (HPLC), and individual HPLC fractions lyophilized and reconstituted in Hanks' buffered saline. Aliquots of these fractions were then pulsed onto ^{51}Chromium-labeled HLA-A2+ B cell lines in the presence of exogenous human $\beta 2$-microglobulin for 1h at room temperature. The resulting peptide-charged target cells were assessed for sensitivity to lysis by a series of oligoclonal CD8+ TIL displaying HLA-A2 restriction. Using this approach, a series of six melanoma-associated T cell epitopes have been identified (termed P1-P6) that are capable of conferring sensitivity to TIL-mediated lysis upon HLA-A2+ B cell targets. These epitopes could be extracted from both primary and metastatic HLA-A2+ melanoma, but not from HLA-A2+ cultured melanocytes or peripheral blood lymphocytes. The expression of these epitopes appeared restricted to melanoma targets since no such determinants could be extracted from HLA-A2+ squamous cell carcinoma, gastric carcinoma, breast carcinoma, colon carcinoma, or B lymphoblastoid cell lines. Further, HLA-A2- melanoma did not express the P1-P6 species, unless they were first transfected with the HLA-A2.1 trans gene. Such HLA-A2 transfected melanoma cell lines were determined to express the P1, P2, and P4 epitopes. Based on the pattern of seven distinct HLA-A2 restricted TIL lines with peptides derived from 5 distinct HLA-A2+ melanoma cell lines, it was determined that P1, P2, and P4 represent "shared" melanoma-associated epitopes since they are expressed by 5/5 HLA-A2+ melanoma and are recognized by 7/7 of the TIL. P3, P4, and P6 were variable in both expression by melanoma cell lines and recognition by TIL. The P1, P2, and P4 determinants may represent immunodominant melanoma epitopes presented in the context of HLA-A2 and appear to represent promising candidates for melanoma vaccine design. Peptides present within the HPLC fractions containing P1, P2, and P4 exhibit Mr from 670-1400 consistent with the nonameric structures typically associated with class I molecules. Individual peptides are currently being sequenced/synthesized in order to deduce the P1, P2, and P4 structures.

MOLECULAR CONTROL OF MACROPHAGE ACTIVATION FOR TUMOR CELL KILLING. Donna M. Paulnock, University of Wisconsin, Madison, WI.

Macrophages frequently are observed as a major component of the cellular infiltrate of experimental tumors. Although these cells have been proposed to play a major role in host defense against neoplasia, the factors determining the successful stimulation of antitumor effector macrophages (MP) remain poorly understood. We previously have identified a series of cellular antigens and genes induced by interferon-gamma (IFN-γ) in MP during the process of activation of cytolytic activity in these cells. Using these reagents, we characterized antigen and gene expression in MP present in developing tumors of the B16-F10 murine melanoma and in peritoneal MP isolated from B16 tumor-bearing mice. These studies revealed that in most cases neither the tumor-associated nor the peritoneal MP expressed the activation-associated antigens of interest during tumor development. In an effort to understand the molecular mechanisms involved in MP activation which control the development of cytolytic MP, we have investigated the regulation of one gene, mag-1, in response to IFN-γ. Genomic fragments of the mag-1 promoter region were inserted into vectors containing the luciferase (LUC) reporter gene. When transiently transfected into cells of the RAW 264.7 cell line, which acquire cytolytic activity in response to IFN-γ stimulation, a 928 bp fragment was found to be sufficient for IFN-γ-mediated induction of LUC activity. Additional analysis of 5' deletion mutants identified sequences within 100 bp of the major transcription initiation site which conferred strong interferon responsiveness. A perfect match to the previously-described Interferon Stimulated Response Element (ISRE) was present within this region and was shown to be essential for both basal and interferon-induced expression. Additionally, a loss of sequences directly upstream of the ISRE resulted in a substantial reduction of transcriptional activity, but only within the context of the native mag-1 promoter. In contrast to the results observed in RAW 264.7 cells, transient transfection assays carried out using the non-cytolytic WEHI-3 MP cell line revealed that all promoter constructs were transcriptionally inactive within these cells. This lack of promoter region activity corroborates our previous observation of the absence of mag-1 gene expression in these cells and suggests that WEHI-3 cells are unable to utilize an ISRE for gene induction after IFN-γ stimulation. The phenotypic and functional similarities between the WEHI-3 cell line and those MP predominating in mice bearing the B16 tumor suggest that MP recruited during tumor development may exhibit regulatory controls that limit their effective activation as tumoricidal cells.

RECOGNITION OF HLA-B27 ANTIGENS BY ALLOREACTIVE T CELLS, José A. López de Castro, Fernando García, Daniel López, Susana Rojo, and José A. Villadangos. Centro de Biología Molecular, Consejo Superior de Investigaciones Científicas, Madrid.

HLA-B27 has been the subject of intense study due to its association with ankylosing spondylitis. Our studies have focused on the molecular basis of HLA-B27 allorecognition. Alloreactive T cells can readily distinguish among naturally occurring HLA-B27 subtypes, although the degree of crossreactivity depends on the responder individual. HLA-B27 subtypes differ among each other by one or few amino acid changes, which are almost always located at one side of the peptide binding site. They share a same structure in the B pocket, which binds a conserved Arg residue at the second position of HLA-B27-bound peptides. HLA-B27 mutants with altered structure in the B pocket can still be recognized by a significant number of alloreactive T cell clones, indicating that structural integrity of the B pocket is not necessarily critical for B27-specific allorecognition. Thus, HLA-B27 polymorphism in and outside pocket B modulates T cell allorecognition, but recognition by many T cell clones was not abrogated by most of the changes examined. Metabolic labeling and radiochemical pool sequencing were used to compare the repertoires of peptides bound to HLA-B27 subtypes and to analyze the influence of mutations in pocket B on the Arg2 motif of bound peptides. The results suggest that many peptides bind to multiple HLA-B27 subtypes, but significant subsets of peptides bound to a given subtype do not bind to other subtypes, or do so with greatly altered efficiency. Several, but not all of the mutations in pocket B were compatible with binding of peptides bearing the Arg2 motif. The results show the importance of polymorphism in and outside pocket B in modulating peptide binding to HLA-B27 and illustrate the role of peptide repertoires in HLA-B27 allorecognition.

Antigen Processing and Presentation and Effector Cell Activation

NZ 100 ACCUMULATION OF ADOPTIVELY TRANSFERRED KILLER CELLS IN METASTASES. P.H. Basse[1], Ulf Nannmark[2], Bengt Johansson[2], M. Hokland[3], R.B. Herberman[1], K. Wasserman[1] & R.H. Goldfarb[1]. [1]Pittsburgh Cancer Institute, Pittsburgh, PA, USA, [2]Anatomiske Instit. University of Gotenburg, Sweden, and [3]Inst. of Medical Microbiol., University of Aarhus, Denmark.

The efficacy of adoptive immunotherapy might depend on the number of effector cells reaching the malignant tissues. We have therefore analyzed the migratory pattern of fluorescently labeled IL-2 activated killer cells in mice bearing 10-12 day B16 lung and liver metastases: A significant fraction of *adoptively* transferred, IL-2 activated NK (A-NK) cells accumulated (in a time and dose-dependent manner) in lung metastases of the B16 melanoma, the MCA 102 sarcoma and the Lewis lung carcinoma lines. Thus, 16 hours after injection, five to ten fold higher numbers of A-NK cells were found in the melanoma lesions compared to the surrounding normal tissue. EM analysis revealed that the A-NK cells were in intimate contact with both tumor cells and tumor endothelial cells. Up to 10% of the lung metastases seemed totally resistant to infiltration even though neighboring metastases were effectively infiltrated. When no IL-2 was injected along with the A-NK cells, significantly ($p<0.01$) fewer cells accumulated in the tumors at 16 h compared to when high doses of IL-2 (50,000 Cetus units) were given i.p. every 4 hours (203±20 vs. 540±46 per sq.mm tumor tissue). The highest infiltration (701±51 A-NK cells per sq.mm) was seen when 50,000 units of IL-2 was given i.p. every 2 h. A-NK cells were more effective in infiltrating the lesions than unpurified 3 day LAK cells. In addition, non-activated splenocytes, as well as various tumor cell lines did not have the ability to accumulate in the malignant lesions. Finally, while substantial infiltration of lung metastases was seen after i.v. injection, significant infiltration of liver metastases was seen only after intraportal injection. Thus, it seems that specific accumulation is seen mainly in tumors formed within the first capillary bed the A-NK cells come in contact with.

We conclude that A-NK cells, in contrast to e.g. non-activated splenocytes, can accumulate specifically in malignant tissues. This might be caused by differences among these cell types with respect to expression of surface adhesion molecules and/or proteases involved in the migration process. Due to their ability to accumulate in tumors upon proper routes of administration, A-NK cells might, in addition to their direct or indirect effector functions, serve as ideal vehicles for targeting cytotoxic drugs or gene-products as e.g. IL-2 or TNF, to malignant foci.

NZ 101 REGULATION OF $CD4^+$ MEMORY EFFECTOR T CELL SUBSET DEVELOPMENT FOLLOWING REEXPOSURE TO ANTIGEN. Linda M. Bradley and Susan L. Swain. Department of Biology and Cancer Center, University of California, San Diego, La Jolla, CA 92093

We have studied the development and function of memory helper T cells (Th) following reexposure to antigen both in vivo and in vitro. Resting $CD4^+$ memory T cells from the spleens of mice primed >6 weeks earlier with KLH in adjuvant produce high titers of IL-2 and IL-3, but only low levels of IL-4 and IFN-γ, and no detectable IL-5 in response to specific restimulation in vitro. In contrast, when previously primed mice are boosted by injection of soluble KLH, splenic $CD4^+$ T cells secrete greatly increased levels of IL-4, and variably increased levels of IL-2, IFN-γ and/or IL-3. While resting memory Th provide helper activity primarily for IgM production by primed B cells in vitro, memory effector Th are excellent helpers for all IgG isotypes, as well as IgM antibody. Memory effector Th activity is transient, attaining maximum levels 3 days after in vivo boosting, and declining rapidly thereafter. Memory effector Th are induced in the spleens of KLH-primed mice that have been depleted of precursor T cells by thymectomy >25 weeks previously, suggesting that they are derived from the resting memory T cell population. Memory effector Th can also be generated in vitro from resting memory Th by culturing $CD4^+$ T cells from KLH-primed, thymectomized mice in the presence of KLH-pulsed antigen-presenting cells and exogenous cytokines. Th1-like effectors with the capacity to secrete high levels of IL-2 and IFN-γ, and low levels of IL-4 develop by 3-4 days after reactivation of primed $CD4^+$ T cells in the presence of rIL-2, in combination with rIFN-γ and anti-IL-4 antibody to block endogenous IL-4 secretion. In contrast, Th2-like $CD4^+$ T cells with the capacity to secrete high levels of IL-4, and low levels of IL-2 and IFN−γ are generated with similar kinetics, when primed $CD4^+$ T cells are restimulated in the presence of rIL-2, together with rIL-4 and anti-IFN-γ antibody to block endogenous IFN-γ secretion. The results demonstrate that memory effector Th differentiate from resting memory Th in response to reexposure to antigen, and suggest that IL-4 and IFN-γ differentially regulate the development of memory effector Th subsets which secrete distinct arrays of cytokines.

NZ 102 ABNORMALITIES IN HLA EXPRESSION ON COLORECTAL TUMOUR CELL LINES: IMPLICATIONS FOR ANTIGEN PRESENTATION, Michael Browning, Peter Krausa, Andrew Rowan & Walter Bodmer, ICRF, Institute of Molecular Medicine, John Radcliffe Hospital, Oxford, UK.

The recognition of cellular antigens by CTL involves the processing and presentation of antigen in association with MHC class I gene products. Failure to express the relevant class I restricting molecule results in failure of recognition of the cell. We have developed a PCR-based method for defining HLA-A locus alleles from genomic DNA, and have used this method to compare the HLA genotype with surface expression of HLA class I in a panel of 30 colorectal tumour cell lines. Abnormalities of HLA expression were detected in 8 cell lines, and resulted from at least 4 independent mechanisms. Three cell lines showed complete lack of class I expression associated with failure to express β2 microglobulin. Two cell lines showed loss of one HLA-A locus gene, whilst 2 other cell lines showed selective loss of expression of one HLA-A locus allele. One cell line failed to express surface HLA class I, in spite of the expression of normal levels of β2 microglobulin. It is not possible to determine whether these abnormalities in HLA expression reflect immunoselection of tumour cells with mutations in HLA expression. However, the failure of these cell lines to express particular HLA molecules could result in the failure of recognition of tumour-antigen bearing cells by MHC restricted CTL.

NZ 103 RT-PCR CLONING OF A RAT B7 cDNA HOMOLOGUE, Elaine Brunschwig and Mark L. Tykocinski, Institute of Pathology, Case Western Reserve University, Cleveland, Ohio 44106

B7 is an interferon-gamma-responsive cell surface costimulator on B-cells and monocytes that binds CD28 and CTLA-4. Human and murine B7 cDNAs have previously been cloned. We now report the reverse transcriptase-polymerase chain reaction (RT-PCR) cloning of a rat B7 cDNA homologue. Rat splenocyte RNA served as a source of RNA, and degenerative PCR primers corresponding to subsequences most conserved between human and mouse B7 were used for amplifying the rat sequence. Primary sequence analyses of the PCR amplified rat product will be described. Availability of rat B7 sequence will now enable gene transfer studies to study the effects of B7 surface expression on tumor immunogenicity in well-developed syngeneic rat tumor cell systems.

NZ 104 ROLE OF ADHESION MOLECULES IN THE STIMULATION OF IL-2-ACTIVATED LYMPHOCYTES.
Anita S.-F. Chong, Xing-Li Jiang, Marlene Lamas and Lloyd H. Graf, Jr. Department of General Surgery and Immunology/Microbiology, Rush-Presbyterian-St. Luke's Medical Center, Chicago, IL 60612 and Center for Molecular Biology of Oral Diseases, University of Illinois, Chicago, IL 60612.

PBLs cultured *in vitro* with IL-2 acquire the capacity to mediate the regression of tumors in experimental animal models and in humans. The ability of IL-2 activated T (LAT) lymphocytes to mediate tumor regression *in vivo* correlates best with their ability to be stimulated by tumor cells to produce cytokines such as TNF-α and IFN-γ.

Our previous studies indicate that cytokine (IFN-γ, TNF-α and TNF-β) production by LAK-T cells require cross-linking of the TCR-CD3 complex. Using immobilized monoclonal antibodies (mAbs), we demonstrated that crosslinking of LFA-1, CD2, CD45 and CD44 molecules enhance cytokine production by LAK-T cells. We have extended these studies and here report that mAbs directed at CD28 and CD7 but not CD31 also enhance cytokine production. In addition, co-immobilized ligands such as fibronectin, laminin, collagen but not hyaluronic acid also enhance cytokine production, presumably by binding and stimulating via VLA-4, 5 or 6 on LAT cells. Another approach we have adopted to further define the contributions of cell adhesion molecules involved interspecific gene transfer of genes for huICAM-1 and LFA-3 into a null mouse melanoma background. Cells expressing huICAM-1 and huLFA-3, but not untransfected parental nor cells transfected with a melanoma-associated antigen, enhanced cytokine production by LAK-T cells. These studies indicate that while none of the adhesion molecules can directly stimulate cytokine production in LAK-T cells, a number of these molecules can function as co-stimulators to enhance cytokine production triggered via the TCR-CD3 complex.

Little is known about the nature of molecules that regulate cytotoxic activity or cytokine production in IL-2-activated LAK-T cells. LANK cells were generated as described for A-LAK cells, then stimulated via adhesion molecules alone or in combination with CD16. Using the same approaches we determined that with the exception of CD45, none adhesion molecules could directly stimulate or co-stimulate cytokine production in LAK-NK cells.

NZ 105 LYMPHOCYTE TRAFFICKING TO THE CERVIX IN LOW AND HIGH GRADE SQUAMOUS INTRA-EPITHELIAL LESIONS,
Nicholas Coleman[1], Stephen B. Fox[2], Colin A. Michie[3], Thomas J. Schall[4] and Margaret A. Stanley[1], Departments of Pathology, [1]University of Cambridge and [2]University of Oxford, U.K., [3]Human Tumour Immunology Group, University College, London, U.K. and [4]Genentech Inc., San Francisco, CA.

High-grade squamous intra-epithelial lesions (HG-SILs) of the cervix are associated with lymphocytic infiltration, whereas low-grade SILs are not. We have examined the phenotype and activation status of the infiltrating cells in HG-SILs, and have investigated mechanisms of lymphocyte trafficking to the site of the neoplastic epithelium. Recruitment of activated lymphocytes in HG-SILs is associated with the induction in the sub-epithelial stroma of a number of vascular adhesion molecules likely to be important in lymphocyte homing. Using the Stamper-Woodruff *in vitro* adhesion assay, we have demonstrated a functional role for these adhesion molecules in lymphocyte entry into the cervix. Additionally, we have obtained evidence for local expression of chemotactic cytokines potentially important in lymphocyte recruitment to the cervix. These data suggest that a complex network of interactions exists in HG-SILs between lymphocytes, keratinocytes and the cellular components of the sub-epithelial stroma.

NZ 106 TUMOR NECROSIS FACTOR SELECTIVELY ENHANCES TUMOR LOCALIZATION OF ADOPTIVELY TRANSFERRED CULTURED SPLENOCYTES IN A MURINE MELANOMA MODEL.
Phillip A. Dean, Patrick S. Ramsey, and Heidi Nelson, Mayo Clinic, Rochester, MN 55905.

Successful application of adoptive immunotherapy depends on the selective distribution of cellular reagents to sites of tumors. Inducible cell adhesion molecules participate in the distribution of lymphocytes to sites of inflammation and likely participate in the distribution of adoptively transferred cells to sites of tumors. We have previously shown that TNFa and IL-1a enhance the tumor vascular expression of MALA-2, the murine equivalent of intercellular adhesion molecule-1, in a murine melanoma model. The current study aimed to test whether the enhancement of MALA-2 using cytokines, translates into enhanced tumor localization of adoptively transferred splenocytes. METHODS: Splenocytes from C3H/HeN mice were cultured for 6 days in media containing 100U/ml IL-2 and 0.5mg/ml 500A2 (anti-CD3). On day 0, C3H/HeN mice (immune competent) were injected subcutaneously and intraperitoneally with the syngeneic murine melanoma cell line K-1735 (transfected with p97). On days 9 and 10, tumor bearing-mice (4-6 per group) were injected twice daily with saline (controls), TNFa ($1x10^5$u/dose) or IL-1a (500ng/dose) and on day 11 were injected via a tail vein with indium^{111}-labeled splenocytes. At 12, 24, and 48 hours after splenocyte injection, tissues were harvested, weighed, and gamma counted to determine %injected dose/g (%ID/g). RESULTS: Tumor localization of splenocytes was increased by TNFa at all timepoints (control 4.99 ± 1.16 vs TNF 7.41 ± 1.20, %ID/g at 24hr, $p=0.03$), but was not effected by IL-1a (control 1.27 ± 0.50 vs TNFa 1.33 ± 0.37, %ID/g 24 hr). Localization of splenocytes to normal organs was not increased by TNFa or IL-1a, but in fact, was decreased by TNFa. CONCLUSIONS: TNFa selectively enhanced localization of splenocytes to sites of tumors without enhancing localization to normal organs. Although IL-1a also increases tumor vascular expression of MALA-2, it does not increase splenocyte localization to tumors, suggesting that other factors are involved in the regulation of splenocyte trafficking. Future studies will investigate whether TNFa can enhance the effectiveness of adoptive immunotherapy.

NZ 107 DIFFERENTIATION OF ANTITUMOR T CELLS BY MICROBIAL SUPERANTIGENS,
Bernard A. Fox, Daniel D. Elsholz, Mark Cameron and Suyu Shu. Surgical Oncology Laboratory, Department of Surgery, University of Michigan, Ann Arbor, MI 48109.

Recently this laboratory has demonstrated that T cells from Tumor draining lymph nodes (TDLN) are sensitized but not fully differentiated, as defined by their failure to mediate therapeutic activity. Maturation of these "pre-effector" cells can be initiated by in vitro sensitization with tumor cells, or in a two step in vitro activation process with anti-CD3 and IL-2. Microbial superantigens (SAg) from staphococcal origin, are a family of small proteins which bind antigen presenting cells and stimulate preferential activation of T cell subpopulations which express select Vβ TCR genes. Different SAg bind and expand distinct Vβ TCR repertoires which may otherwise represent only a small percentage of the total T cell population. In an attempt to dissect the T cell population(s) mediating specific antitumor activity, a panel of microbial SAg were used to stimulate pre-effector cells obtained from TDLN. TDLN isolated from animals bearing 12 day MCA-106 or MCA-205 tumors were activated by two days of culture with SAg and subsequent three day expansion in 10-12 U/ml IL-2. Six of the 8 SAg tested, stimulated greater than 4 fold expansion of T cells in the 5 day culture (mean of 4 to 6 experiments). Subsequent experiments focused on three SAg (SEA, SEC1, and SEE) which, by flowcytometric analyses, selectively expanded distinct Vβ TCR subsets of the T cell repertoire of TDLN. The predominant Vβ TCR usage (>10% expression) of SAg-activated TDLN were: Vβ 3 and 11 for SEA, Vβ 8.2 and 8.3 for SEC1, and Vβ 11 and 13 for SEE. SAg-activated TDLN were analyzed for in vitro cytotoxicity and therapeutic efficacy. Similarly to CD3-activated TDLN, SAg-activated TDLN were not cytotoxic for fresh tumor cells in 4 hr ^{51}Cr-release assays. Adoptive transfer of SEC1-activated TDLN ($1-1.2 \times 10^7$ cells/mouse) were the most effective, significantly reducing metastases compared to IL-2 alone ($p<0.05$) in 4/4 experiments (81% mean reduction in pulmonary metastases). SEA and SEE -activated TDLN demonstrated a weak effect which was inconsistent between experiments. These preliminary results suggest that some Vβ T cells may preferentially respond vigorously to MCA-205 and that SAg may be useful reagents for dissecting the elements of the T cell response to tumors.

NZ 108 IMMUNIZATION AGAINST TUMOR AND MINOR HISTOCOMPATIBILITY ANTIGENS BY NATURALLY PROCESSED PEPTIDES.

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[1]Department of Tumor Biology and [2]Department of Immunology, Karolinska Institutet, Box 60 400, S-104 01 Stockholm, Sweden.

Material eluted from RMA lymphoma or B6 spleen cells at low pH was fractionated by reversed-phase HPLC. The fractions were tested for ability to restore the sensitivity to CTL of the processing / presentation defective mutant line RMA-S. This allowed identification of three fractions carrying B6 minor histocompatibility antigens (MiHa) recognized by A.BY anti B6 CTL, and one fraction carrying a tumor antigen recognized by B6 anti RMA CTL. By parallel runs of material from cell lysates over MHC class I affinity columns, two MiHa were defined as Kb restricted, and one MiHa and the tumor antigen as Db restricted. These restriction patterns could be conferred utilizing the human peptide presentation defect celline T2 transfected with either Db or Kb as read out target celline. Isolated fractions loaded onto RMA-S cells could be used to prime anti MiHa and tumor CTL in vivo. They could also be used for in vitro restimulation of spleen cells from mice that had been primed either by antigen loaded RMA-S, or by wild type RMA and B6 cells. The CTL generated by these methods were specific for the loading antigen, but they recognized the antigen also on the "physiological" target, i e RMA or B6 lymphoblasts. This system based on RMA-S as an immunization and target antigen reporter cell may be used for dissection of complex CTL responses, e g in studies of clonal composition and epitope dominance, or for studies of tumors that are poor stimulators of immunity. The results also confirm that the previously reported escape of RMA-S from restricted CTL against MiHa and tumor antigens is due to impaired peptide presentation, even if this defect is not absolute; a significant sensitivity of native RMA-S to CTL induced by peptide loaded RMA-S illustrates that isolated antigen can be used to manipulate the afferent arm of the response to "non-immunogenic" tumors carrying subthreshold doses of antigens.

NZ 109 IDENTIFICATION OF HLA-A2.1-RESTRICTED T-CELL EPITOPES USING TANDEM MASS SPECTROMETRY,

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Endogenous peptides bound to HLA-A2.1 were isolated and separated by reverse-phase HPLC. A portion of each fraction was used to sensitize target cells for lysis by a panel of peptide dependent HLA-A2.1-restricted murine xenoreactive CTL clones. These clones exhibited 5 distinct patterns of peptide specificity. Several clones were chosen for further analysis. The reconstituting fractions for these clones were analyzed by microcapillary HPLC-electrospray ionization-tandem mass spectrometry. This allowed the total number and the masses (m/z) of all of the peptides in each fraction to be determined. These reconstituting fractions contained approximately 50 - 100 different peptides. In order to further subdivide these peptides, the reconstituting fractions were split into equal parts and further separated by RP-HPLC using either HFA or HFBA as an organic modifier. These fractions were again tested for their ability to sensitize target cells for lysis by CTL. Mass spectrometric analysis of these reconstituting fractions showed that approximately 25 - 30 peptides were now contained in each fraction. By comparing the peptides found in the reconstituting fractions from the HFA and HFBA HPLC separations, we were able to limit the number of peptides that may be responsible for epitope reconstitution to two or three candidate peptides. These candidate peptides are currently being sequenced by tandem mass spectrometry. The epitope for one CTL clone has been identified as having the sequence ALWGFFPV(I or L). It is important to note that the identification and sequencing of this epitope was accomplished in complex HPLC fractions in which this peptide was not dominant. Thus, the coupling of this mass spectrometric technique with T-cell sensitization assays provides a unique, powerful, and generally applicable approach to the analysis of T-cell epitopes. This should greatly facilitate the identification of peptide antigens associated with cellular transformation and viral infection.

NZ 110 **SYNTHETIC PEPTIDES DERIVED FROM B CELL SPECIFIC ANTIGENS (CD19 / CD20) GIVE RISE TO PEPTIDE SPECIFIC CTL.**

Erik Hooijberg[1], Marjan Visseren[2], Paul van den Berk[1], Petra Romeyn[1], Anke Petra Jellema[1], Annemarie Hekman[1], en Kees Melief[2].
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Malignant B cells express CD19 and CD20 antigens as do normal B cells. We have chosen these antigens as targets for immunotherapy of B cell malignancies. Since no CTL epitopes of these B cell specific antigens are known we made use of the binding motifs to murine MHC class I molecules as found by Falk et al (1991). A computer search (J. D'Amaro, University Hospital, Leiden, The Netherlands) revealed the following numbers of peptides derived from murine and human CD19 and CD20 antigens predicted to bind to K^b or D^b molecules.

	binding to K^b predicted / actual		binding to D^b predicted / actual	
murine CD19	3	1[a]	6	1[b]
human CD19	7	1[a]	5	1[b]
murine CD20	1	1	9	2 + 1[c]
human CD20	4	1	9	2 + 1[c]

a,b,c; same sequence in the human and the murine peptide.

Synthetic peptides derived from CD19 or CD20 predicted to bind to K^b or D^b were used to test binding to MHC molecules on RMA-S cells. Peptides capable of binding to K^b or D^b at the lowest concentration (1 μM/ml) applied in the RMA-S assay have been used to immunize mice and generate specific class I restricted CTL. We have isolated; 1) murine CTL specific for a peptide derived from human CD20; 2) murine CTL specific for an autologous peptide derived from murine CD20 and 3) murine CTL specific for a peptide from murine/human CD19. These CTL are cytotoxic to cells loaded with the appropriate synthetic peptide. In the near future we will perform experiments to see whether or not these CTL are able to kill; 1) murine cells transfected with human CD20 cDNA; 2) autologous murine B cell tumors; or 3) autologous murine B cells or murine cells transfected with cDNA encoding human CD19. Furthermore we will transfect murine cells with cDNA encoding human CD19 or CD20 and use these to generate CTL recognizing peptides in MHC class II molecules.

NZ 111 LOSS OF CD44 (PGP-1) ON CTL-P IN THE LYMPH NODES OF SENDAI VIRUS-INFECTED MICE OCCURS PRIOR TO TRAFFICKING OF THESE CELLS TO THE INFECTED LUNG,

Samuel Hou and Peter C. Doherty, Department of Immunology, St. Jude Children's Research Hospital, Memphis, TN 38105.

We have characterised the cell surface expression of several adhesion molecules on the lymphocytes in the broncho-alveolar lavage (BAL) and the draining mediastinal lymph nodes (MLN) of intranasally infected C57BL/6J mice. LFA-1 expression on the BAL cells paralleled the degree of virus-specific cytotoxicity, peaking on day 10 postinfection. CD44 (Pgp-1) expression was elevated throughout the timecourse of the experiment in the BAL lymphocytes. CD45RB expression on the BAL cells paralleled that of LECAM-1 in that a huge influx of CD45RB/LECAM negative cells is observed in the lung at the time of peak cytotoxicity and subsequent viral clearance. However, there was no partitioning of the CD45RB+/- cells into effectors or bystanders, both populations contained effector CTL. Cell surface expression of these adhesion molecules were mostly unchanged in the MLN. We clearly demonstrate the predominant LECAM-1- phenotype of the effector CTL population in the lung and precursor CTL in the MLN and preliminary results indicate that loss of CD44 expression on the LECAM-1- CTLp population in the MLN may be a prerequisite for the movement of this population of cells to the lung.

NZ 112 **PEPTIDES BOUND TO HLA-B7 DETERMINED BY MASS SPECTROMETRY**, Eric L. Huczko, Wanda Bodner, David Benjamin, Kazuyasu Sakaguchi, Nian Zhu Zhou, Jeffrey Shabanowitz, Robert Henderson, Ettore Appella, Donald F. Hunt and Victor H. Engelhard, Beirne Carter Center and Department of Chemistry, University of Virginia, Charlottesville, VA 22901

Microcapillary HPLC/electrospray ionization/tandem mass spectrometry was used to sequence 18 peptides eluted from the human class I molecule HLA-B7. 16 of these were 9 residues long, while 2 were 10mers, and one was an 11mer. All peptides contained a small hydrophobic residue at the carboxyl terminus, 14 contained proline at position 2, and 11 contained a positive charge, usually arginine, at position 3. Alanine or arginine was found at position 1 in 16 sequences. Direct binding experiments using synthetic peptides indicated that these conserved features were important determinants for specific interaction with HLA-B7, and suggested a similar mode of interaction regardless of peptide length. Modeling of peptides in the binding cleft of a predicted HLA-B7 structure, derived from the known crystal structure of HLA-B27, suggests explanations for the observed residue preferences. The B, D, and F pockets are involved in selection of residues at position 2, 3, and 9. The size and nature of the F pocket is similar to that of some other class I molecules and allows for the observed range of several small hydrophobic residues at the carboxyl terminal position. Proline appears to be preferred at position 2 because the B pocket of B7 is drastically reduced in size and hydrophilicity compared to B27 due to the substitution of tyrosine for cysteine 67. Arginine at position 3 participates in extensive van der Waals interactions in the D pocket, and forms a salt link with aspartate 114. These are the first data to suggest a role for the D pocket in specific peptide binding. These results provide additional support for a general mode of peptide interaction common to several class I MHC molecules, but demonstrate additional constraints on peptide binding to HLA-B7 that impose additional restrictions on peptide heterogeneity.

NZ 113 ISOLATION AND CHARACTERIZATION OF TUMOR-SPECIFIC PEPTIDES

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One of the most critical questions in cancer biology is why the immune system fails to eliminate tumors that arise *de novo*. Recent studies have provided strong evidence that this failure owes to an inability to respond to tumor-specific antigens. We have demonstrated that the injection of tumor cells genetically engineered to produce certain lymphokines can activate inadequately functioning helper T cells and generate tumor-specific cytotoxic T lymphocytes (CTL) that provide systemic immunity to the parental tumor. Tumor antigens to which CTL respond have been difficult to characterize because they are not generally recognized by antibodies and therefore cannot be isolated by immunoprecipitation.

Using the method of acid elution and reversed-phase high performance liquid chromatography (RP-HPLC), we recently isolated tumor-specific antigens from the MHC class I groove of two murine H-2d tumors, CT26, a colon carcinoma and Renca, a renal cell carcinoma. Tumor-specific CTL, which are used to identify RP-HPLC fractions that contain these peptides, were generated in vivo using our tumor-vaccine system. With this approach we were able to detect 1 immunodominant tumor-specific peptide out of 40 total RP-HPLC fractions for each tumor, using heterogeneous populations of CTL initially primed in vivo with a tumor vaccine genetically engineered to secrete the cytokine granulocyte-macrophage colony-stimulating factor (GMCSF). One minor peptide was also recognized for each tumor. Interestingly, for CT26, the major peptide repeatedly elutes from other CT26 tumors in fraction number 25, and the minor peptide in fraction number 32. For Renca, the major peptide elutes in fraction 33, and the minor in fraction number 36. Furthermore, these same peptides are detected by different populations of CTL generated in different mice of the same MHC haplotype using the same tumor vaccine. Recently, the RP-HPLC fractions were analyzed by several methods employing mass spectroscopy. The results revealed that each fraction contained at least one peptide with a molecular weight in the range of 1200 to 1500 daltons, which is approximately the molecular weight of an 8 or 9 amino acid peptide. Our data therefore suggests that there is only one or two immunodominant peptides presented by a population of tumor cells that have been grown *in vivo*. The characterization of these peptides will be discussed.

NZ 114 INDUCTION OF B CELL COSTIMULATORY FUNCTION BY RECOMBINANT MURINE CD40 LIGAND, Mary K. Kennedy, Kendall M. Mohler, Kurt D. Shanebeck, Peter R. Baum, Richard J. Armitage, William C. Fanslow, Melanie K. Spriggs, and Kenneth H. Grabstein, Immunex Corp., Seattle, WA 98101

Activated, but not resting B cells, can costimulate APC-dependent T cell proliferation and can function as stimulators in a primary MLR. B cells were activated in the presence of either LPS or membrane-bound recombinant murine CD40 ligand expressed on fixed CV1/EBNA cells. LPS- or CD40 ligand-activated, but not control-cultured B cells provided potent sources of: 1) costimulation for anti-CD3 dependent proliferation of syngeneic CD4+ T cells; and 2) stimulation of allogeneic CD4+ T cells in a primary MLR. These stimulatory activities of CD40 ligand-activated B cells were comparable to or greater than those of LPS-activated B cells. Soluble CTLA-4:Fc had a significant inhibitory effect on the stimulatory activities of both B cell populations. In addition, the enhancement of B cell stimulatory function by CD40 ligand or LPS correlated with the induction of cell surface expression of B7.

NZ 115 **Micropocket usage in HLA-A2 presentation of melanoma peptides to CD8+ tumor infiltrating lymphocytes (TIL).**

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We have previously identified a series of six T cell epitopes derived from human melanomas that are recognized in the context of the HLA-A2 class I molecule by CD8+ cytolytic T lymphocytes (i.e. TIL). These epitopes (termed P1-P6) appear to represent melanoma-restricted peptides approximately 8-10 amino acids in length. Further, in peptide-pulsing experiments, P1-P6 were capable of conferring sensitivity to TIL-mediated lysis upon a B cell line expressing a homogeneous population of HLA-A2 class I molecules (C1R.A2). In order to more fully characterize the binding site requirements of the P1-P6 determinants, we constructed a series of HLA-A2 site-directed mutant molecules with a modified antigen binding site (ABS). Each mutation affects a single amino acid residue within one of the six (A-F) micropockets constitutive to the HLA-A2 ABS (positions 5, 58, 66, 70, 74, 95, 97, 116, 156, 158, 177). These mutant genes were then transfected into the C1R (HLA-A, B null) cell line and positive transfectants selected. P1-P6 peptides were subsequently pulsed onto each of these C1R transfectants and the resultant sensitivity to HLA-A2 restricted, melanoma-specific TIL assessed in 4h cytotoxicity assays. P1, P2, and P6 appeared to be recognized efficiently by TIL when presented by any of the HLA-A2 mutant class I molecules. P3 was perturbed by mutations in the A (position 5) and B (position 66) micropockets, while P4 was not presented effectively by HLA-A2 molecules with mutations in the C (position 74) and F (position 116) micropockets. P5 presentation appeared principally affected by mutations in the C (positions 70, 74, and 95) but was also inhibited to a minor degree by mutations in the A (position 5) and D (position 156) micropockets. These data support heterogeneous usage of multiple micropockets by the individual P1-P6 melanoma peptides. Further, the P1, P2, and P6 species may bind with the greatest affinity to HLA-A2 since no single mutation examined within the ABS appeared to affect their presentation to the TIL effector cells.

NZ 116 DIFFERENTIAL REGULATION OF HLA-CLASS I GENES BY INTERFERON IN VIVO AND IN VITRO

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Modulation of HLA-antigens on peripheral blood lymphocytes, monocytes and hematopoietic precursors during IFNα therapy was investigated in 18 patients with myeloproliferative syndrome. After one month of IFNα therapy an increased number of monocytes and hematopoietic precursor cells, but not of lymphocytes expressed HLA-DQ antigens. In addition, a strong induction of HLA-class I antigens was found on both hematopoietic progenitors and normal peripheral blood mononuclear cells. By daily injections of IFN in the first month of therapy stimulation was continuously increasing suggesting a major effect of IFNα on hematopoietic progenitors with sustained enhanced expression of HLA-class I antigens during differentiation of myelomonocytic cells. HLA-class I antigen expression was consistently augmented by IFNα in all patients irrespective of their haematologic response. Differential in vivo regulation of HLA-class I antigens by IFN was demonstrated by comparison of HLA-A2 with HLA-B antigen expression.

In vitro expression of the HLA-B7 and -Bw64 genes was significantly more inducible by interferon (IFN) than the genes coding for the HLA-B27, HLA-B51, HLA-B38, HLA-B39, HLA-Cw3 and HLA-A2 antigens after transfection into mouse L cells. Modification of the 5' ends of the HLA-B7 and HLA-B27 genes before transfection in mouse L cells revealed the presence of enhancer sequences responding to interferon treatment in the 5' untranslated region of the HLA-B7, but not of the HLA-B27 gene and suggested further independently acting enhancer elements downstream of the transcription initiation site.

These findings may indicate specific regulatory mechanisms of HLA class I antigen expression possibly influencing T-cell recognition in immune response.

NZ 117 INDUCTION OF AUTO-TUMOR CYTOTOXICITY IN BLOOD LYMPHOCYTES BY CYTOKINE PRE-TREATED EX-VIVO TUMOR CELLS,

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Ovarian and lung carcinomas express low amounts of class I and ICAM-1. Treatment with IFNγ and TNFα increased the expression of these molecules. Untreated tumor cells were lysed by the autologous blood lymphocytes in 6/52 cases. They could rarely induce cytotoxic function in the 16 h MLTC. When cytokine treated tumor cells were used in the MLTC the cytotoxic function of blood lymphocytes was activated in 11/18 ovarian and 9/13 lung carcinomas. The activated lymphocytes damaged both the untreated and the cytokine treated tumor cells. Cold target competition experiments suggested that the untreated and cytokine treated targets were lysed by the same effectors. Involvement of class I and ICAM-1 of the tumor cells in the induction and the effector phase was tested by blockade with α-class I (W6/32) and α-ICAM-1 (LB-2) mAbs. Presence of either of these two mAbs in the MLTC inhibited the activation of cytotoxic function. In the effector phase, sensitivity of the pre-treated targets was reduced by the LB-2 mAb with 89% (5 tests) and by the W6/32 mAb with 42% (8 tests). This suggests that activated lymphocytes can exert lysis without involvement of class I molecule. Our results also show a quantitative difference in the requirement of class I molecules on the targets for the functional interaction with lymphocytes in the afferent and efferent phases.

NZ 118 RAPID GENERATION OF MULTIFUNCTIONAL EFFECTOR T LYMPHOCYTES FOR CANCER IMMUNOTHERAPY,

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Our work on cancer therapy is based on the capacity of bispecific antibodies to redirect cytolytic T lymphocytes (CTL) onto gastro-intestinal tumor cells. At the clinical level, this approach involves the generation of effector T lymphocytes prepared ex vivo. In our concept, the ex vivo activation procedure must be simple (to reduce upscaling logistics), safe (by avoiding toxic stimuli), and short (to prevent blast formation and altered homing of reinfused cells). Activation via the CD28 pathway appears particularly attractive in this context because: a) T-lymphocytes are selectively stimulated; b) it does not imply TCR/CD3 complex occupancy nor induces its modulation; c) anti-CD28 monoclonal antibody (mAb) can be used directly in solution; d) as it is known that this pathway enhances markedly lymphokine production, multifunctional effector T-lymphocytes can be produced.

Cytolytic activity and lymphokine production were measured after activation protocols including IL-2, anti-CD28 mAb, the phorbolester PDBu, DAG (1,2-diactanoyl-sn-glycerol) and combinations thereof. CD28+PDBu generated highest lytic activity within 24-36 hr from normal PBL harvested by leukapheresis. Comparable results were obtained by substituting the toxic phorbolester with DAG, a natural ligand of PKC. In addition, early, strong and sustained TNF-α and IFN-γ gene expression was achieved with CD28+DAG (or phorbol-ester), as well as increased TNF-α production in cell culture supernatants. Using cell sorting experiments we observed that CD28+DAG stimulated selectively the CD4 subset to produce TNF-α. Also, the CD8+/CD45R0- cells were less responsive than the CD8+/CD45R0+ cells. Thus, CD28 activated T-lymphocytes used in conjunction with bispecific antibodies may allow both the selective delivery of lymphokines (such as TNF-α and/or IFN-γ) and the targeting of CTL at the tumor site.

T Cell Stimulatory Tumor Antigens

NZ 200 IDENTIFICATION OF THE MELANOMA ASSOCIATED ANTIGENS RECOGNIZED BY MOABs NKI-beteb AND HMB-45, G.J. Adema, A.J. de Boer, A.M. Vogel and C.G. Figdor, Department of Immunology, The Netherlands Cancer Institute, Amsterdam, The Netherlands

The glycoproteins recognized by moAbs NKI-beteb (gp 100 and gp7) and HMB-45 (gp10) are amongst the best markers for human melanoma available to date. Although moAbs directed against these antigens are very suitable for diagnostic purposes, they are less suitable for therapeutic purposes since the antigens are primarily expressed intracellularly. However, peptides derived from the antigens, if presented by MHC molecules, may well be capable of evoking cellular immune responses.

Using a rabbit polyclonal antiserum against gp100 we isolated a cDNA clone containing an insert of 2.1 kb. This cDNA detected RNA species of 2.5 kb and 4.2 kb on Northern blots containing total RNA isolated from melanoma cells and melanocytes but not from any other cell type tested. Expression of the cDNA in either cos-7 cells or gp100 negative melanoma cells resulted in NKI-beteb as well as HMB-45 immunoreactivity. This indicates that NKI-beteb and HMB-45 both recognize the protein(s) encoded by this cDNA. Biochemical characterisation of the protein recognized by NKI-beteb in transfected cos-7 cells demonstrated that the cDNA clone encodes gp100 as well as gp7.

We are currently determining the sequence of this cDNA clone. In addition, we are generating CTLs which recognize peptides derived from gp100 in an MHC restricted manner. These CTls will be tested for anti-tumor activity in an in vivo model system.

NZ 201 CLONING OF THE GENE ENCODING TROP-2, A POTENTIAL TARGET FOR IMMUNOTHERAPY HIGHLY EXPRESSED ON THE CELL SURFACE OF HUMAN CARCINOMAS, Saverio Alberti, Manuela Stella, Roberta Dell' Arciprete, Cecilia Bucci°, Michele Nutini, Anna Maria Naglieri, Enrica Riva and Mara Fornaro, Institute "Mario Negri Sud", Santa Maria Imbaro (Chieti), Italy. °: Department of Cellular and Molecular Biology and Pathology, University of Napoli, Italy.

The gp50/Trop-2 cell surface glycoprotein is expressed at high levels by trophoblast cells and by the majority of human carcinomas. On the other hand, it is scarcely represented in most normal tissues with the exception of multistratified epithelia. We speculate that this molecule may play a relevant role in tumor cell growth and invasion and may represent a target for novel diagnostic and immunotherapeutic strategies. To clone the gene encoding gp50/Trop-2 we adopted an episomal vector-based expression cloning procedure, modified by us to reach transfection of 100% of the COS cells used, efficient selection of positive cells by cell sorting in flow and an overall sensitivity of screening of cDNA libraries of 1 positive clone dispersed in 10^5 irrelevant ones. We constructed a cDNA library in the CDM8 vector using mRNA from the OVCA-432 cell line, which expresses gp50/Trop-2 at high levels. From this library we isolated a clone encoding gp50/Trop-2. This clone contains a 2.4 kb cDNA, which transfects gp50/Trop-2 and hybridizes with a single 2.5 kb mRNA species from positive sources, including genomic transfectants expressing gp50/Trop-2. Southern blot analysis shows that Trop-2 is likely a single copy gene in human cells. The DNA sequence of the coding strand of the Trop-2 gene does not show significant homology to any other sequence available from data banks. However, quite surprisingly, the template strand contains an entire gene of the Trop-1 family, tumor markers usually co-expressed with gp50/Trop-2. Even more surprisingly the template strand transfects COS cells for a protein apparently undistinguishable from Trop-2 on serological grounds, as confirmed by in vitro gene transcription and translation. To define the regulatory elements of the two complementary chains of the Trop-2 gene we have cloned and sequenced the genomic Trop-2 gene. The genomic Trop-2 gene is intronless and transfects again for Trop-2 in COS cells in both orientations. We are currently defining possible post-transcriptional processing of the Trop-2 transcripts and we are determining by mutagenesis the DNA sequences responsible for the observed patterns of expression.

NZ 202 TISSUE-RELATED ANTIGENS ON MELANOMA AND MELANOCYTES ARE RECOGNIZED BY HLA-A2-RESTRICTED CYTOTOXIC T CELL (CTL) CLONES FROM MELANOMA PATIENTS. Andrea Anichini, Cristina Maccalli, Roberta Mortarini, Arabella Mazzocchi, Paola Squarcina, Meenhard Herlyn[1] and Giorgio Parmiani, Division of Experimental Oncology D, Istituto Nazionale Tumori, Milan, Italy and [1]The Wistar Institute, Philadelphia, PA, USA.

$CD3^+$, $CD8^+$, $WT31^+$ CTL clones, acting by a T cell receptor- and HLA class I-dependent mechanism, were isolated from peripheral blood and tumor site of two $HLA\text{-}A2^+$ melanoma patients with the aim of verifying the possible recognition of neoplastic and normal cells of the melanocyte lineage. Some of these CTL clones lysed not only the autologous melanoma but also allogeneic HLA-A2-matched normal melanocytes isolated from newborn foreskin. Lysis of normal melanocytes and of neoplastic cells was HLA-A2-restricted as indicated by blocking experiments with HLA-A2-specific monoclonal antobodies. Lysis of $HLA\text{-}A2^+$ melanocytes was not dependent on the presence of bovine or human serum in the culture medium and was seen on proliferating as well as on growth arrested melanocytes. The HLA-A2-restricted CTL clones lysed all but one of 9 allogeneic $HLA\text{-}A2^+$ melanomas but none of 9 $HLA\text{-}A2^-$ melanomas. In addition, the same CTL clones did not lyse any of 21 normal or neoplastic cells of non-melanocyte lineage, including autologous B lymphoblastoid cell lines, fibroblasts, and carcinomas, even though HLA-A2 was expressed on 14 of these cells. These results indicate that at least a proportion of the "common" melanoma antigens defined by autologous T cells, and seen in association with HLA-A2, are tissue-related and expressed on normal and neoplastic cells of the melanocyte lineage.

NZ 203 EVALUATION OF THE JOINING REGION SEGMENT OF $p210^{BCR\text{-}ABL}$ CHIMERIC PROTEIN AS A POTENTIAL LEUKEMIA-SPECIFIC ANTIGEN TO ELICIT CLASS I MHC-RESTRICTED CTL RESPONSES. W. Chen, S.G. You, M.L. Disis and M.A. Cheever, Div. of Oncology, Dept. of Medicine, Univ. of Washington School of Medicine, Seattle, WA 98195

The hallmark of chronic myelogenous leukemia is the transposition of the *c-abl* protooncogene (*ABL*) from chromosome 9 to the specific breakpoint cluster region (*bcr*) of the *BCR* gene on chromosome 22. The transposition results in the formation of a new *BCR-ABL* fusion gene which encodes a 210 kD chimeric protein with tyrosine kinase activity. The segment of protein encoded by the joining region of the *BCR-ABL* transcript is composed of a unique sequence of amino acids which are expressed only by malignant cells. Our previous studies have demonstrated that immunization of mice with peptides corresponding to the joining region of $p210^{BCR\text{-}ABL}$ protein can stimulate class II MHC-restricted $CD4^+$ T cell responses specific for both the immunizing peptide and the $p210^{BCR\text{-}ABL}$ protein. The purpose of the current ongoing study is to determine whether the joining region segment of $p210^{BCR\text{-}ABL}$ protein can similarly elicit class I MHC-restricted $CD8^+$ CTL responses in mice and humans. In initial murine studies, Balb/c mice were immunized with synthetic peptides corresponding to the joining region of $p210^{BCR\text{-}ABL}$ protein. T cells were restimulated periodically *in vitro* with syngeneic antigen-presenting cells plus peptides. The $CD8^+$ T cell lines and clones elicited could specifically lysis syngeneic targets coated with the immunizing peptides, and were class I MHC-restricted. The peptide-specific T cell clones were specific for the joining region segment. Whether the peptide-specific T cells can lyse murine leukemia cells transferred by the BCR-ABL fusion gene will be evaluated. In initial human studies, synthetic peptides corresponding to the joining segment of $p210^{BCR\text{-}ABL}$ protein were analyzed for the ability to bind to class I MHC molecules. The T2 cell line which expresses unstable "empty" class I molecules (HLA-A2) was used to assess peptide binding. Results showed that particular peptides corresponding to the joining segment of $p210^{BCR\text{-}ABL}$ protein can bind to HLA-A2, but binding depends on precise construction of peptides, and not all potential peptides can bind. The observation that BCR-ABL peptides can bind to the human HLA-A2 molecules demonstrates that the generation of human CTL to BCR-ABL is possible. Ongoing studies are evaluating whether joining region peptides that bind to HLA-A2 can be used to elicit peptide-specific CTL by primary *in vitro* sensitization using purified dendritic antigen presenting cells.

NZ 204 EVALUATION OF HER2/NEU PROTEIN AS A POTENTIAL TARGET FOR CLASS I MHC-RESTRICTED CTL.

ML Disis, JW Smith, AE Murphy, W Chen, MA Cheever, Division of Oncology, University of Washington, Seattle WA 98195.

A major issue in developing specific T cell therapy for human malignancy is defining appropriate antigenic targets recognizable by autochthonous T cells. The aim of the current studies is to determine whether HER2/neu protein expressed by human breast cancer is an appropriate CTL target. HER2/neu is a transmembrane protein with intrinsic tyrosine kinase activity, and most likely functions as a growth factor receptor. The HER2/neu proto-oncogene is amplified and over-expressed in a variety of malignancies and is associated with poor prognosis in breast cancer. The encoded HER2/neu protein is a normal self protein and is not unique to malignant cells. Thus, self tolerance may prevent generation of immune CTL. However, HER2/neu is markedly overexpressed in malignancy and therefore any CTL elicited might lyse tumor preferentially and not destroy normal tissue. HER2/neu contains 1260 amino acids (aa). It has an extracellular binding domain (ECD) of approximately 650 aa, with 40% homology to epidermal growth factor receptor (EGFR), a highly hydrophobic transmembrane anchor domain (TMD), and a carboxyterminus cytoplasmic domain (CD) of approximately 580 aa with 80 % homology to EGFR. CTL normally recognize peptides derived from cellular proteins which have been processed and presented to the T cell receptor in the groove of Class I MHC. We chose HLA-A2 as a prototype human class I MHC model for study. The binding motif has been described [Falk, K et al. Nature 351, 290-296 (1991)] and the mutant cell line, T2, can be used as an assay system for peptide binding. In initial experiments, we analysed the normal sequences of HER2/neu for 9 aa segments with the potential to bind to HLA- A2. 19 peptides containing at least one of the dominant anchor residues were chosen for synthesis. The range of homology to EGFR was 0-89% (median 46%). 9 peptides were included from the ECD, 3 from the TMD, and 7 from the CD. Only 11/19 peptides could be constructed due to insolubility of the aa sequences chosen. Of the 9 peptides constructed, 6 have been shown to bind to HLA-A2 in the T2 binding assay. T2 fails to appropriately process peptide and as a result has low expression of HLA-A2 (30-50%). When incubated with peptides able to bind A2, the level of Class I MHC stabilizes on the cell surface and can be measured by immunofluorescent staining. Binding of peptides was determined by an increase in Class I surface expression to 60-85%. Non-binding peptides had no effect on class I MHC expression. Ongoing studies are evaluating whether the HER2/neu peptides that bind to HLA-A2 can be used to elicit peptide-specific CTL by primary *in vitro* immunization using homozygous HLA-A2 antigen presenting cells.

NZ 205 MHC-RESTRICTED AND UNRESTRICTED RECOGNITION OF EPITHELIAL TUMOR MUCIN BY T CELLS.

Nieves Domenech, Julie Magarian-Blander, J. Darrell Fontenot, Dawen Bu and Olivera. J. Finn. Department of Molecular Genetics and Biochemistry, University of Pittsburgh, School of Medicine, Pittsburgh PA, 15216

Mucins are large, heavily glycosylated molecules expressed and secreted by ductal epithelial cells and tumors of the same origin. The deduced protein sequence of both pancreatic and breast mucin consists of multiple copies of a 20-amino acid tandem repeat. We identified an epitope expressed in each repeat which serves as a target for tumor specific CTL.

Our previous work demonstrated that anti-mucin cytotoxicity was specific but MHC-unrestricted. We have now established mucin specific CTL lines and clones derived from breast and pancreatic cancer patients using mucin-transfected autochthonous and allogeneic EBV-B cells. The results of the functional analysis of those cells confirm that anti-mucin reactivity can be antigen specific and MHC-unrestricted. In as much as we consider this to be due to the highly repetitive nature of the mucin CTL epitopes, and the ability of a large number of epitopes to engage multiple TCR simultaneously, we have also generated autochthonous and allogenic targets transfected with cDNA vectors which encode mucin carrying only two CTL epitopes. We are now investigating the potential of these cells to stimulate only autochthonous but not allogeneic mucin specific CTL.

We have also synthesized a series of 9 aa long overlapping peptides which span the mucin polypeptide tamdem repeat sequence. Exogenus addition of some of those peptides restores the expression of HLA class I on acid-treated APC. These results mean that mucin peptides are capable of binding, at least, some class I alleles. Class I negative cells expressing defined transfected class I alleles are now being used to determine how restricted this finding is. At the same time we are isolating the peptides bound by Class I and Class II molecules from mucin-transfected EBV-B cells analyzing them for the presence of mucin peptide sequences.

The results of these studies will provide comprehensive information about processing and presentation of this unusual antigen to the immune system. We expect this information to be applicable to the treatment of epithelial cancers.

NZ 206 EVIDENCE FOR BIOCHEMICAL HETEROGENEITY OF GP96 HEAT SHOCK PROTEIN / TUMOR REJECTION ANTIGEN , Anna M Feldweg and Pramod K Srivastava, Department of Pharmacology, Box 1215, Mount Sinai School of Medicine, New York, NY 10029

Gp96 tumor rejection antigens are nearly identical to ERp99, grp94, hsp100 and hsp108. Significant differences among these molecules are : (i) mouse gp96 cDNA is identical to endoplasmin/ERp99 in the coding but not in the 5'–untranslated region; (ii) the 5' flanks of human grp94 and gp96 have similar but not identical sequences ; (iii) a human hsp108 gene is similar but not identical to human gp96. These persistent differences raise the question if gp96, hsp100, hsp108, ERp99 and grp94 represent a multigene family of related genes or if the differences are attributable to allelic variation of a single gene.

Three serological reagents were used to analyze the complexity of gp96 : (i) anti- murine gp96 rabbit antiserum, (ii) anti-NH2 terminal peptide HS-1 rabbit antiserum (from M Green) (iii) anti-murine grp94 rat mAb. Iodinated gp96 from the Meth A sarcoma was precipitated with each antibody until no further precipitable material could be detected by SDS-PAGE. The unprecipitated material was then precipitated with the other two antibodies. It was observed that : (i) rabbit anti-gp96 and anti-HS-1 antisera precipitate all the gp96 and completely pre-clear each other; (ii) *a significant proportion of gp96 remains unprecipitated by the anti - grp94 mAb. This can however be precipitated fully by the anti-gp96 or anti-HS-1 antisera*; These observations suggested that the anti-grp94 mAb was recognizing a sub-population of gp96 molecules. The possibility that these results may reflect differences in the relative affinities of the mono- and polyclonal antibodies was ruled out by repeating the pre-clearings by solid-phase immunoaffinity columns. Our results suggest that gp96, like its cytosolic counterpart hsp90, may exist in two similar but distinct isoforms.

NZ 207 EFFICIENT PROCESSING AND PRESENTATION of H-2D^b- AND H-2K^b-RESTRICTED CYTOTOXIC T LYMPHOCYTES (CTL) EPITOPES EXPRESSED AT DIFFERENT LOCATIONS IN SIMIAN VIRUS 40 (SV40) LARGE T ANTIGEN. Tong-Ming Fu, Robert H. Bonneau, Mary J. Tevethia and Satvir S. Tevethia, Department of Microbiology and Immunology, Pennsylvania State University College of Medicine, Hershey, PA 17033.

Cytotoxic T lymphocytes (CTL) recognize 8 or 9 amino acid peptide fragments derived from viral or tumor antigens in association with major histocompatibility complex (MHC) Class I molecules on the surface of viral infected or tumor cells and results in the specific lysis of these target cells. The main objective of this study was to determine the effect of the epitope location on antigen processing and presentation. One of the five CTL epitopes of SV40 T-Ag, Site I (residues 205-215), and a herpes simplex virus (HSV) glycoprotein B (gB) epitope (residues 498-505) were inserted at amino acid positions 350 and 650 of SV40 T-Ag by synthetic oligonucleotide fragment insertion. The T-Ag constructs used for this purpose contain an *EcoRI* linker introduced at T-Ag residues 350 and 650, respectively. The resultant T-Ag constructs, named pSite I/350, pSite I/650, pgB/350 and pgB/650, were confirmed by DNA sequencing and transfected into C57BL/6 mouse embryo fibroblasts, from which stable T-Ag-transformed cell lines were derived. The expression of recombinant T-Ag in these cell lines was verified by Western blot and by immunoprecipitation analysis. The effect of epitope location on antigen prosessing and presentation was examined by a standard cytotoxicity lysis assay (^{51}Cr release assay) by using epitope-specific CTL clones. The epitope-specific CTL clones used in the study were H-2D^b-restricted Y-1 (or K-11) for recognition of the SV40 T-Ag Site I and H-2K^b-restricted 2D5 for recognition of the HSV gB epitope. The results showed that SV40 Site I and HSV gB epitope are efficiently expressed at residues 350 and 650 of SV40 T-Ag, and recognized by epitope-specific CTL clones. Our results indicate that both an H-2D^b-restricted epitope (SV40 T-Ag Site I) and H-2K^b-restricted epitope (HSV gB epitope) can be processed, presented and recognized by the epitope-specific CTL clones from the same locations in SV40 T antigen.

NZ 208 **ONCOGENE DERIVED PEPTIDES. A NEW CLASS OF TUMOR REJECTION ANTIGENS?** *Gustav Gaudernack, Tobias Gedde-Dahl III, Beate Fossum, Bente H. Olsen, Jon Amund Eriksen and Erik Thorsby. Institute of Transplantation Immunology, The National Hospital and The University of Oslo, Norway*

ras proto-oncogenes activated by point mutations within codons 12, 13 or 61 are frequently found in human tumors. Proteins derived from mutated p21 ras may give rise to immunogenic peptides following intracellular processing. Using cells from several healthy donors we have demonstrated that synthetic peptides derived from mutated p21 ras can elicit specific T cell responses in vitro after repeated stimulations. Both the peptides and the corresponding recombinant p21 ras proteins could be efficiently presented to the T cell clones by EBV transformed cell lines, carrying the appropriate HLA molecules. For one of these peptides we could show that three different T cell clones which were restricted either by HLA-DR2, -DQ7 or -DP3 molecules, recognized partly overlapping epitopes contained within the same peptide. Memory T cell responses towards such mutated ras derived peptides could also be detected in some cancer patients. In a cellular peptide competition assay and/or in a direct binding assay using purified HLA molecules, we could show that most of the synthetic peptides bound to HLA-DR1 and -DR2, and that all bound to DQ6. These observations indicate that binding to HLA of mutated ras derived peptides may be a relatively general phenomenon, perhaps reflecting an important surveillance function in eliminating cells carrying potentially deleterious ras mutations. Immunotherapy targeted against a common specific genetic alteration of neoplastic cells may thus be possible.

Our results also suggest a strategy for active immunization to obtain tumor rejection in patients carrying ras mutations, based on highly immunogenic artificial tumor cells constructed by loading autologous irradiated EBV transformed cell lines with the appropriate synthetic peptide.

NZ 209 CYTOTOXIC T LYMPHOCYTES WITH RECOGNITION SPECIFICITY FOR ERBB-2 EXPRESSING TUMOR CELLS, Bernd Groner, Winfried Wels, Nancy Hynes and Dirk Moritz, Friedrich Miescher Institut, P.O. Box 2543, CH 4002 Basel, Switzerland

The amplification of the erbB-2 proto-oncogene is a frequent mutation in breast-, ovarian- and lung carcinomas and causes the overexpression of a growth factor receptor with tyrosine specific protein kinase activity. The enhanced receptor expression on the surface of tumor cells, when compared to normal epithelial cells, provides a target for directed tumor therapy. In order to enable cytotoxic T lymphocytes to recognize and eliminate erbB-2 overexpressing cells, their recognition specificity was manipulated in vitro. For this purpose we used insights into the CTL components important for target cell recognition and signal transduction. The α/β chains of the T cell receptor (TCR), responsible for target cell recognition by most CTL, are associated with the proteins of the CD3 complex (γ, δ, ϵ and ζ). The ζ chain of the CD3 complex plays a central role in the induction of cytolysis. We have attempted to circumvent the recognition specificity of the α/β chains of the TCR and confer a target cell recognition function directly to the ζ chain. For this purpose we derived a single chain antibody construct (scFv) from mRNA of a hybridoma cell line producing monoclonal antibody against the extracellular domain of the erbB-2 receptor. The scFv domain was fused with the cDNA of the ζ chain to yield a chimeric scFv-ζ gene, which was introduced into established CTL lines via retroviral gene transfer. The expression of the scFv-ζ protein was detected in infected CTL by immunoprecipitation with antibodies specific for the scFv and the ζ chain. The scFv-ζ protein was present as a transmembrane protein and scFv-ζ homodimers as well as scFv-ζ ζ heterodimers were detected. The killing capacity of the manipulated CTL has been evaluated on erbB-2 expressing target cells.

NZ 210 **IN-VITRO INDUCTION OF PRIMARY CTL RESPONSE AGAINST WILD TYPE OR MUTANT p53 PEPTIDES.** **Jos G.A. Houbiers[1,2], H.W. Nijman[1,3], S.H. vd. Burg[2,1], J.W. Drijfhout[1], P. Kenemans[3], C.H.J. vd. Velde[2], A. Brand[1], W.M. Kast[1], C.J.M. Melief[1].** Dept. [1]Immunohaematology & Blood Bank and [2]Surgery, University Hospital Leiden, The Netherlands, Dept. [3]Obstetr. & Gynecology, Free University Hospital, Amsterdam

The p53 tumor-suppressor gene is mutated and/or overexpressed in a substantial proportion of human tumors and therefore an attractive target for immunotherapy of cancer. Several different peptides of wild type and mutant p53 have been selected for HLA-A2.1 binding using the HLA "upregulation" phenomenon upon exogenously adding of a binding peptide to the human processing defective 174CEM.T2 cell line (T2; abstract Nijman H.W., *et al.*). Post-Ficoll peripheral blood mononuclear cells (PBL) of an HLA-A2.1 donor were added to peptide loaded T2 cells (as antigen presenting cells) in medium containing pooled human serum, the same HLA-A2.1 binding p53 peptide (A8), but no Interleukin 2. On day 7 and 14 viable responding cells were restimulated. On day 21 the bulk responders were cloned by limiting dilution and growing clones were expanded.

The most efficient protocol variant resulted in highly specific, CD8+, CTL clones which lyse HLA-A2.1 target cells sensitized with A8 peptide. The CTL clones lyse in loading peptide concentration and effector-to-target ratio dependent way down to 10ng/ml and 0.3:1 respectively. Application of various target cells and monoclonal antibody cytotoxicity blocking proved the HLA-A2.1 restriction of the clones. Amino acid replacement studies showed the fine specificity and probed the importance of epitope position 5, (6, 7) and 8 for HLA-A2.1 restricted CTL recognition. We also succeeded in generating CTL clones directed against other (mutant) p53 peptides. CTL clones capable of lysing tumor cells with the relevant p53 mutation or with p53 overexpression bear the potential for cellular immunotherapy of cancer. It is concluded that our protocol with T2 cells as APC is useful for *in-vitro* primary immune response induction and that autoreactive CTL precursors for epitopes of wild type p53 are present in healthy donors.

NZ 211 p53 PEPTIDES RECONSTITUTE HLA-A2 EXPRESSION AND CONFER SUSCEPTIBILITY TO TIL-LYSIS, Gerhard H. Leder[1], Walter J. Storkus[1], György Stuber[2], Susanne Modrow[3] and Michael T. Lotze[1], Departments of Surgery and Molecular Genetics and Biochemistry and the Pittsburgh Cancer Institute, University of Pittsburgh, Pittsburgh, PA 15261[1]; Department of Tumor Biology, Karolinska Institute, Stockholm, S-10401, Sweden[2]; Institut für Medizinische Mikrobiologie und Hygiene, Universität Regensburg, 8400 Regensburg, Germany[3]

Nonameric peptides conforming to the recently defined MHC class I peptide binding motifs are considered to be the predominant antigenic epitopes recognized by the T-cell receptor. Peptide binding motifs have recently been determined for a series of MHC class I molecules, including the HLA-A2 allele (Falk K. et al., (1991) Nature 351:290). Based on these predicted requirements for peptide binding to the HLA-A2 class I molecule, 21 peptides (9-11 amino acid long) derived from the human p53 sequence were identified and 18 synthesized. The ability of these peptides to bind to the HLA-A2 class I structure was examined by testing their potential to reconstitute BB7.2 reactive HLA-A2 complexes on 2 different HLA-A2 positive B-cell lines (K4B and T2) that had previously been acid (pH3.3) treated. This procedure has previously been shown to reliably identify peptides binding to different MHC class I motifs (Storkus W. et al. submitted for publication). Of the 18 synthetic peptides, 10 reproducibly induced significant reconstitution of the HLA-A2 complex. Four of 18 peptides did not reconstitute the HLA-A2 complex and an additional 4 peptides showed weak and variable ability to reconstitute the HLA-A2 complex. Binding to an HLA-A2 peptide motif would only be a minimum requirement for a peptide to serve as a T-cell epitope. To test directly for T-cell reactivity with p53 peptides four of the HLA-A2 binding peptides were pulsed onto a K4B B-cell line that subsequently was assayed for susceptibility to melanoma derived TIL-lysis in a Cr^{51}-release assay (p53 is overexpressed and/or mutated in 85% of all melanomas). Two of the peptides were found to be competent to confer elevated melanoma TIL lytic activity to B-cell targets. This degree of lysis is comparable to that observed for the autologous melanoma target. Currently we are examining the potential of these p53 peptides to stimulate a primary *in vitro* CD8+ T-cell response.

NZ 212 Gp96 IS AN ATPase, Zihai Li & Pramod K Srivastava Mt Sinai Sch of Med, Box 1215, New York, NY 10029

Immunization of mice with gp96 elicits protection against a challenge with the tumor from which gp96 is isolated but not another tumor (1). Gp96 from normal tissues does not elicit protection against any tumors tested (2). The specificity of immunogenicity of gp96 does not reside in the DNA sequence or glycosylation of gp96. In light of our demonstration that gp96 is a heat shock protein (hsp) and that some hsps are known to bind peptides we have proposed (3) that gp96 acts as a chaperonin for peptides, which confer on it the specificity of its immunogenicity. Further, as gp96 molecules reside largely in the endoplasmic reticulum (ER), we suggested (3) that it acts as a peptide-acceptor in the ER lumen, and that it may be accessory to charging of MHC class I with peptides.

As peptide-chaperoning/sequestering is likely to be ATP-dependent, we looked for and identified ATP-binding Walker sequences in gp96. When extracts of metabolically ^{32}P-labelled cells are precipitated with anti-gp96, anti-hsp70 and anti-MHC class I antisera, ^{32}P is found associated with gp96 and hsp70 but not class I. ATP and ADP are found associated with gp96 and hsp70, but not with class I on TLCs of the precipitates. The results indicate that gp96 binds ATP in vivo.

An ATPase activity is observed in gp96 preparations. The activity is inherent in gp96 and not associated with it, as observed by loss of the ATPase activity on specific immunodepletion of gp96 from active preparations. The ATPase activity has a K_m of 8 μM, a temperature optimum of 42 C and a wide pH optimum. The activity is Mg++ and Ca++ dependent, is not stimulated by exogenous peptides, but is significantly stimulated by other proteins such as casein. These observations are consistent with a role for gp96 in an energy-depenedent process in the ER lumen.

1. Srivastava et al. 1986 PNAS 83, 3407. 2. Udono et al. this meeting.
3. Srivastava & Maki 1990 Curr Top Micro Immun. 167, 109

NZ 213 IDENTIFICATION OF POTENTIAL CYTOTOXIC T CELL (CTL) EPITOPES FOR HLA-A2.1 USING A PROCESSING DEFECTIVE HUMAN CELL LINE, 174CEM.T2 (T2), AND A SCORING SYSTEM DEDUCED FROM THE HLA-A2.1 PEPTIDE MOTIFS.

H.W. Nijman[1,3], J.G.A. Houbiers[1,2], S.H. vd. Burg[2,1], M.P.M. Vierboom[1], A. Brand[1], C.J.H. vd. Velde[2], P. Kenemans[3], W.M. Kast[1], C.J.M. Melief [1].
Dep. of [1]Immunohaematology & Blood Bank and [2]Surgery, University Hospital, P.O. Box 9600, 2300 RC LEIDEN, The Netherlands; [3]Dep. of Obstetrics and Gynecology, Free University Hospital, De Boelelaan 1117, 1007 MB AMSTERDAM, The Netherlands.

For identification of potential CTL epitopes of human proteins we used a human processing defective cell line T2. T2 expresses "unstable" HLA-A2.1 molecules which can be stabilized by exogenously added peptides. This stabilization can be detected as an increase in HLA-A2.1 expression, measured by immunofluorescence using the mouse monoclonal antibody BB7.2 and FITC labeled goat anti-mouse $F(ab)_2$ antibody. The synthetic peptides used for HLA-binding studies were selected using a scoring system deduced from the HLA-A2.1 specific motifs. Of the influenza matrix protein and p53 protein mainly high scoring peptides upregulated the HLA-A2.1 expression on the T2 cell line. Twenty-five different wild type and four mutant p53 HLA-A2.1 peptides have been selected in this way. Of one of the binding p53 peptides we replaced one at a time every residue by alanine or arginine. Position 2,3 and 9 seem to be essantial for binding in HLA-A2.1. The binding p53-epitopes are being used for induction of a primary CTL response with peptide loaded T2 cells as the antigen presenting cell (see abstract of J.G.A. Houbiers, et al.).
We conclude that the combination of our scoring system and the processing defective T2 cell line is a good tool in selecting potential epitopes for (primary) CTL induction.

NZ 214 T CELL RECEPTOR (TCR) V GENE USAGE IN MELANOMA SPECIFIC TUMOR INFILTRATING LYMPHOCYTES (TIL).

Michael I. Nishimura, Yutaka Kawakami, Patrick Charmley[1], Bert O'Neil, Joel Shilyansky, John R. Yannelli, Steven A. Rosenberg, and Leroy E. Hood[2]. Surgery Branch, National Cancer Institute, Bethesda, MD 20892, [1]Virginia Mason Research Center, Seattle, WA 98101, [2]Department of Molecular Biotechnology, University of Washington, Seattle, WA 98195.

CTL can be isolated from human melanoma biopsies that specifically lyse autologous melanoma in vitro and can be effective therapeutic agents for patients with advanced disease. Recent evidence indicates that HLA-A2 restricted, melanoma specific TIL recognize melanoma obtained from different HLA-A2 positive patients suggesting the presence of one or more common melanoma antigens. Furthermore, analysis by other groups of the TCR repertoire of TIL in fresh melanoma biopsies suggests limited TCR V gene usage in TIL. One serious limitation in analyzing the TCR repertoire in fresh tumors has been the inability to correlate TCR usage with immune function. Therefore, the TCR repertoire was determined in TIL cultures which specifically lyse autologous melanoma in vitro and in many cases, mediate regression of metastatic lesions in patients with advanced disease. The TCR repertoire in cultured melanoma specific TIL (n=15) was diverse, with each TIL containing an average 6.2 ± 2.9 of the 23 Vα and 7.3 ± 2.9 of the 23 Vß families. Furthermore, there is no correlation between the TCR V gene usage and HLA haplotype, clinical response, or length of culture. The large TCR diversity observed in TIL implies that there is no restricted TCR V gene usage to a single melanoma antigen. This diversity may be due to multiple melanoma associated antigens, each capable of eliciting CTL responses, and/or presentation of melanoma associated antigens by multiple HLA molecules in a single patient can lead to a heterogeneous CTL response. A principle goal of this study remains to determine which TCR V genes contribute to in vitro and in vivo TIL function. While there is no direct demonstration that the presence of a particular TCR V gene contributes to TIL function, indirect evidence suggests that certain TCR V genes may be involved lysis of human melanoma. Several Vα and Vß gene segments are present in the majority of the TIL cultures tested. Vα1, Vα2, Vα7, Vα21, and Vα22 appear to be the most frequently utilized Vα gene segments and Vß13, Vß14, and Vß18 appear to be the most frequently utilized Vß gene segments in the TIL lines tested. Northern blot analysis indicates that Vα2 and Vα7 are predominant TCR rearrangements in some bulk TIL populations implying their involvement in melanoma specific lysis. Although results in this study suggest which TCR V gene segments are involved in immune responses to human melanoma, analysis of clonal melanoma reactive CTL is necessary to determine which clonotypes are important in lysis of human melanoma.

NZ 215 CELLS TRANSFORMED BY THE EJRAS ONCOGENE EVADE DESTRUCTION BY TUMOR-SPECIFIC CTL.

Rienk Offringa, R. Toes, R. Blom, C.J.M. Melief and W.M. Kast, Dept. of Immunohematology and Blood Bank, University Hospital Leiden, The Netherlands.

Mouse embryo cells (C57BL/6, H-2^b) transformed by the E1a and E1b genes of Human Adenovirus type 5 (Ad5MEC) are highly immunogenic. These cells, although forming large tumors in nude mice, are not tumorigenic in syngeneic immunocompetent animals. Ad5MEC are efficiently killed by specific CTL clones in vitro and, after adoptive transfer of CTL, in vivo. Examination of Ad5MEC-reactive CTL clones revealed two types of H-$2D^b$-restricted CTL, the respective specificity of which is (1) an Ad5E1a peptide (SGPSNTPPEI) and (2) a peptide that is apparently encoded by an Ad5E1a-induced cellular gene.
Cells that, in addition to Ad5E1a/E1b, express an activated H-ras oncogene (EJras) are highly tumorigenic in syngeneic immunocompetent mice. Furthermore, tumors of these cells in nude mice were not eradicated by adoptive transfer of Ad5E1a-specific CTL. Ad5+ras MEC are, however, efficiently killed by these CTL in vitro, indicating that the tumor cells do present the highly immunogenic E1a-peptide. Interestingly, Ad5+ras MEC express and secrete considerable amounts of active Transforming Growth Factor β (TGF-β). Secretion of this immunosuppressive agent may enable the cells to evade destruction by Ad5E1a-specific CTL in vivo. Moreover, Ad5+ras MEC were found to lack the second, cell-encoded CTL-epitope (see also abstract of C.J.M. Melief et al.). We are, at present, determining the relative contribution of these two properties of Ad5E1+ras MEC to the escape from T cell immunity.

NZ 216 EXPRESSION OF THE MAGE-1 GENE BY HUMAN HEMATOLOGICAL MALIGNANCIES, Daniel Olive*, Hervé Chambost*, Francis Brasseur**, Pierre Coulié**, Anne-Marie Stoppa*, Daniel Baume*, Jacques Camerlo, Patrice Mannoni*, Thierry Boon** and Dominique Maraninchi*, *INSERM U.119 and Institut Paoli Calmettes, Marseille, France ; ** Ludwig Institute for Cancer Research Brussels Branch, Belgium

High-dose r-IL2 alone induce partial or complete remission in some cases of acute myeloid leukemias (AML) .The mechanisms of the antileukemic effect observed in vivo are not clearly explained .The expression of a tumor rejection antigen by some leukemic blasts could lead to a specific antileukemic response that might be amplified by the administration of r-IL2. However there is no evidence until now for the existence of cytotoxic T lymphocytes (CTL) specifically directed against leukemic blasts Recently a gene called MAGE-1 has been identified (van der Bruggen et al.) that directs the expression of a tumor rejection antigen Gene MAGE-1 is expressed in a significant proportion of melanomas and breast tumor samples, as well as in other types of tumors . We looked for expression of gene MAGE-1 by hematological malignancies using PCR with MAGE-1 specific primers . We analyzed 45 fresh leukemia samples and 10 cell lines derived from hematological malignancies. This panel included acute(n=36) and chronic (n=4) leukemias from all lineages (myeloid, lymphoid T and B) at different stages of differenciation, as well as multiple myeloma and Waldenstrom disease. All the fresh samples proved to be negative for the expression of gene MAGE-1, but cell lines K 562, HEL and HL-60 expressed MAGE-1. The positivity of these cell lines is probably not related to the culture conditions since other leukemic B , T and myeloid cells lines were negative. This suggests that a larger number of leukemia samples should be analyzed . The expression of gene MAGE-1 by some hematological cell lines could provide an in vitro model for the immunogenicity of leukemic blasts.

NZ 217 EVALUATION OF THE TUMOR INFILTRATING LEUKOCYTES IN BREAST CARCINOMAS ACCORDING TO THE c-erbB-2 ONCOPROTEIN OVEREXPRESSION, Serenella M. Pupa, Sylvie Ménard, Salvatore Andreola, Barbara Pozzi, Rosaria Bufalino and Maria I. Colnaghi, Istituto Nazionale per lo Studio e la Cura dei Tumori, Milan, Italy

We recently reported (Int. J. Cancer: 49, 44-49, 1991) that c-erbB-2 oncoprotein (neu) overexpression in breast carcinoma is strongly associated ($p=10^{-6}$) with the presence of lymphoplasmacytic infiltration (LPI) in the tumor and that LPI is indicative of a good prognosis only in the oncogene-positive subgroup. On the contrary, in the neu-negative group, there is a trend for LPI positivity to be associated with a poor prognosis. When LPI was analyzed in association with the necrosis, a strong association ($p=10^{-4}$) was found in the neu-negative cases, whereas in the neu-positive group this association was only borderline. These observations suggest the presence of 2 types of LPI: one corresponding to a specific anti-tumor response in the neu-positive group and another one consisting of an aspecific infiltration induced mainly by tumor necrosis. To verify this hypothesis, the phenotype of the LPI detected in 67 primary breast carcinomas was characterized by immunohistochemistry performed on cryosections using a panel of 8 MAbs directed against different leukocyte subpopulations. The results were analyzed according to the neu overexpression. In keeping with the previously observed association between neu and LPI, the frequency of neu-positive tumors was high (55%) in this series. The leukocyte phenotypic analysis indicates that neu-positive tumors are more frequently infiltrated by monocytes and granulocytes than neu-negative ones. This data, apparently in constrast with our hypothesis, may be explained by the fact that neu overexpression is frequently associated with the expression of other oncogenes such as c-fms. CSF-1 released by the tumor cells might be responsible for mono/granulocytic infiltration.

NZ 218 HLA-A2 RESTRICTED LYSIS OF HUMAN RENAL CELL CARCINOMA BY TUMOR-INFILTRATING LYMPHOCYTES, Dolores J. Schendel, Bernd Gansbacher, Ralph Oberneder and Oscar G. Segurado, Institute of Immunology and Department of Urology, University of Munich, Germany and Sloan-Kettering Institute for Cancer Research, New York, NY 10021

Primary renal cell carcinoma (RCC), like melanoma, belongs to the small group of human tumors in which partial or complete remission has been observed in some patients following treatment with various forms of immunotherapy. CTL showing MHC-restricted lysis of RCC have not been easily found among tumor-infiltrating lymphocytes (TIL), leading to the suggestion by some that responses to immunotherapy are mediated by non-MHC-restricted cells. We isolated MHC-restricted, CD8-positive CTL from a TIL population cultured from primary RCC; these CTL represent a majority of the short-term cultured population. The CTL lysed autologous tumor cells but not normal kidney cells or targets sensitive to LAK or NK cells. In contrast, LAK cells isolated from the peripheral blood lysed autologous tumor and normal kidney cells and allogeneic tumors. The TIL could be expanded optimally using an autologous tumor line retrovirally transduced with the human gene encoding IL-2. The TIL utilized a restricted number of Vα gene families, suggesting that they recognize a limited number of MHC-peptide complexes. HLA-A2 was identified as an MHC restriction molecule for presentation of one unknown tumor-derived peptide to these CTL. Only some allogeneic HLA-A2 RCC tumors were lysed, indicating that heterogeneity in MHC or peptide influences CTL recognition. These studies demonstrate that RCC tumors express antigenic determinants that can be recognized by highly specific CTL and open the possibility to define the nature of RCC-derived peptides which can generate an effective immune response.

NZ 219 T CELL RECEPTOR VARIABLE GENE USAGE IN MELANOMA SPECIFIC TIL CLONES AND LINES. Joel Shilyansky, Michael I. Nishimura, John R. Yanelli, Yutaka Kawakami, Bert O'Neil, James J. Mulé, Steven A. Rosenberg. Surgery Branch, National Cancer Institute, Bethesda, MD 20892.

Tumor infiltrating lymphocytes (TIL) obtained from human melanomas are capable of melanoma specific reactivity *in vitro* and can mediate tumor regression *in vivo*. In order to develop more effective therapeutic reagents and to further understand the T cell response to tumors, it is important to determine which clonotypes are involved in melanoma antigen recognition. This is particularly relevant since recent experimental evidence suggests the existence of shared melanoma antigen(s). Thus, a TIL line derived from one patient may be reactive against melanomas obtained from other patients who share HLA restriction elements. Analysis of T cell receptor (TCR) repertoire was previously performed on bulk TIL lines. These studies could not define the clonotypes responsible for melanoma specific lysis, but suggested that some variable (V) regions were found at increased frequency among the TIL lines. We determined the TCR variable gene segments used by four clonal and two oligoclonal TIL. All the TIL lines studied are CD8+ and specifically lyse autologous or HLA matched melanomas. TIL F11-21 (Vβ15), F2-2 (Vα21), A10 (Vα2.2, Vβ4), TIL 5 (Vα1, Vβ7) obtained by limiting dilution cloning, each expresses a single TCR, confirming their clonal nature. Line C10-1 (Vα14, Vα23, Vβ13) derived by limiting dilution and 1200 (Vα2, Vα9, Vβ10, Vβ22) obtained from a 61 day culture, each expresses two TCRs. Three TIL cultures (1200, F2-2 and TIL 5) are HLA-A2 restricted, but each uses a different TCR to recognize melanoma antigen. In summary, we found no limited V gene usage. This pattern was seen even among three HLA-A2 restricted TIL. These findings suggest that multiple TCR clonotypes may be reactive against a melanoma specific antigen. Alternatively, there may be multiple melanoma specific antigens presented on the surface of tumor cells in the context of different HLA haplotypes.

NZ 220 T CELL RECOGNITION OF HODGKIN'S LYMPHOMA ANTIGENS, AP Sing, RF Ambinder, PD Greenberg, Dept. of Medicine and Immun., Univ. of Washington, Seattle, WA. 98195.and Johns Hopkins Univ., Baltimore, MD. 21231

One obstacle that interferes with the general application of immunotherapy is *the difficulty identifying* target antigens on tumor cells that are not expressed on normal tissues and can elicit T cell responses. The expression of proteins encoded by oncogenic viruses associated with the development of malignancy provides potential target antigens for an immune response. The Epstein-Barr virus (EBV) is a transforming virus associated with several malignancies including Hodgkin's Disease (HD). Recent studies have revealed, in a subset of HD patients, the presence of a clonal EBV genome in the malignant Reed-Sternberg (R-S) cell, and detected expression of several potentially immunogenic EBV proteins.

Previous studies in healthy seropositive people have demonstrated an immunodominant response to specific EBV proteins, EBNA-3A and EBNA 3C. Unfortunately, neither of these proteins is expressed in R-S cells. However, R-S cells do express LMP-1 and LMP-2, both of which can elicit immune responses in normal people. Thus, manipulating and/or augmenting the response to LMP may have therapeutic benefit in a subset of HD patients. To determine whether there is a quantitatively deficient or qualitatively restricted immune response that prevents effective recognition or elimination of the malignant cells, we are now characterizing the cytotoxic T cell (Tc) response to LMP-1 and LMP-2 in EBV seropositive HD patients. Polyclonal cell lines have been established that have cytotoxic activity against autologous EBV transformed lymphoblastoid cell lines (LCLs). These lines are now in the process of being cloned by limiting dilution to determine the specificity and magnitude of this response for individual EBV encoded proteins.

Despite expression of EBV proteins in R-S cells and a potentially intact EBV-specific immune response, this maignant cell could escape immune surveillance by alternative mechanisms. For example, R-S cells could express Class I genes poorly or could have defects in the processing machinery resulting in inadequate presentation of EBV encoded proteins. To address these issues we are evaluating CTL reactivity to an *in vitro* maintained R-S cell line, L-428, derived from an HD patient. Endogenous expression of Class I alleles is being quantified and the response to inducing agents such as IFN-γ is being determined. Since this line is EBV negative, EBV genes will be introduced via infection with recombinant vaccinia viruses, and the ability of the cell line to process and present EBV proteins to Tc will be assessed. The results from these studies should elucidate the mechanism of immune escape and may lead to a potential therapeutic intervention.

NZ 221 RECOGNITION OF RAS PEPTIDES BY CLASS I RESTRICTED T CELLS

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Activated $p21^{ras}$ exhibiting a single amino acid substitution at either position 12 or 61 is associated with a variety of human malignancies. Class II MHC-restricted $CD4^+$ T cell responses specific to mutated $p21^{ras}$ have been demonstrated (1). Corresponding recognition by class I MHC-restricted $CD8^+$ T cells would suggest that p21 might be a tumour rejection antigen and a target for therapy in humans. Ras peptides with a $H\text{-}2K^b$ binding motif were synthesised and class I binding was measured (2) using mutant RMA-S cells which were shown to have a peptide loading defect resulting in the expression of a significant number of empty MHC molecules. Among several Ras derived peptides which bound to $H\text{-}2K^b$ one corresponded to amino acid 60-67 of the protein sequence. Peptides containing either wild type residue 61 (glutamine) or a transformation associated mutation (glutamine to lysine change) were found to bind to K^b. CTL lines were generated from C57 Bl/10 mice by stimulation with Ras peptides and class I restricted cytotoxicity was observed. These CTL lines were able to specifically recognise RMA targets pulsed with the immunizing Ras peptides. Experiments are being performed to test whether peptide specific CTL can recognise target cells expressing ras endogenously, which would suggest the use of ras as a target in immunotherapy of certain cancers.

1. Peace, D.J. (1991) J.Immunol. 146: 2059-2065
2. Stauss, H.J. (1992) PNAS 89: 7871-7875

NZ 222 **MULTIPLE SHARED HLA-A2.1 ASSOCIATED PEPTIDE EPITOPES FOR MELANOMA-SPECIFIC CTL,**

Craig L. Slingluff, Jr., Andrea L. Cox, Robert A. Henderson, Donald F. Hunt, Victor H. Engelhard, Departments of Surgery, Chemistry, and Microbiology, University of Virginia, Charlottesville, VA 22908

Identification of shared melanoma antigens recognized by human CTL has potential implications for understanding oncogenesis and for modulating the host immune response to melanoma. In the present study, HLA-A2.1-associated peptides were extracted from human melanoma cells and were used to study epitopes for HLA-A2.1-restricted human melanoma-specific cytotoxic T lymphocytes (CTL). CTL generated from human tumor-involved lymph node cells lyse autologous melanoma and four different HLA-A2.1+ human melanoma lines; they fail to lyse all other targets tested. HLA-A2.1 molecules were isolated from 10^{10} melanoma cells by detergent solubilization and immunoaffinity chromatography. Peptides bound to the MHC molecules were acid-eluted and fractionated by reversed-phase HPLC. Individual fractions were added to the HLA-$A2.1^+$ antigen-processing mutant, 721.174XCEM.T2 (T2), and several of these fractions were reproducibly capable of reconstituting epitopes for these melanoma-specific CTL. Using combinations of three different HPLC separation conditions, a minimum of six distinct peptide epitopes for melanoma-specific CTL were repeatedly demonstrated. All six epitopes were reconstituted from peptide extracts from each of two distinct allogeneic HLA-A2.1+ melanomas; therefore, they are believed to represent shared epitopes for melanoma-specific CTL. They may represent peptides derived from six distinct endogenous proteins or they may represent a smaller number of endogenous proteins with more than one epitope derived from proteolysis of each. By using microcapillary HPLC/tandem mass spectrometry, peptides present in reconstituting fractions on two different HPLC gradients have been identified. These include a nonamer peptide that matches the amino-acid sequence of monophenyl mono-oxygenase, a tyrosinase involved in melanin production. Sequencing, synthesizing, and testing these peptides is expected to permit identification of the peptide epitopes for melanoma-specific CTL, which will enable development of novel peptide-based synthetic tumor vaccines, and optimization of immunotherapy regimens for melanoma.

NZ 223 A DELAYED TYPE HYPERSENSITIVITY RESPONSE TO HUMAN PAPILLOMAVIRUS TYPE 16 (HPV16) PROTEINS, M.A. Stanley and M.A. Chambers, Department of Pathology, University of Cambridge, Tennis Court Road, Cambridge, United Kingdom.

We have established an animal model incorporating the exclusively epithelial nature of HPV16 to investigate the immune response to viral proteins *in vivo*.

Immortal non-tumorigenic Balb/C keratinocyte cell lines containing a range of HPV16 ORFs have been grafted onto syngeneic mice using a transplantation technique that permits the reformation of a differentiated epithelium on a granulation tissue bed. In this way viral antigens may be presented to the immune system in a manner comparable to the natural infection. A DTH response was studied a minimum of 7 days post grafting by challenging the ear with recombinant vaccinia virus expressing either the viral E6, E7 or L1 protein, or E7 protein alone. Mice showed a statistically significant ear swelling response to the challenge protein expressed by the grafted cell line when compared to the relevant control challenge. This response was not seen in mice grafted with an irrelevant cell line.

It has been determined that inflammation at the site of secondary protein challenge is necessary to achieve a specific DTH response. Furthermore the response has been found to decrease in a dose dependent manner with decreasing number of grafted cells.

This model has been used to determine the nature of effector cells involved in the response and also the potential of low concentrations of priming antigen to induce specific immunological suppression.

NZ 224 IMMUNOLOGICAL RECOGNITION OF 61 CODON MUTATED P21 RAS PROTEINS AND PEPTIDES. Anne-Françoise Tilkin, Nicole Ngo-Giang-Huong, Benoit Déprez*, André Tartar*, Michèle Kayibanda, Jean-Paul Lévy, and Jean-Gérard Guillet, INSERM U152, 27 rue du Faubourg Saint-Jacques, 75014 Paris (France), *Service de Chimie des Biomolécules, Institut Pasteur, Lille (France).

The activation of ras protooncogenes is usually due to a point mutation within codon 12 or 61 which induces single aminoacid substitutions. To know if such substitutions induce new antigenic determinants which are recognized by the immune system, different ras proteins and peptides : wild-type or mutated in codon 61 were synthesized and inoculated in different strains of mice. Among the five substitutions tested (61 Gln -> Leu, Lys, Arg, His or Gly), two (Gln -> Leu and Gln -> His) induce specific T helper responses in the H-2^K haplotype. Furthermore, we have established T $CD4^+$ hybridoma cell lines which recognize the mutated peptides (Gln -> His) or (Gln -> Leu) presented by the I-A^K H-2 antigen. These T hybridoma cell lines allow us to performe competitions experiments which establish that wild-type peptides at the contrary to mutated peptides are not able to fix to the I-A^K H-2 molecules. Furthermore, processing experiments realized with wild-type or mutated proteins have shown that (61 Gln -> Leu) mutated peptide could be a "natural" peptide produced by the processing of (61 Gln -> leu) mutated protein.

The study of the immune protection against ras tumours induced by inoculation with ras tumour cells in mice is now beginning with ras tumour cells produced by transfection of ras mutated oncogenes in murine tumour cell lines and by transfection of IA^K α and ß in murine ras tumours.

NZ 225 HEAT SHOCK PROTEINS HSP70, HSP90 AND GP96 ELICIT TUMOR - SPECIFIC IMMUNITY TO THE TUMORS FROM WHICH THEY ARE ISOLATED, Heiichiro Udono and Pramod K Srivastava, Dept of Pharmacology, Box 1215, Mount Sinai School of Medicine, New York, NY 10029

Gp96 heat shock proteins (hsp) elicit tumor-specific protective immunity to the tumor from which they are isolated but not to other tumors (1,2). We demonstrate that ***gp96 obtained from normal liver and spleens of BALB/c does not elicit protective immunity against Meth A and CMS5 sarcomas.***

As hsp90 has also been shown to elicit tumor-specific immunity (3), we compared the relative immunogenicity of hsp90 and gp96. It was observed that ***hsp90 isolated from Meth A cells elicits tumor-specific immunity at a lower efficiency than gp96*** : almost ten fold more hsp90 than gp96 is required to elicit immunity to comparable challenge with Meth A cells.

In order to explain the specific immunogenicity of hsps against antigenically distinct tumors, we have proposed that hsps act as chaperones of antigenic peptides (4). As hsp70 has been shown to bind peptides (5,6) we immunized mice with hsp70 from Meth A and challenged mice with 80,000 Meth A cells. It was observed that ***hsp70 also elicits tumor-specific immunity***. The immunogenicity of hsp70 is significantly higher than that of hsp90 and comparable to that of gp96.

These observations suggest that hsps encounter peptides not only in the lumen of the ER, (as in case of gp96), but also in other cellular compartments. The possible mechnaisms of this encounter as well as the cellular immunological circuitry which elicits specific protective tumor immunity in response to vaccination with hsps will be discussed.

1. Srivastava et al. 1986 PNAS 83, 3407. Palladino et al. 1987 Cancer Res 47, 5074. 3. Ullrich et al. 1986 PNAS 83, 3121 4. Srivastava and Maki 1990 Curr Top Microbiol & Immunol. 167, 109 . 5. Pierce et al. 1990 Curr Top Micro Immun 67, 83.6. Flynn 1989 Science 245, 385.

NZ 226 A MUTANT p53 TUMOR SUPPRESSOR PROTEIN IS A TARGET FOR PEPTIDE-INDUCED $CD8^+$ CYTOTOXIC T CELLS, Michael Yanuck,*# David Carbone,† C. David Pendleton,* Taku Tsukui,* John D. Minna,† and Jay A. Berzofsky, *Molecular Immunogenetics and Vaccine Research Section, Metabolism Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892 USA, #Howard Hughes Medcial Institute-NIH research scholar, and the †Simmons Cancer Center, University of Texas Southwestern Medical Center, Dallas, TX 75235-8593 USA

We asked whether mutations in the p53 tumor suppressor gene, that lead to transformation and frequently overexpression of the mutant p53 protein, might serve as tumor antigens recognizable by cytotoxic T lymphocytes (CTL). CTL recognize processed peptide fragments of any endogenous protein, after these peptides are carried to the cell surface by major histocompatibility (MHC) molecules. Thus, a tumor antigen does not have to be expressed as an intact protein on the cell surface to be recognizable by CTL, as long as the antigen is processed in the cytoplasm and peptide fragments can bind to class I MHC molecules. p53-specific CTL were generated by immunizing BALB/c mice with spleen cells pulsed with a peptide, corresponding to a 21 amino acid sequence encompassing a mutation in the mutant p53 gene product from a human lung carcinoma. The CTL recognize peptide-sensitized targets as well as fibroblasts transfected with the mutant p53 gene, endogenously expressing the mutant protein. MHC restriction studies show that the peptide is presented by the K^d molecule, and blocking with monoclonal antibodies indicated that the CTL were $CD8^+$. Thus, these results demonstrate that endogenously synthesized mutant p53 in a tumor cell can render the cell a target for specific CTL, and that these CTL can be generated by peptide immunization. These findings may point the way toward an approach to selective immunotherapy against tumors.

NZ 227 IL-12: A NOVEL T AND NK STIMULATORY FACTOR; IMPLICATIONS FOR CANCER IMMUNOTHERAPY. H.J.Zeh,III W. J. Storkus, M. Brunda*, M. Gately, H. Tahara, M.T. Lotze. Dept of Surgery, University of Pittsburgh, Pittsburgh, PA 15213, *Hoffmann-LaRoche, Nutley, NJ., 07110.

IL-12 is a recently described heterodimeric cytokine able to cause the proliferation of activated T and NK cells. IL-12 is produced primarily by stimulated macrophages. Michael Brunda and colleagues at Hoffmann-LaRoche have recently demonstrated a profound antitumor effect of IL-12. Studies are now underway in our laboratory to better define IL-12's role in the antitumor response. **Methods.** PBMC isolated from normal volunteers were cultured for five days in the presence of high dose IL-2 (6000u/ml), low dose IL-2 (120U/Ml), or IL-12 (120U/ML) added either at the initiation or at the end of the culture period. At the end of culture, cells were tested for their ability to proliferate and to lyse AK562 and Daudi. TIL's isolated from primary and metastatic melanoma lesions have also been examined for their ability to proliferate in the presence of IL-12. TIL's grown in IL-2 or a combination of IL-2 and IL-4 or IL-7 were placed in 96 well plates at a concentration of 5 x 10^5 cells/ml. After 49 hours of incubation in concentrations of IL-12 ranging from 0-1000 units/ml the cells were pulsed with 0.5μCi of thymidine and incubated for an additional 24 hours. **Results.** IL-12 was able to augment the LAK activity of PBMC **preactivated** with IL-2, 3 $LU_{30}/10^6$ compared to 16 $LU_{30}/10^6$. IL-12 was also able to augment the LAK activity induced by low doses of IL-7. LAK activity in all cultures (IL-2, IL-7, IL-12, and combinations of the three) were inhibited between 40%-52% by TGF-β at a concentration of 5ng/ml. IL-12 was able to support IL-2 expanded TIL's derived from three patients with melanoma in a dose dependent manner e.g. TIL 1128 S.I. = 0 at 0 U/ml; S.I. = 1.5 at 0.6 U/ml; S.I = 3.4 at 1.0 U/ml; S.I. = 30 at 15 U/ml; S.I. = 27 at 250 U/ml). We have also recently introduced each of the two genes encoding both chains (p35,p40) of murine IL-12 into the NIH 3T3 in order to examine its in vivo antitumor efficacy. Preliminary studies have indicated that the local secretion of IL-12 is able to inhibit the establishment of melanoma. Further studies are now underway in our lab in order to establish the most efficacious route of IL-12 administration.

Immune Unresponsiveness

NZ 300 IL-10 IS PRODUCED BY MELANOMA CELLS AND MAY HAVE A ROLE IN IMMUNOSUPPRESSION MEDIATED BY MELANOMA, Qiyuan Chen*, Vincent Daniel*, Darryl W. Maher# and Peter Hersey*, *Immunology and Oncology Unit, Mater Misericordiae Hospital, DMCSB, Royal Newcastle Hospital, Newcastle, NSW 2300, and #Ludwig Institute for Cancer Research, PO Box Royal Melbourne Hospital, Melbourne, Victoria 3050, Australia.

Previous studies have shown that IL-10 may modulate immune responses towards the humoral arm by inhibiting production of cytokines involved in cell mediated responses. This property of IL-10 has been implicated in the pathogenesis of certain infections and prompted us to examine its possible role in immunomodulation of immune responses against tumor cells. In the present studies it was found that mRNA to IL-10 could be demonstrated in 66% of melanoma cell lines by PCR analysis and in supernatants of a small proportion of the cell lines by Eliza assay. The mol. wt. of ^{35}S labelled IL-10 secreted by melanoma cells was similar to that reported in previous studies. Both IL-10 and supernatants from melanoma cell lines were found to inhibit TNF-α and IFN-γ production by PHA stimulated blood lymphocytes and these effects could be reversed in part by MAbs to IL-10. IL-10 and the melanoma cell supernatants also inhibited mixed lymphocyte cultures and interleukin-2 production but although MAb to IL-10 partially reversed the effects of IL-10, they had no effect on the inhibitory effects of the supernatants. This suggested that other tumor derived inhibitors were involved. These studies show that IL-10 is detectable in the majority of cell lines established from melanoma and indicate that its production by these cells may be important in modulating immune responses against melanoma.

NZ 301 T CELLS INFILTRATING RENAL CELL CARCINOMA AND NON-HODGKINS B CELL LYMPHOMAS ARE SELECTIVELY UNRESPONSIVE TO IL2 AND/OR ANTI-CD3, James H. Finke, Seiji Kudoh, Jeannine P. Alexander, Pat Rayman, Mark G. Edinger, Raymond R. Tubbs, Oscar F. Hidalgo, Eric Klein, Thomas A. Hamilton, and Ronald M. Bukowski. The Cleveland Clinic Foundation, Cleveland, OH 44195

While some tumor infiltrating lymphocytes (TIL) are tumor reactive it appears that a significant portion display a selective unresponsiveness. The proliferative and cytokine producing potential of TIL isolated from human renal cell carcinomas (RCC) and non-Hodgkins B cell lymphomas was compared to the responsiveness of peripheral blood T lymphocytes. TIL from both tumor types displayed a poor proliferative response to IL2 (1000 U/ml) and cross-linked anti-CD3 antibody. The proliferative unresponsiveness of TIL was not due to shift in kinetics since the response of TIL was depressed on days 3, 6 and 9 of culture. The lack of proliferation by TIL was not due to decreases in IL2Rβ or CD3 surface expression. The induction of IL2Rα surface expression on TIL was depressed relative to peripheral T cells. However, the low expression of IL2Rα did not account for the proliferative unresponsiveness since TIL that expressed normal levels of IL2Rα did not proliferate. When compared to peripheral T cells the induction of IL2 and IFNγ mRNA as well as secreted product was normal in T cell derived from RCC. Thus, the unresponsiveness of TIL from RCC is selective. In contrast, the unresponsiveness of TIL from non-Hodgkins B cell lymphomas includes IL2 and IFNγ gene expression. Following stimulation with anti-CD3 TIL did not express any mRNA for either IL2 or IFNγ. These results suggest that a significant portion of T cells in RCC and B cell lymphomas display a selective unresponsiveness and the degree of this altered state exhibited by TIL may vary depending on the tumor type.

NZ 302 **$CD4^-CD8^+$ CTL IN MICE LACKING β_2-MICROGLOBULIN,** **Rickard Glas, Petter Höglund, Claes Öhlén and Klas Kärre, Dpt of Tumorbiology, Karolinska Institute, 10401 Stockholm, Sweden.**

Allogeneic irradiated A/Sn spleen cells or P815 tumor cells were injected into mice heterozygous(β_2m+/-) or homozygous(β_2m-/-) for the β_2m knock-out mutation. Spleen cells were restimulated in an MLTC and used as effectors in a cytotox assay after 5 days. CD4-CD8+ effector cells killing P815 and A/Sn Con A blasts were generated from β_2m+/- as well as the β_2m-/- mice. The CD4-CD8+ effectors from β_2m+/- showed the expected specificity for A/Sn or P815, whereas the CD4-CD8+ effectors from the β_2m-/- had a broader specificity, and crossreacted with targets expressing self $H\text{-}2^b$ with β_2m. In untreated β_2m+/- and β_2m-/- mice there is a population of CD4-CD8+ cells, although very small in the latter (1-2% as detected by FACS). By day 5, after the in vitro restimulation, this population can be expanded by 5-10 fold. These results suggests that β_2m-/- contain a small pool of CD8+ CTL precursors that have been selected on β_2m free MHC heavy chains.

NZ 303 PRODUCTION OF CYTOKINES BY MALIGNANT MELANOMA, Peter Hersey, Qiyuan Chen, Melanie Smith and Tam Nguyen, Immunology and Oncology Unit, David Maddison Building, Royal Newcastle Hospital, Newcastle, NSW 2300, Australia.

Several previous reports have suggested that melanoma cells may produce cytokines known to be involved in immune responses. In view of this we have carried out "cytokine mapping" on mRNA from 16 melanoma cell lines using reverse transcription and PCR amplification. IL-1α and IL-1ß were detected in 6/8 and 7/8 primary melanoma and 1/8 and 3/8 lines from metastatic melanoma. IL-6 and IL-8 were detected in 7/8 and 7/7 primary melanoma respectively and 2/6 and 6/7 lines from metastatic melanoma. 4/6 primary and 0/5 metastatic melanoma expressed mRNA for GM-CSF. 4/6 of metastatic melanoma but no primary melanoma expressed receptors for interleukin-2. Finally 4/8 primary melanoma and 6/8 metastatic melanoma were found to express mRNA for IL-10. mRNAs for IL-2, IL-4, IL-5 and TNF-ß were not detected. It is evident from these results that melanoma cells produce a wide spectrum of different cytokines, that the ability to do so varies according to the stage of the disease, and that their production of cytokines has the potential to markedly influence the immune response against melanoma. In particular, IL-10 production from melanoma cells (not previously reported) may cause a qualitative change in the immune response to that of TH-2 or suppressor response. GM-CSF production on the other hand may result in the local accumulation of APC and favour tumor rejection, IL-8 and IL-6 may be growth factors for the tumor. Studies on the significance of these findings are continuing.

NZ 304 CYTOKINE EXPRESSION IN HUMAN RENAL CELL CARCINOMAS; LACK OF IL-2 EXPRESSION AND SELECTIVE EXPRESSION OF IL-10 MRNA IN TUMOR TISSUE.
Rolf Kiessling, Hiroshi Nakagomi, Pavel Pisa, Eva K. Pisa, Yasuyoshi Yamamoto, Eva Halapi, Karin Backlin and Claes Juhlin. Dept. of Immunology, Karolinska Institutet, Box 60400, 104 01 Stockholm. Dept. of Surgery, Akademiska Hospital, 751 85 Uppsala.
One of the key functional parameters of an immune response is the local production of cytokines. Using a PCR-assisted mRNA amplification assay, we assessed in renal cancer tissue the constitutive expression of interleukin-1α (IL-1α), interleukin-2 (IL-2), interleukin-3 (IL-3), interleukin-4 (IL-4), interleukin-6 (IL-6), interleukin-10 (IL-10), tumor necrosis factor-α (TNF-α), interferon-γ (IFN-γ), granulocyte-macrophage colony stimulating factor (GM-CSF), and granulocyte colony stimulating factor (G-CSF). We compared the cytokine mRNA expression in freshly isolated samples of renal carcinomas, renal cancer cell lines established from the tumor samples, peripheral blood mononuclear cells (PBMC) and non-tumor kidney tissue isolated from the same patients. We found IL-10 mRNA expression only in the tumor samples, while renal cancer lines, PBMC and non-tumorous kidney tissue were devoid of this cytokine. One third of the tumor samples but none of the normal kidney samples also expressed G-CSF. IL-6, TNF-α and IFN-γ mRNA were expressed non-selectively in tumors, PBMC as well as in normal renal tissue. IL-2, IL-3 and IL-4 were not detected in any of the tissues analysed. Established renal cancer lines exhibited expression of IL-1α, IL-6, TNF-α and GM-CSF. Culture of tumor-derived cells with anti-CD3 monoclonal antibody resulted in expression of IL-2, IL-3 and IL-4 mRNA. In contrast, none of these cytokines became expressed by culture in recombinant human IL-2 alone. These findings have important implications for the possible in vivo role of IL-10 as a suppressor of local anti-tumor response.

NZ 305 IMMUNE PRIVILEGE AND THE FAILED ELIMINATION OF RETINOBLASTOMA BY SPECIFIC CYTOTOXIC T CELLS.
Bruce R. Ksander, D.C. Geer, J.M. O'Brien, D.M. Albert, and T.G. Murray, Dept of Ophthalmology, Univ of Wisconsin, Madison, WI., Depts of Immunology and Ophthalmol., Univ of Miami, Miami, Florida 33101.
The expression of SV40 *Tag* within the retina of transgenic mice produces heritable retinoblastoma (Rb) with histological, ultrastructural, and immunohistochemical features identical with human Rb. Transgenic mice are produced with *Tag* driven by the promoter of the luteinizing hormone β-subunit gene. Rb tumor cells, but not other tissues, express *Tag* mRNA by northern blot analysis. Tumors are first observed at 2 months of age, but initial growth is monotonous and tumors do not fill the vitreous cavity until 5-6 months. Normally tumors derived from *Tag* transgenes either, grow rapidly in *Tag* tolerant mice, or fail to grow in mice that induce specific cytotoxic T cells (Tc). By contrast, we have shown previously that tumors within immune privilege sites in the eye induce specific Tc, but the local microenvironment prevents Tc from eliminating tumors. Since Rb develops within the immunologically privileged subretinal space, we hypothesize that Rb induces specific Tc, but the immune privilege environment prevents Tc from eliminating tumors.

To test this hypothesis, spleen cells (H-$2^{b/d}$) were recovered from: (a) Rb mice, (b) *Tag* immunized mice (positive control), and (c) normal mice (negative control) and restimulated for 6 days in vitro with x-irradiated H-2^b *Tag*+ fibroblasts. Lymphocytes were assayed for cytotoxic activity in a standard 4hr chromium release assay against several H-2^b targets: (a) *Tag*+ cells, (b) *Tag*- cells, and (c) Rb tumor cells. As expected, spleen cells from *Tag* immunized mice lysed specifically *Tag*+ targets. Interestingly, Rb spleen cells were primed for a *Tag* specific Tc response and lysed *Tag*+, but not *Tag*- targets. However, Rb tumor cells (mRNA *Tag*+) were not lysed by lymphocytes recovered from either Rb, or *Tag* immunized mice. Tumor cells can escape Tc recognition by either mutation of epitopes recognized by Tcr, and/or down-regulation of class I molecules. In support of the latter, we observed that Rb cells expressed no H-$2K^b$ (as detected by FACS analysis). Moreover, IFN-γ treated Rb cells up-regulated H-$2K^b$ and were lysed by Rb and *Tag* primed lymphocytes. Our interpretation of these results is that immunogenic Rb cells induce specific Tc, however, the immunologically privileged environment within the eye prevents Tc from eliminating completely all tumor cells resulting in slow tumor growth. Under these conditions Tc select for class I deficient mutant Rb cells that form rapidly growing progressive tumors.

NZ 306 IMMUNE CONTROL OF TUMOR GROWTH IN SV40 T ANTIGEN TRANSGENIC MICE,
Xuehai Ye, James McCarrick, Lorraine Jewett, Robert Riccardi, Roberto Weinman and Barbara Knowles, The Wistar Institute, Philadelphia, PA19104
Mice of RIP-Tag4 lineage express SV40 T antigen (SV40 Tag) transgene exclusively in pancreatic β-cells. RIP-Tag4 mice are thought to be non-tolerant to SV40 Tag, presumably due to a delayed onset of transgene expression. Although the mice are fully capable of generating an active humoral or cellular response against the transgene product upon immunization with SV40 Tag, they all succumb to SV40 Tag-induced islet tumors at 8-10 months of age. We find that the tumor growth in RIP-Tag4 mice can be significantly delayed by immunization with SV40. However, immunization is only effective if the mice are exposed to SV40 Tag prior to the time of endogenous expression. SV40 immunization of RIP-Tag2 mice, another transgenic lineage which express SV40 Tag neonatally in β-cells, showed little effect on tumor development. This finding suggests that tissue-specific unresponsiveness is induced upon endogenous expression. We also found that the levels of MHC class I antigen are lower on insulinoma cells than normal β-cells. These tumor cells are poor targets for SV40 Tag-specific CTLs but become good targets after treatment with IFN-γ, suggesting that a selective advantage for decreased class I expression may also contribute to the tumor progression. Taken together, our results suggest that SV40 Tag-induced islet tumors avoided active host immune response by a combined effect of inducing tissue-specific unresponsiveness and a passive selection of those tumor cells with lower levels of class I expression. To further investigate the regulatory mechanism of class I expression in islet tumors, a detailed molecular analysis was carried out on several insulinoma cell lines derived from the tumor. We demonstrated that the expression is controlled at the transcriptional level and, moreover, we showed that the suppression of class I expression is mediated by the loss of a certain transcriptional factor(s) that binds specifically to the R1 element of H-2 enhancer A. The same mechanism may also be responsible for the low level of class I expression in normal β-cells.

Alloimmunity, Autoimmunity and GVH

NZ 307 DISCREPANCY BETWEEN *IN VIVO* AND *IN VITRO* ANTIGENIC RESPONSES OF CD8+ T LYMPHOCYTES IN TCR TRANSGENIC MICE,
Béatrice Gaugler, Claire Langlet, Nathalie Auphan, Anne-Marie Schmitt-verhulst, Annick Guimezanes, Centre d'Immunologie INSERM-CNRS de Marseille-Luminy, Case 906, 13288 Marseille Cédex 9, FRANCE.
Alloreactive, K^b-specific CTL clones, KB5.C20 and BM3.3 which differ in their requirements for the CD8 co-receptor, recognize also different endogenous peptides in association with K^b. Possibly related to its low dependency on CD8, BM3.3 is able to recognize its peptide in a crude peptide extract while KB5.C20 needs HPLC fractionation of the mixture. Taken together, these results indicate that BM3.3 has a higher "affinity" for cell surface expressed K^b molecules than KB5.C20.
Two lines of transgenic mice have been established, which express respectively the KB5.C20 and BM3.3 TCRs. In both Tg lines, the majority of $CD8^+$ TCR-Tg^+ mature T cells are cytolytic only after *in vitro* stimulation with H-2^b expressing cells. CTL induction occurs in the absence of $CD4^+$ Th cells, and $CD8^+$ CTL secrete IL2.
No anti-H-2^b CTL activity was detected after *in vivo* immunization of KB5.C20 TCR Tg mice, in contrast to the cytolytic activity of T cells from BM3.3 TCR Tg or from non-transgenic mice immunized in the same conditions. T lymphocytes from KB5.C20 TCR Tg mice injected with H-2^b cells are neither activated (lack of pgp-1 expression), nor anergized since they respond *in vitro* to H-2^b stimulation in a manner similar to non-immunized KB5.C20 TCR Tg mice. These results will be discussed in terms of the *in vivo* recognition of Kb-associated specific peptides recognized by each of the clone.

NZ 308 T CELL RECEPTOR BETA CHAIN VARIABLE REGION REPERTOIRES IN HUMAN ALLO-REACTIVE RESPONSES, Susan L. Hand, Bruce Lee Hall, and Olivera J. Finn, Department of Molecular Genetics and Biochemistry, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261

T cell receptor (TCR) beta chain variable region (Vβ) gene repertoires utilized in specific alloresponses were analyzed to determine whether particular Vβ genes were selected. The primary focus was on alloresponses to class II HLA-DR1. Two sources of DR1-reactive cells were employed: T cells lines established from cells infiltrating rejecting human renal allografts, and mixed lymphocyte reactions (MLR) established using peripheral blood lymphocytes (PBL) from normal individuals. A semiquantitative, polymerase chain reaction (PCR) technique was used to analyze Vβ repertoires. For the MLR, repertoire analysis was performed at a number of serial time points, and selection was observed with time *in vitro*. In the majority of cases, selection was for one or two predominant Vβ which remained dominant for the duration of the culture.

To determine the consistency of Vβ selection in a given response, two separate sensitizations of one responder to the same $DR1^+$ stimulator were established. Reproducibility was observed at the levels of DR1 fine-specificity and TCR Vβ usage. Moreover, both populations appeared to be predominated by T cells expressing identical TCR β-chains. MLR were established in which the same responder was paired with four different $DR1^+$ stimulators. The identities of the Vβ's used in responses to the same HLA molecule expressed on different cells was not predictable, but there did seem to be a preference for utilization of Vβ with relatively high homologies.

Vβ repertoire heterogeneity of MLR was compared with that of renal allograft-derived T cell lines. Although the apparent heterogeneity of the two types of populations began at comparable levels, the Vβ repertoires of graft-infiltrating cells became restricted more rapidly *in vitro*. This supports the concept that cells isolated from grafts contain previously activated populations of alloreactive T cells.

NZ 309 **HUMAN CD8 TRANSGENE EXPRESSION ENHANCES RECOGNITION OF HLA CLASS I MOLECULES BY MURINE T CELLS: EXPERIMENTAL ANIMAL MODEL FOR HUMAN CLASS I ANTIGEN PRESENTATION,** Drake LaFace, Mikael Vestberg, Rakesh Srivastava, Young Yang, Linda Sherman and Per Peterson, Department of Immunology, The Scripps Research Institute, La Jolla, CA 92037.

Primary in vitro immune responses to allogeneic class I MHC antigenic determinants are known to induce strong cytotoxic T-lymphocyte (CTL) reactivity. In contrast, reactivity to xenogeneic HLA class I antigens by mouse lymphocytes is negligible as murine CD8 interacts poorly with human MHC class I (HLA). Therefore, we have produced several lines of transgenic mice expressing human CD8 accessory molecules to further reconstitute a human antigen presenting system in the mouse. Primary, in vitro cytotoxic T lymphocyte (CTL) responses to xenogeneic HLA class I antigenic determinants were utilized to assay the human CD8 transgene function. The expression of transgenic human CD8α or CD8α+β molecules resulted in a markedly enhanced capacity for mounting a specific xenogeneic response to HLA-A2.1 antigenic determinants, to a level comparable with allogeneic responses. Limiting dilution analysis indicated that the CTL precursor frequency of HLA-A2.1 specific responder cells was markedly increased in human CD8 transgenic mice. The results show that human CD8 enables murine T-cell receptors to efficiently recognize human MHC class I molecules.

The human CD8 transgenic mice have been bred to HLA-A2, -B7 and -B27 transgenic mice to determine the ability of human CD8 to enhance tumor and viral antigen presentation by HLA restricting elements to murine T-cells. This experimental model will provide important insights for anti-tumor and anti-viral immunotherapy regimens.

NZ 310 IL-7 INVOLVEMENT DURING THE ACUTE PERIOD POST ALLOGENEIC BONE MARROW TRANSPLANTATION. R.B. Levy, M. Jones, J. Paupe, *Brian L. Hamilton and R. Riley. University of Miami School of Medicine, Miami, FL 33101. *Children's Hospital, Oakland, CA 94609.

Graft vs. host (GvH) induced immune dysfunction/disease (GvHD) are major complications following clinical allogeneic bone marrow transplantation (BMT). The precise cellular interactions/signals occurring during the acute period post allogeneic BMT have not yet been clearly defined. We have been examining the events occurring following allogeneic B10.D2 ($H\text{-}2^d$) BMT into lethally irradiated BALB/c ($H\text{-}2^d$) recipients and previously demonstrated the marked expansion of B10.D2 donor T-cells within 3-5 days post-transplant. Notably, as a result of the Mls endogenous superantigen disparity present in this combination, the majority of TcRα,β donor cells present after one week were Vβ3 TcR+. Regardless of whether conventional and/or superantigens activate donor T-cells, lymphokines including IL-2 are likely to help drive and expand anti-host i.e. alloreactive populations. Since IL-7 is capable of inducing proliferation of alloantigen primed T-cells, we hypothesized that this molecule contributes to the alloreaction, i.e. GvHR occurring post-BMT. We observed that IL-7, as well as IL-2 was capable of inducing proliferation by T-cells from BMT recipients within 10 days of transplant. IL-7 induced proliferation was T-cell dependent and moreover, inhibitable by anti-IL-2, as well as anti-IL-7 mAb. Notably, IL-7 specific mRNA was found by PCR analysis in the spleens of allogeneic BMT recipients at this time. Interestingly, after induction of proliferation by IL-7 and IL-2, no IFN-γ could be detected in cultures of spleen cells from BMT recipients within one week of transplant. However, following TcR engagement with anti-CD3 or anti-Vβ3 mAb, IFN-γ secretion was detected. Addition of IL-7, together with anti-Vβ3 mAb induced greater levels of IFN-γ secretion vs. anti-Vβ3 mAb stimulation alone. In total, these findings demonstrate that IL-7 can 1) drive donor T-cell proliferation within two weeks post-allogeneic BMT and 2) together with anti-TcR ligation, induce the release of IFN-γ. Since IL-7 mRNA was also detected in the spleens of control BMT transplants presumably as a consequence of lymphohematopoietic reconstitution, we propose that when alloreactive donor T-cells are also present following BMT, IL-7 can function as an important co-stimulator in the expanding donor anti-host, i.e. GvH alloreactive T-cells.

NZ 311 UNCONTROLLED CELL PROLIFERATION AND ENERGY RESTRICTION. W.S. Lynn, S. Wilson and D. Coppenhaven. University of Texas, M.D. Anderson Cancer Center, Science Park-Research Division, Smithville, Texas and University of Texas Medical Branch, Galveston, Texas

To reverse uncontrolled cell growth using either murine transplanted tumors or retrovirus-induced proliferation murine models, we previously showed that both caloric restriction (60%) and an interferon inducer (polyinosine-cytosine) were required (Clin. Biotech. 3:39-45, 1991). Both agents appear to block energy utilization, one by removing substrates and the other by removing energy production (mitochondrial suppression). To ascertain if these agents could also reverse chronic inflammatory cell proliferation, these agents were given (for several weeks to NZB/NZW hybrid autoimmuna mice which accumulate massive numbers of chronic inflammatory cells in their renal glomerulae, with resultant proteinuria and early death due to renal failure. These treatments did eliminate the proteinurea and the hydropic tubular degeneration but did not remove the accumulated glomerular phegocytes (crecents). With return to ad lib feeding, the proteinuria and tubular swelling also return.

These findings indicate that this type of antigrowth therapy is effective in vivo only in rapidly proliferating cells - cells which can express the tumor suppressor gene, p53, and activate apoptosis.

NZ 312 DEVELOPMENT OF A NOVEL CTL POPULATION,
Susan A. McCarthy and Michael S. Mainwaring, Departments of Surgery, and Molecular Genetics and Biochemistry, University of Pittsburgh School of Medicine, Pittsburgh, PA 15213

We have previously identified a novel functional population of $CD8^+$, but anti-CD8 resistant, MHC class I allospecific CTL. These CTL have unusual activation requirements in that their efficient generation in vitro requires multivalent cross-linking of cell surface CD8 on their precursors by anti-CD8 mAb. These CTL are potent in vivo effectors that can mediate allograft rejection even in the absence of conventional, anti-CD8 sensitive CTL. In the present study we demonstrate that anti-CD8 resistant pCTL/CTL are detectable only in peripheral lymphoid tissues of adult mice, and not in the adult thymus. We found no evidence for a suppressive effect preventing the activation of such pCTL/CTL in the adult thymus, and no evidence that peripheral T helper cells and/or accessory cells could facilitate a thymic anti-CD8 resistant CTL response in adult mice. These results indicate that anti-CD8 resistant pCTL/CTL are absent or functionally inactive in the adult thymus. In contrast, in very young mice, anti-CD8 resistant pCTL/CTL are readily detectable in the thymus, but not in the peripheral lymphoid tissue. These results indicate that the generation of anti-CD8 resistant pCTL/CTL is developmentally regulated. Neonatal thymic anti-CD8 resistant pCTL may emigrate to the periphery as a cohort, leaving the adult thymus deficient in this T cell functional population. Alternatively, the neonatal thymic anti-CD8 resistant pCTL population may have a limited life span, and may be unrelated to the adult peripheral anti-CD8 resistant pCTL population. In that case, adult peripheral anti-CD8 resistant pCTL/CTL may represent either an extra-thymic T cell lineage or a post-thymic maturation stage. We are currently investigating these possibilities.

NZ 313 SCLERODERMA WITH ANTI-TOPOISOMERASE I ANTIBODIES IS ASSOCIATED WITH AN HLA-DRw11 ALLELE.
Penelope A. Morel, Huang J. Chang, Susan L. Saidman, David J. Tweardy and Thomas A. Medsger Jr. Dept. of Medicine, University of Pittsburgh School of Medicine, Pittsburgh PA 15213.

Systemic sclerosis (SSc, scleroderma) is an autoimmune disease in which strong HLA associations have not been described. Anti-topo I antibodies are associated , in general, with SSc patients with diffuse cutaneous involvement, while anti-centromere antibodies (ACA) are found in individuals with limited cutaneous involvement. We have studied 38 SSc patients with anti-topo I antibodies, 27 with ACA and 38 SSc patients with neither antibody. DNA was extracted from peripheral blood lymphocytes and the DRB1, DQA1 and DQB1 genes were amplified using the polymerase chain reaction. Amplified products were analyzed by dot blot analysis using a panel of sequence specific oligonucleotide probes. A significant association was observed in the anti-topo I positive patients with DRw11 ($p = 6 \times 10^{-8}$; RR 20.7). The distribution of DRw11 alleles in these patients was significantly different from the controls and an increase in the frequency of the DRB1*1104 allele was observed. No DR association was observed in the ACA positive group and the role of DQ in this form of SSc will be discussed.

The topo I protein has an area of homology with a retroviral p30gag protein and this region of the protein is recognized by sera from SSc patients. Topo I protein has been purified and these preparations are being tested for their ability to stimulate T cells from SSc patients with auto antibodies to this protein. The significance of immune responsiveness to such proteins in autoimmunity will be discussed.

NZ 314 **ALTERATIONS IN SIGNAL TRANSDUCTION MOLECULES IN T LYMPHOCYTES FROM TUMOR-BEARING MICE**,
Augusto C. Ochoa, Hiromoto Mizoguchi, John J. O'Shea, Cynthia M. Loeffler, Dan L. Longo, Clinical Services Program, PRI/DynCorp, and Biological Response Modifiers Program, National Cancer Institute-Frederick Cancer Research and Development Center, Frederick, MD 21702

Impaired immune responses have been reported frequently in cancer patients or in tumor-bearing mice, but the mechanisms of the tumor-induced immune defects remain poorly understood. In an in vivo murine colon carcinoma model (MCA-38), animals bearing tumor longer than 26 days develop CD8+ T cells with a marked impairment of cytotoxic function, decreased expression of the TNFα and granzyme B genes and decreased ability to mediate an antitumor response in vivo. Proliferation and lymphokine production by CD4+ T cells remained normal. T lymphocytes from tumor-bearing mice expressed T-cell antigen receptors (TCR) characterized by a reduction in the CD3γ chain and by the complete absence of the ζ-chain, which was replaced by an Fc$\epsilon\gamma$-chain. Additionally, expression of the tyrosine kinases $pp56^{lck}$ and $pp59^{fyn}$ were markedly reduced. Thus, the switch in the TCR isoform and the alterations in the levels of components of the signal transduction pathway could be the basis of immune defects in tumor-bearing hosts.

NZ 315 ENGINEERING THE INFUSED T CELL CONTENT OF HLA DISPARATE MARROW ALLOGRAFTS BY ELUTRIATION TO PROMOTE GVL.
R.Quinones, P.Dinndorf, A.Hinkle, B. Taylor, S.Karandish, N.Luban, G.Reaman. Children's National Medical Center and George Washington University, Washington, D.C. 20010. The graft versus leukemia (GVL) effect of allogeneic marrow transplantation does not directly correlate with the severity of graft versus host disease (GVHD). Altering the infused marrow's T cell content, either quantitatively or qualitatively may allow the separation of GVL from GVHD. We have developed an extended cycle elutriation (ECE) process, separating $\geq$3 logs of T cells from a stem cell enriched fraction. Separated, unstimulated T cells can be:(1) directly added back to control the number of infused T cells or (2) fractionated and/or activated to become potential effectors of GVL. We have used ECE to control infused T cell content ($7.5x10^4$ T cells/kg) of HLA disparate marrow in order to promote GVL without severe GVHD in 15 patients with refractory leukemia. In this group at high risk of severe GVHD, 7 had GVHD (6,$\leq$ grade II; 1, grade III). Only 3 patients relapsed, each at very high risk of relapse. Other complications included infection (12), graft failure (1), and fatal lymphoproliferative disease (1). Actuarial disease free survival is 40% at 2 years (155 to 1250 days, median 1020). Our data show that in the setting of HLA disparate BMT, ECE allows controlled infusion of unfractionated T cells without severe GVHD. These infused T cells may mediate a GVL effect, as evidenced by a lower relapse rate in our patients compared to that reported for recipients of T cell depleted grafts. In preclinical studies, we have enriched the T cell fraction for $CD3^+$,$CD4^-$,$CD8^-$ T cells, putative effectors of GVL, and shown them capable of activation. We demonstrate that ECE provides a system for clinical trials of engineering the infused T cell content of extensively T cell depleted marrow to provide a GVL effect.

NZ 316 EFFECT OF CALCITRIOL ON GVHD. By Abdus Salam, M.Waer and M. Vandeputte, Rega Institute,Minderbroederstraat-10,University of Leuven,B-3000,Leuven Belgium.

The specific effector cells responsible for GVHD are still controversial. While alloreactive T cells are the prime mediator of GVHD, the role of other cells like NK cells have strongly been implicated especially in acute GVHD. The ex vivo or in vivo depletion of donor NK-cells by anti NK antibody is reported to have increased survival of mice undergoing mild to moderate GVHD. But-in vivo depletion of NK cells from recipient mice may also play an important protective role in GVHD. This issue has been addressed here by looking at the influence of Calcitriol on GVHD in TBI treated mice.

Calcitriol an active metabolite of Vit.D_3 maintain s calcium homeostasis through Bone,Kidney and Intestine. It plays an important role as immuno modulator of NK cell function in vivo. The increased NK cells activity has been observed at 24 and 48 hour's after Calcitriol administration in vivo though decreased NK cell function is found at 4 hour's after the same. The recipient F_1 mice (Balb/C X C57bl) are constructed by giving 950 rads total body irradiation and to which bone marrow of Balb/C is given.One group of recipient is injected with anti NK1.1 antibody I.P before being transplanted with BM from Balb/C which does not express NK1.1 marker specific for NK cells. The NK cell depleted group has been shown to have increased survival than the non depleted control group.

NZ 317 **CONTRIBUTION OF CD4$^+$, CD8$^+$, AND NK1.1$^+$ CELLS TO ANTILEUKEMIA (GVL) AND ANTIHOST (GVH) REACTIONS AFTER ALLOGENEIC BONE MARROW TRANSPLANTATION,** **Robert L. Truitt, Cathleen M. McCabe, Michael B. Weiler, and Bryon D. Johnson, Medical College of Wisconsin, Milwaukee, WI 53226**

The antileukemia or graft-vs-leukemia (GVL) effect associated with allogeneic bone marrow transplantation (BMT) is a dramatic example of immunologic reactivity against neoplastic disease. It is critical that we understand the cells involved and how they interact in order to successfully manipulate the reaction. Using mouse models, we have shown that multiple T-cell and non-T-cell effector systems are involved in the GVL effect. Some, but not all, contribute to the graft-vs-host (GVH) syndrome. In recent studies, we have used monoclonal antibodies to deplete donor populations, singly or in combination, in order to examine the contribution and interaction of donor CD4$^+$, CD8$^+$, and NK1.1$^+$ cells after BMT in MHC-matched [B10.BR→AKR] and haplotype mismatched [SJL→(SJLxAKR)F_1] donor-host combinations. Host mice were conditioned with lethal total body irradiation (11 Gy). The severity of GVH disease was CD4-dose-dependent, and complete depletion of CD4$^+$ donor T-cells eliminated GVH disease. In contrast, the effect of CD8-T-cell-depletion on clinical GVH disease was modest with little survival advantage. For optimal GVL reactivity (against acute T-cell leukemia/lymphoma of AKR origin), both CD4 and CD8 T-cells were required; however, GVH disease was more severe when both T-cell subsets were present in the transplant inoculum. Donor-derived NK1.1$^+$ cells contributed to the pathologic manifestations of GVH disease after MHC-matched BMT, but did not contribute significantly to the GVL effect. Recent studies on combined depletion of donor lymphoid subsets suggest that NK1.1$^+$ donor cells suppress the beneficial GVL effect attributed to CD8$^+$ T-cells. NK1.1-associated immunosuppression was dependent on the coadministration of CD4$^+$ T-cells. These results impact upon the design of clinical strategies to prevent or modulate GVH disease, especially by T-cell-subset-depletion, without loss of the beneficial antileukemia response.

Gene Therapy of Tumors

NZ 400 MECHANISM OF IMMUNE RESPONSE INDUCED BY CYTOKINE GENE MODIFIED CMS-5 TUMOR CELLS, Rajat Bannerji and Eli Gilboa, Program in Molecular Biology, Memorial Sloan-Kettering Cancer Center, New York, NY 10021.

Intradermal injection of IL-2 or IFN-gamma gene modified CMS-5 tumor cells leads to rejection of the tumor and the induction of antitumor immunity, which correlates with the generation of tumor specific spleen derived CTLs. The role of T cells in rejection of the cytokine secreting tumor cells, and induction of immunity against a challenge with parental tumor cells was investigated in T cell deficient athymic nude mice. Unexpectedly, IL-2 secreting tumor cells failed to form tumors in nude mice suggesting that T cells do not participate in the rejection of the gene modified tumor cells. Immunodepletion studies in immunocompetent mice have shown that neither T cell subset is important in the elimination of cytokine secreting tumor cells. However, both CD4 and CD8 T cells are required at the time of immunization for the induction of immunity and at the time of challenge for rejection of the tumor. Immunohistochemical studies have shown that no T cells are present at the site of immunization, although, as expected, they did infiltrate the site of tumor challenge. NK cells infiltrated the IL-2 secreting tumor and macrophages infiltrated the IFN-gamma secreting tumor. Implications to the mechanism of CTL induction will be presented.

NZ 401 GENE THERAPY OF MELANOMA: CHARACTERIZATION OF RECURRENT MELANOMA FROM MICE TREATED WITH AN IL-2-SECRETING ALLOGENEIC CELL CONSTRUCT THAT EXPRESSES MELANOMA ASSOCIATED ANTIGENS, Edward P. Cohen,[1] Tae Sung Kim[1] and Mary K. L. Collins[2] [1]University of Illinois College of Medicine, Chicago, IL 60680 and [2]Institute of Cancer Research, London, SW3 6JB, UK

The survival of C57BL/6 mice (H-2^b) bearing established B16 melanomas (H-2^b) was prolonged if the animals were treated with an IL-2-secreting cell construct that expressed melanoma associated antigens along with allogeneic class 1 determinants (H-2^k). The allogeneic construct was prepared by transfecting LM mouse fibroblasts (H-2^k) with genomic DNA from B16 cells, followed by transduction of melanoma antigen-positive transfected cells with a retroviral vector carrying the gene for IL-2. The IL-2-secreting construct was non tumorigenic in H-2^b mice; however, tumors invariably formed in mice injected with viable IL-2-secreting B16 cells. Recurrent tumors in mice treated with the allogeneic cell construct were melanomas that were resistant to further immunotherapy with the same construct used initially. The recurrent tumors were sensitive to immune effector cells from mice immunized with constructs that differed in immunogenic properties, as determined by both in vivo and in vitro determinations of anti melanoma immunity. Anti melanoma cytotoxicity in mice immunized with the allogeneic construct was mediated by CD8 and NK effector cells. Low MHC antigen-expressing variants of B16 cells were detected in the population of resistant cells; they were responsive to treatment with interferon-gamma. The use of transfection-competent allogeneic cells that form cytokines raises the possibility that a universal recipient cell construct can be prepared for tumor therapy. Supported by HHS CA55651.

NZ 402 ASSESSING THE EFFICACY OF TUMOR CELLS ENGINEERED TO SECRETE CYTOKINES, AS ANTI-TUMOR VACCINES. Mary K.L. Collins, Poulam M. Patel, Claudia L. Flemming, Gary Box* and Suzanne A. Eccles*, Section of Cell and Molecular Biology and *Section of Immunology, Institute of Cancer Research, Chester Beatty Laboratories, 237 Fulham Road, London SW3 6JB, U.K.

Retroviral vectors have been used to obtain derivatives of the murine, transplantable fibrosarcoma FS29 which secrete IL2, IL4 or γIFN. Cytokine-secreting tumor cells showed considerably reduced tumorigenicity in syngeneic animals. Immunization of syngeneic mice with cells secreting IL4 or γIFN protected animals against delayed challenge with unmodified FS29 tumor cells. The host cells which invaded cytokine-secreting tumors have been characterised and will be described. This suggested that patient tumor cells, obtained from resected tumor material and engineered to secrete cytokines, might be used as a vaccine to protect patients from recurrent disease. The feasibility of this approach has been assessed using a second tumor, the rat fibrosarcoma HSN, which has a high incidence of metastasis to the liver. Rats, from which a primary HSN tumor had been excised, were injected with live cytokine-secreting cells. These animals showed significantly reduced incidence of metastasis compared to animals which received irradiated parental or cytokine-secreting tumor cells. The implications of these data for potential human therapy will be discussed.

NZ 403 THE EFFECT OF TUMOR CLASS I MHC EXPRESSION ON THE GENERATION OF TWO DISTINCT CYTOLYTIC ANTI-TUMOR RESPONSES

Hyam I. Levitsky and Drew M. Pardoll, Department of Oncology, Johns Hopkins University School of Medicine, Baltimore, MD.

Previous studies using murine tumor models have shown that significant tumor specific immunity can be generated by tumor vaccines engineered to secrete various cytokines. Of over ten cytokines tested, GM-CSF production by tumor vaccine stands out as the most effective tumor/cytokine combination for the induction of long lived immunologic rejection of a systemic tumor challenge. This rejection is largely mediated by tumor specific, MHC class I restricted $CD8^+$ cytoxic T cells (CTL). Consequently, immunologic selective pressure for tumor loss of class I MHC molecules would be expected to result in the failure of cytolytic recognition by CD8+ CTL resulting in tumor outgrowth. This prediction was tested using an MHC class I negative variant of the B16 melanoma, known as B78H1. This tumor has no detectable surface staining of MHC class I molecules by FACS analysis, and expression is not inducible by gamma-interferon treatment. The genes for murine MHC class I molecules (Db and Kb) were introduced by transfection and positive polyclonal populations were obtained by cell sorting. Class I positive and negative vaccines were then created by retroviral transduction with a vector encoding for GM-CSF. Surprisingly, mice were able to reject a class I negative tumor challenge, but only if vaccinated with class I negative, GM-CSF producing vaccine. If MHC class I positive, GM-CSF producing vaccine was used, protection against the class I negative challenge was lost, although mice so vaccinated were still protected against the class I positive challenge. In vivo lymphocyte subset depletion experiments demonstrated that while the response to both class I positive and negative challenges required the presence of $CD4^+$ helper T cells, the rejection of class I positive challenge was dependent on $CD8^+$ T cells, while the rejection of the class I negative tumor was dependent on $NK1^+$ cells. Both of these cytolytic effector cells could be generated when the vaccine displayed no class I MHC expression, whereas only the class I restricted, $CD8^+$ response occured when the vaccine was class I positive, suggesting inhibition of NK cell activation by class I positive vaccine. Finally, depletion of $NK1^+$ cells prior to a class I positive challenge occasionally resulted in outgrowth of tumor that had lost MHC class I expression, further suggesting that these cells function as a safeguard against this mechanism of tumor evasion of immunologic recognition.

NZ 404 DECREASED TUMORIGENICITY OF MURINE NEUROBLASTOMA AFTER RETROVIRAL-MEDIATED IL-2 GENE TRANSFER AND EXPRESSION. Emmanuel Katsanis, Paul J. Orchard, Maria A. Bausero, Keith. B. Gorden, R. Scott McIvor and Bruce R. Blazar, Department of Pediatrics, Institute of Human Genetics and the Department of Laboratory Medicine and Pathology, University of Minnesota, Mineapolis, MN 55455.

To examine the influence of localized IL-2 production on tumorigenicity of murine neuroblastoma, neuro-2a cells were transduced with the retroviral vector LIL-2SN. Two G418 resistant sublines, N-2a/c3 (2.5 ± 0.4 U/ml/10^6 cells/24 h) and N-2a/c2 (44.6 ± 8.8 U/ml), were studied as representative low and high IL-2 producers. Retroperitoneal (r.p.) injection of 10^6 control neo R virus transduced, N-2a/LN cells, into syngeneic A/J mice invariably led to death of all animals (median survival time [MST]=27 days). Mice receiving N-2a/c3 cells had prolonged survival (MST=39 days; $p<0.001$), but all died of progressive disease. The survival of mice inoculated with N-2a/c2 was strikingly improved (MST=166 days; $p<0.001$). Depletion of asialo G_{M1}^+ cells increased mortality for both N-2a/c3 and N-2a/c2 lines ($p<0.001$), compared to untreated mice, indicating that asialo G_{M1}^+ cells play a major role *in vivo* in eliminating IL-2 producing neuro-2a cells. We evaluated whether IL-2 secretion by N-2a/c2 conferred a systemic and/or local anti-tumor effect on mice receiving N-2a/LN cells injected at a distance or at the same site. Inoculation of 10^5 N-2a/LN cells into the left flank led to death of all mice (MST=48 days). Concurrent injection of 10^5, 10^6, or 10^7 N-2a/c2 cells into the contralateral or 10^5 cells to the ipsilateral flank did not delay tumor progression, while co-injection of 10^6 N-2a/c2 cells at the same site delayed time to death ($p<0.001$). Furthermore, immunization with N-2a/c2 cells failed to elicit a protective immunity to challenge with parental tumor cells. N-2a/c2 tumors were isolated from r.p. inoculated mice dying between days 70-85. These cells remained G418 resistant but had decreased production of IL-2 (5-11 U/ml) compared with the N-2a/c2 line propagated *in vitro* during the same period (49 U/ml). Southern blot analysis of emerging tumors revealed an intact proviral structure with consistent 5′ and 3′ flanking regions that varied from that of the N-2a/c2 line. The N-2a/c2 line was therefore composed of 2 clones, one of which expressed low levels of IL-2 and was positively selected *in vivo*. These data indicate that endogenous IL-2 production in the vicinity of the emerging tumor results in a dose dependent anti-tumor protective effect. In neuro-2a cells which express low levels of MHC class I molecules on their surface, these effects appear to be mediated primarily by asialo G_{M1}^+ cells.

NZ 405EXPRESSION OF IL-3 BY TUMORS ENHANCES DEVELOPMENT OF TUMOR REACTIVE CTL BY A CD4 CELL DEPENDENT MECHANISM

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We have investigated the effects of IL-3, a cytokine involved in maturation of hematopoetic cells, on generation of immune effectors capable of killing tumors. By expressing cytokines within the tumor the concentration of cytokine is highest at the site of the tumor challenge. The tumor we have used, line 1, expresses low levels of class I, but can be induced to high levels by treatment with interferons. This allows us to specifically assess CTL against the tumor, by testing effectors against class I low and class I high line 1. We have made line 1 transfectants that express IL-3 or IL-2. Both IL-3 or IL-2 enhance rejection of the tumors.

To determine the lytic potential of the T cells developed early in the rejection of the cytokine expressing tumors, we extracted Tumor Infiltrating Lymphocytes (TIL) from the tumors using paramagntic beads and antibodies to Thy 1. TIL from line 1 tumors transfected with either IL-3 or IL-2 expression vectors show a dramatically enhanced CTL response to untransfected parental line 1 when compared to TILs isolated from parental tumors. IL-2, but not IL-3, expression by the tumors enhanced the killing of YAC-1 cells. To investigate the cells required for generation of cytotoxic effectors, mice were depleted of CD4 or CD8 cells by treatment with monoclonal antibodies. CD8 depletion eliminated the cytotoxic effectors in all cases. Interestingly, CD4 depletion abrogated the IL-3 mediated CTL response, but not the IL-2 mediated response. These results strongly suggest that IL-3, unlike IL-2, works to generate CTL by a mechanism which requires CD4 cells.

As line 1 expresses low levels of class I in vitro, we wished to determine if the IL-3 and IL-2 transfected tumors were induced to express high class I in vivo. To investigate this, we recovered line 1 cells, or the cytokine expressing line 1 cells, from tumors growing in vivo. As determined by flow cytometry, the tumor cells from the parental tumors, and the IL-2 or IL-3 transfectants all express high class I. This is consistent with the role of CTL in the rejection of the line 1 expressing IL-2 or IL-3. Furthermore, we have used PCR to demonstrate that interferon-gamma is detectable in the IL-3, but not parental tumors, suggesting that T cells play a role in the induction of class I in the IL-3 expressing tumors.

NZ 406 ENHANCED EXPRESSION OF MHC MOLECULES AND STIMULATION OF AUTOLOGOUS TUMOR INFILTRATING LYMPHOCYTES FOLLOWING TRANSDUCTION OF MELANOMA CELLS WITH IFN-γ GENES, Masahiro Ogasawara and Steven A. Rosenberg, Surgery Branch, NCI, NIH, Bethesda, MD 20892

Gene Therapy for cancer is being tested in clinical trials by using tumor infiltrating lymphocytes (TIL) or tumor cells modified by the insertion of genes coding for IL-2 or TNF-α. In the present study, we investigated the feasibility of transducing human tumor cells with genes coding for IFN-γ or IFN-α, which are two other cytokines that can enhance host antitumor immune responses.

Tumor cells from twelve melanoma and two renal cell carcinoma patients were transduced with IFN-γ retroviral vectors. In both IFN-γ secreting and non-secreting tumor lines, the cell surface expression of HLA class I and class II molecules increased following transduction. However the magnitude of the increase in MHC expression appeared to be greater in tumor lines secreting IFN-γ. Northern blot analysis showed IFN-γ transcripts only in IFN-γ transduced cells. The amount of RNA transcribed correlated roughly with IFN-γ secretion.

Two melanoma cell lines were successfully transduced with an IFN-α retroviral vector. Melanoma cells transduced with the IFN-α gene transcribed IFN-α RNA and secreted large amounts of IFN-α. In contrast to cells transduced with the IFN-γ gene, the expression of HLA class II molecules was not increased in IFN-α -transduced cells.

Finally, we tested the ability of HLA.DR+ melanoma cells, which had been transduced with the IFN-γ gene, to stimulate specific cytokine release by autologous CD 4+ TIL. Both GM-CSF and IFN-γ were secreted when the lymphocytes and tumor cells were cultured together but not when they were cultured alone or with control tumor cells. These results suggest that the HLA.DR molecules newly expressed on the transduced cells promoted antigen presentation and T cell responses against the transduced tumor cells. The insertion of IFN-γ genes into melanoma cells may be useful either for active immunization against melanoma or for the generation of TIL to be used in adoptive immunotherapy.

NZ 407 RETROVIRAL MEDIATED GENE TRANSFER OF IL-2 DECREASES TUMORIGENICITY IN MURINE B CELL LYMPHOMA. Orchard P.J.[1,2], Katsanis E.[1], Gorden K.[1], May C.[1], McIvor R.S.[2] and Blazar B.R.[1,2] *[1]Department of Pediatrics, Division of Bone Marrow Transplantation and [2]Institute of Human Genetics, Department of Laboratory Medicine and Pathology, University of Minnesota, 55455*

The potential to increase immune responsiveness against otherwise minimally immunogenic malignancies by the transfer of cytokine genes into tumor cells has been of great interest. We have examined the effect of endogenous production of Interleukin-2 (IL-2) on BDL-2, a murine B cell lineage lymphoma, by transducing BDL-2 with a retrovirus (LIL2SN) we have constructed containing the human IL-2 cDNA under transcriptional regulation of the Moloney long terminal repeat, the SV40 internal promoter and the neomycin phosphotransferase gene. BDL-2 clones transduced with LIL2SN were isolated by limiting dilution in G-418, and have been shown to secrete 0.2 - 88.5 U IL-2/10^6 cells/mL/24 hours (mean 29.5 U/mL) by ELISA. No changes in phenotype (MHC class I, CD2, CD5, B220, ICAM-1 or surface IgG) or variation in the rate of proliferation were observed following retroviral transduction and expression of IL-2. Intravenous (iv) or intraperitoneal (ip) injections of this IL-2 secreting BDL-2 clone in syngeneic Balb/c mice resulted in significantly increased median survival time (MST) when compared to controls ($p< 0.03$ and $p< 0.001$, respectively). Immunization with irradiated IL-2 secreting tumor cells subcutaneously (sc) resulted in enhanced survival ($p< 0.0002$) following live tumor challenge with the parental BDL-2 line 14 days later. In a minimal residual disease model, iv injection of 10^5 BDL-2 cells followed on day 12 by sc administration of 10^7 irradiated IL-2 secreting cells resulted in a significant ($p< 0.01$) improvement in MST. In vitro ^{51}Cr release assays demonstrated sensitivity of BDL-2 to both natural killer (NK) populations and activated cytotoxic T cells. In vivo depletions of $CD4^+$ cells (RL 172 antibody) $CD8^+$ cells (2.43) and NK cells (anti-asialo GM_1) were performed. Depletion of $CD8^+$ and NK cells resulted in decreased survival in mice inoculated with IL-2 secreting tumor cells iv, compared to controls and to mice depleted of $CD4^+$ cells. This implicates both cytotoxic T cells and NK cells as important in the resistance of Balb/c mice to a IL-2 secreting BDL-2 tumor line in vivo.

NZ 408 **EXPRESSION OF SYNGENEIC MHC CLASS II GENES IN MELANOMA CELLS INHIBITS METASTATIC DISEASE.** S. Ostrand-Rosenberg and Noelle Patterson, Dept. of Biology, University of Maryland, Baltimore, MD 21228.

Previous studies have established that transfection of syngeneic MHC class II genes into constitutively class II^- mouse sarcoma cells produces an immunogenic tumor (SaI/A^k) which is rejected by the autologous host, and which effectively immunizes the host against a challenge of wild type class II^- tumor. We have hypothesized that the SaI/A^k transfectants induce protective immunity because they function as antigen presenting cells (APC) for endogenously synthesized tumor peptides, and thereby stimulate tumor-specific T_h cells, by-passing the need for professional APC. In the present study we demonstrate that immunization with MHC class II gene transfected tumor also protects the autologous host against subsequent metastatic disease. The C3H-derived ($H\text{-}2^k$) K1735 melanoma gives high levels of spontaneous (subcutaneous inoculation) and experimental (intravenous inoculation) metastastases in syngeneic C3H mice. In order to test the protective potential of MHC class II^+ K1735 cells, wild type K1735 tumor cells were transfected with syngeneic A_a^k, A_b^k MHC class II genes, and/or neo^R gene, and 3 clones expressing high levels of $I\text{-}A^k$ molecules selected ($K1735/A^k$ clones). At tumor doses ranging from 10^3 to 5 X 10^5 i.v., the class II^+ transfectants give 5-10 fold fewer lung metastases than their wildtype class II^- or neo^R alone counterparts. C3H mice inoculated subcutaneously with class II^+ K1735 cells have significantly lower frequencies of primary tumors and spontaneous metastases, as compared to mice receiving wild type K1735 inocula. We have also tested the ability of $K1735/A^k$ cells to immunize against wild type tumor. Autologous C3H mice immunized with fixed class II^+ K1735 cells and challenged i.p. 1-3 months later with wild type K1735 tumor have greatly reduced primary tumor growth and spontaneous metastasis formation relative to naive, unimmunized recipients. These studies indicate that transfection and expression of syngeneic MHC class II genes significantly reduces the metastatic potential of a mouse melanoma, and provides an immunization stategy for protecting against subsequent metastatic disease.

NZ 409 THE RETROVIRAL VECTOR MFG ALLOWS HIGH EFFICIENCY TRANSDUCTION OF HUMAN PROSTATE CANCER CELLS: IMPLICATIONS FOR GENE THERAPY OF PROSTATE CANCER. Martin G. Sanda, Sujatha Ayyagari, Liz Jaffee, Drew M. Pardoll, Richard C. Mulligan, and Jonathan W. Simons, Oncology Center and Brady Urological Institute, Johns Hopkins Hospital, Baltimore, MD, and the Whitehead Institute, MIT, Cambridge, MA.

We addressed the feasibility of gene therapy for human prostate cancer using MFG, an amphotrophic and replication defective retroviral vector lacking the gag, pol, and env genes. First, conditions optimizing transduction efficiency using MFG-lacZ (containing the gene encoding beta-galactosidase) were identified with transduction of four long term human prostatic adenocarcinoma cell lines. DEAE-dextran dose and virus-target coincubation titration demonstrated optimum transduction efficiency at 80ug/ml of DEAE-Dextran with 5 hours of target exposure to viral supernatant. Using these conditions, 21-82% transduction efficiency was achieved. We then used these conditions to transduce prostate cancer cells from patients undergoing radical prostatectomy. Cultured cells derived from surgical specimens were confirmed to be of prostatic epithelial origin by immuno-histochemical detection of luminar epithelium specific cytokeratin 18, and by detection of Prostate Specific Antigen (PSA) secretion by these cells. 200 - 300 fold expansion was achieved in vitro prior to senescence of cultured cells. Efficient transduction by MFG-lacZ of such primary culture prostate cancer cells from 7 consecutive patients (transduction efficiency range, 4.3% to 50%; median, 15.2%) demonstrates the feasibility of using MFG in genetic therapy for prostate cancer.

NZ 410 TRANSFER AND EXPRESSION OF THE HUMAN INTERLEUKIN-4 GENE INTO PRIMARY STROMAL AND CARCINOMA CELLS FROM LUNG CANCER PATIENTS, Jill M. Siegfried[1], Jay D. Hunt[1], Barbara A. Pippin[2], Rodney J. Landerneau[2], William F. Jacobs[3], and Michael T. Lotze[2], Departments of [1]Pharmacology and [2]Surgery, University of Pittsburgh, Pittsburgh, PA 15261 and [3]Genetic Therapy, Inc., Gaithersburg, MD 20878. Transfer of an expression vector for the interleukin-4 (IL-4) gene into cells derived from human tumor tissue provides a means of generating a custom tumor vaccine. A vaccine could be produced by transducing tumor-derived stromal cells with the IL-4 vector, and co-injecting tumor cells with these stromal cells, or by transducing the tumor cells themselves. Tumor cells, though difficult to culture, would provide a permanent source of IL-4 producing cells; stromal cells, though of limited lifespan, are more readily and rapidly attainable. We have developed a protocol for culturing cells from non-small cell lung tumors by using a combination of a growth factor-rich conditioned medium and a feeder layer of murine embryonic fibroblasts. We have produced tumor cultures from 25% of non-small cell tumors, and stromal cultures from over 80% of specimens. Several of these cultures were used for transduction by the retroviral vector G1NaSVi4.25, containing the human IL-4 cDNA driven by the SV40 promoter, and the G418-resistance gene driven by a retroviral LTR. Infection of cells by viral titers of 2-5 x 10^4 PFU/ml yielded transfer efficiencies of 3.3 to 32.0 transfectants per 10^4 cells in 6 of 8 attempts. Successful transfers were performed with tumor cells derived from a squamous cell carcinoma, a adeno-squamous carcinoma, and stromal cultures from 4 other lung tumors, including a melanoma metastatic to the lung. Following selection by G418, IL-4 producing cells were isolated. IL-4 titers exceeded 50 U per ml in a 24 hr collection. Transfer of the IL-4 gene was demonstrated by PCR amplification from reverse-transcribed RNA, followed by Southern blotting with a cloned human IL-4 sequence to confirm hybridization. Gene transfers were performed between 18 and 60 days after acquisition for stromal cells and within 150 days for tumor cells. We are attempting transfers within 60 days for tumor cells.

NZ 411 CYTOKINE-TRANSFECTED 3T3 FIBROBLASTS INHIBIT THE PROGRESSION OF MURINE RENAL CANCER. Robert H. Wiltrout[1], Kristin L. Komschlies[2], Jose L. Franco[1], Eilene Gruys[1], Timothy T. Back[2], Drew M. Pardoll[3], Laura C. Post[1], and Robert G. Fenton[1]. [1]Biological Response Modifiers Program and [2]BCDP, PRI/DynCorp, NCI-Frederick Cancer Research and Development Center, Frederick, MD 21702, and [3]The Department of Medicine, Johns Hopkins University, Baltimore, MD 21205.
Several recent studies have shown that mouse tumors that have been engineered to produce IL4 are rejected. Because the use of autologous cytokine-producing tumors in humans may prove logistically difficult, we are studying the ability of cytokine-transfected fibroblasts to mediate anti-tumor effects. Previous studies (Golumbek et al., Science 254:713, 1991) have shown that the injection of IL4-transfected murine renal cancer (Renca) induced the rejection of wild-type Renca (Renca/WT). Our studies confirm that observation and demonstrate that IL4-transfected BALB/c 3T3 fibroblasts (3T3/IL4) also mediate anti-tumor effects against Renca/WT in vivo. Specifically, the subcutaneous (sc) co-injection of 10^6 3T3/IL4 or IL4-secreting Renca (Renca/IL4) cells with 10^5 Renca/WT cells resulted in 100% and 85% tumor-free survival, respectively, as compared to 8-20% survival in normal mice injected with Renca/WT or with Renca/WT plus normal 3T3 cells. Rechallenge of the tumor-free survivors with Renca/WT cells resulted in the tumor-free survival of 100% and 67% of mice that initially received 3T3/IL4 and Renca/IL4 cells admixed with Renca/WT, respectively. These results suggest the induction of systemic immunity. Further, the repeated sc or ip administration of Renca/IL4 reduces the number of experimental Renca/WT pulmonary metastases by 50-90%. Preliminary studies suggest that 3T3/IL4 has similar effects. The repeated administration of Renca/IL4 to mice bearing advanced Renca/WT extended survival from 30 days to about 56 days. Thus, these results demonstrate that systemic effects can be induced by both Renca/IL4 cells and 3T3/IL4 fibroblasts, and suggest that cytokine-secreting fibroblast lines may circumvent some of the logistical and ethical considerations of gene therapy by cytokine-producing autologous tumor cells.

NZ 412 TUMOR DERIVED IL-2 PREVENTS NON-RESPONSIVENESS TO A MURINE FIBROSARCOMA, Karen S. Zier, Silvia Salvadori, and Bernd Gansbacher, Departments of Medicine and Microbiology, The Mount Sinai School of Medicine, New York, N.Y. 10021.
We made retroviral constructs containing IL-2 cDNA. Infection of CMS5 fibrosarcoma cells and selection in G418 resulted in the isolation of clones of tumor cells which produced IL-2. Whereas in vivo injection of parental tumor cells resulted in progressive tumor growth, tumor cells secreting high levels of IL-2 were rejected. Furthermore, the immunosuppression associated with the inoculation of parental tumor cells was not seen. In order to study the basis for the development of non-responsiveness to parental tumor cells we analyzed the mechanisms which influence cell mediated anti-tumor responses. First, our results indicated that immunization with IL-2 secreting tumor cells resulted in the generation of an IL-2 responsive population not seen in tumor bearing animals. Second, functional studies demonstrated that the proliferation of spleen cells from immune animals could be inhibited by restimulation with parental CMS5 cells. This suppression was not due to cell death and could not be transferred by culture supernatants, suggesting that cell to cell contact might be required. In contrast, there was vigorous proliferation to IL-2 secreting CMS5 cells. Third, although in vitro culture of spleen cells from immune animals without tumor cells yielded potent effector cells, restimulation with parental tumor cells resulted in decreased lysis. These results are consistent with the hypothesis that antigen presentation by tumor cells unable to provide required secondary signals inhibit T cell responses by inducing anergy. A source of local IL-2 enables CTL activation and prevents non-responsiveness.

MHC Unrestricted Effector Cells

NZ 413 SPECIFIC RECOGNITION AND REJECTION OF THE H-2 DEFICIENT CELL LINE LR.4 BY C57BL/6J MICE. **Guillermo Alfaro, Gabriel Nava and Emma Verástegui. Departamento de Inmunología, Instituto de Investigaciones Biomédicas UNAM. México 04510, D.F., México.**

Lack of expression of products of the MHC (H-2) in the cell line LR.4 is the consequence of deletions located in B2m and IAβ, and extensively methylated class I genes. LR.4 cells grew and killed different inbred strains of mice: BALB.b (H-2^b), BALB/c and DBA/2 (H-2^d), and BALB.k and C3H (H-2^k). However, C56BL/6J (H-2^b) mice grew and rejected the tumor in > 99% of the cases. Southern blots using DNA probes specific for the α, β and γ subunits of the TcR and for IEα or IEβ demonstrated that these were not the antigens involved in the rejection of the malignant cells. Furthermore, animals which rejected the tumor developed a protective and specific immune response. Immunization with thymocytes from BALB.b also induced a protective immune response against LR.4 cells; however, differences between the two types of immune response were detected based upon the patterns of reactivity of the antibodies which were induced. Since class I and class II molecules are required for antigen presentation, it is likely that the antigen(s) responsible for this phenomenon in C57BL/6J mice belong to a group of membrane proteins which are expressed in normal and in malignant cells. Finally, it should be mention that old mice (87 weeks old) failed to reject the tumor and to develop a protective immune response.

NZ 414 THE BETA SUBUNIT OF MITOCHONDRIAL H+ TRANSPORTING ATP SYNTHASE: TUMOR COUNTER RECEPTOR FOR NAIVE NK CELL MEDIATED CYTOTOXICITY. Ballabh Das, Mary Mondragon, Minoo Sadeghian, Shi-Zhen Tao and Allen Norin, Departments of Medicine and Anatomy & Cell Biology, SUNY Health Science Center Brooklyn, New York 11203

The process by which cytolytic lymphocytes destroy target cells is not completely understood. In previous studies we used a direct cellular adsorption approach to identify a protein of approximately 51.5 KD on the K562 surface as a potential target structure for NK cell mediated cytolysis. The purified 51.5 KD protein bound to and competetively inhibited the killing of the K562 cell line by freshly isolated lymphocytes. N-terminal amino acid sequence analysis of this purified protein revealed 100% homology with the beta subunit of mitochondrial H+ transporting ATP synthase. The purified 51.5 KD K562 protein reacted (on western blots) with antiserum against the ATP synthase of rat liver mitochondria and also with antibodies against a synthetic peptide corresponding to the ATP binding site. Human erythrocytes, lymphocytes and the K562 cell line were incubated with the latter antiserum and then subjected to indirect flow cytometry analysis. A strong fluorescent signal was obtained only with the K562 cell line (about 90% positive cells). Erythrocytes were negative and lymphocytes showed a weak signal (about 5% positive). These results clearly demonstrate expression of the beta subunit of H+ transporting ATP synthase on the surface of K562 cells. ^{51}Cr labelled K562 target cells were incubated with various dilutions of the anti peptide serum and then incubated with freshly isolated human lymphocytes. Concentration dependent inhibition of NK cytotoxicity was observed with 100% blockage of killing at 1:20 dilution of antibody (effector target ratio, 25:1). These results demonstrate for the first time that the beta subunit of mitochondrial H+ transporting ATP synthase is expressed on the surface of tumor cells and that this molecule serves in an important adhesion and/or triggering capacity for NK cell mediated cytotoxicity. (Supported by USPHS grant CA47548).

NZ 415 ROLE OF THE $\alpha1/\alpha2$ DOMAIN IN THE IN VIVO NK RESPONSE TO LYMPHOMA GRAFTS, Petter Höglund, Margareta Waldenström and Klas Kärre, Department of Tumor Biology, Karolinska Institute, Box 60 400, S-104 01 Stockholm, Sweden

We have previously shown that introduction of an allogeneic MHC class I gene (D^d) into the germline of C57Bl/6 (B6) mice (K^b, D^b) brings about a redefinition of the in vivo NK cell repertoire. A panel of H-2^b lymphomas the grew readily in the syngeneic strain (B6), was rejected by NK cells in the H-$2D^d$ transgenic strain D8. The rejection could be abrogated by transfection of the lymphoma cells with the D^d gene, a finding consistent with the "missing self" hypothesis for NK mediated rejection of H-2 deficient or mismatched grafts. We have now started an analysis of which part of the D^d molecule that was responsible for the protection from elimination in the D8 strain,. This was tested by grafting lymphoma cells transfected with chimeric H-2 genes. The results indicated that the $\alpha1/\alpha2$ domains of D^d is necessary for protection, while the $\alpha3$ domain in association with the $\alpha1/\alpha2$ domains of a non-protective H-2 molecule (L^d) did not protect from elimination by D8 NK cells.

NZ 416 HLA-B7 VARIANTS WITH PEPTIDE BINDING GROOVE MUTATIONS FAIL TO INHIBIT HUMAN PERIPHERAL BLOOD NATURAL KILLER CELLS

CT Lutz, ZB Kurago and KD Smith, Departments of Pathology, Oral Pathology, and Microbiology, University of Iowa, Iowa City, IA 52242

Natural killer (NK) cells lyse hematopoietic target cells that express little or no MHC class I. We are systematically testing how well transfected HLA-B7 variant molecules protect HLA-A,B,C-negative 721.221 human B lymphoblast from NK lysis. 721.221 transfectants were tested with freshly isolated peripheral blood mononuclear cells from several HLA typed donors in a standard 5 hour ^{51}Cr release assay. NK killing was compared with killing by cloned alloreactive cytolytic T lymphocytes (CTL). Compared with vector only controls, transfected *wild-type* HLA-B7 protected 721.221 targets from NK killing by 40-60% . Three out of four HLA-B7 variants with mutations pointing up in the putative T cell receptor contact site protected 721.221 from NK killing, equivalent to *wild-type* HLA-B7. Likewise, none of these mutations in up pointing HLA-B7 residues affected most alloreactive CTL clones. In contrast, many HLA-B7 peptide binding groove variants did not protect against NK lysis. Nonprotective variants had mutations in B pocket residues 9, 63, 66 and in D/E pocket residues 114 and 156. Likewise, these peptide binding groove variants were not recognized by most (≥ 6 of 7) alloreactive CTL clones. All variants were killed by some CTL, indicating that HLA-B7 structure was not globally disrupted by the mutations tested. As measured by a panel of mAb, levels of cell surface HLA-B7 varied little and did not correlate with protection from NK killing. This suggests that both NK cells and CTL recognize specific peptide/MHC complexes.

NZ 417 HSP72 - An Antigenic Determinant For Non-MHC Restricted Cytotoxic Effector Cells. Gabriele Multhoff, Claus Botzler, Marion Wiesnet, Markus Essler and Rolf D. Issels, Institut für Klinische Hämatologie der GSF, Marchioninistr. 25, D-8000 München 70, Germany.

We established an in vitro model to study the immunological effects of heat exposure upon human malignant cells. Interestingly, we detected a positive surface staining of heat-shock-protein 72 (HSP72) on heat treated Ewing's Sarcoma (ES) cells, on osteosarcoma cells, on PBL of patients suffering from acute leucemia and on HEK 293 cells, transformed with the E1A sequence of adenovirus, using HSP72 specific monoclonal antibodies. In contrast, PBL of healthy human blood donors or EBV transformed B-LCL did not show any surface expression of HSP72 after heat treatment. The MHC class I expression of the malignant cells was decreased after heat shock, whereas that of PBL of healthy human blood donors was not influenced. We could also demonstrate that CD8+ and CD57+ NK-like effector cells can recognize an antigenic HSP72 epitope on the surface of malignant cells after heat shock. The specificity of these effector cells was defined in a Cell Mediated Lympholysis-assay (CML) using either untreated or heat treated ES cells, K562 cells and B-LCL as target cells. The effector cells showed strong lysis of heat treated ES cells and K562 cells, whereas the lysis of untreated ES cells and B-LCL was weak. By the use of HSP72 specific mAb for blocking experiments, the lysis of heat treated ES cells was inhibited, whereas MHC class I (W6/32) or HLA DR (L243) specific mAb had no influence on the lysis pattern of ES cells. Our results strongly suggest a role for a heat inducible HSP72 epitope on human malignant cells acting as an antigenic determinant for CD8+/CD57+ NK-like effector cells.

NZ 418 A 38.5KD TUMOR PROTEIN IS A UNIQUE RECOGNITION STRUCTURE FOR NAIVE HUMAN NK CELLS. Allen J. Norin, Ballabh Das, Mary Mondragon, Minoo Sadeghian, Shi-Zhen Tao. Departments of Medicine and Anatomy & Cell Biology, SUNY Health Science Center, Brooklyn, New York 11203

NK cells provide spontaneous defense against some types of neoplastic cells and virus infected cells. Although a number of tumor surface molecules have been reported that may play a role in NK cell mediated cytotoxicity, no receptor-counter receptor pair has been identified that is specific to this cell to cell interaction. The objective of this study was to identify a unique tumor membrane protein(s) (TMP) that binds to NK cell surface receptor(s). For this purpose a new technique was developed which utilized a direct adsoprtion method where biotin-labelled solubilized TMP from K562 cells were reacted with viable lymphocytes. Specifically adsorbed TMP were eluted and analyzed by SDS-PAGE and western blot. $CD3^+,5^+,16^-$ (T cells) and $CD3^-CD5^-CD16^+$ (NK cells) enriched subpopulations of lymphocytes were obtained by fractionating unstimulated peripehal blood lymphocytes with immunomagnetic beads. These fractionated cells were incubated with biotinylated K562 TMP in RPMI 1640 medium with 15% fetal bovine serum (to block nonspecific protein adsorption). Washed cells were solubilized and bound K562 proteins identified on western blots. Of approximately 20 adsorbed K562 proteins, a 38.5KD band bound specifically to NK cells whereas a 46KD protein bound specifically to T-cells. K562 38.5KD protein was purified to apparent homegeneity by preparative PAGE and further studied in functional and binding assays. The purified 38.5 KD protein bound exclusively to NK cells compared to T cells. Furthermore, the soluble 38.5KD K562 protein inhibited NK cell mediated cytotoxicity in a concentration dependent manner. Control K562 membrane proteins of 41KD and 80KD were purified by the same techniques. Neither of these proteins bound to lymphocytes nor blocked NK cell activity. Amino acid sequence analysis of two internal peptides indicate that the 38.5 KD K562 is a novel molecule. Initial studies suggest that the NK cell receptor of the 38.5 KD K562 protein is a molecule of 72KD. These studies demonstrate the involvement of a unique pair of counter receptor-receptor proteins in naive NK cell cytotoxicity. (Supported by USPHS grant CA47548).

NZ 419 CHARACTERIZATION OF NOVEL GENES EXPRESSED IN NK CELLS, RP Schall, CA Dahl, TP Leary, OH Wesly, RB Herberman and PM Sondel, Dept of Human Oncology, Univ of WI, Madison, WI 53719 and Pittsburgh Cancer Institute, Pittsburgh, PA 15213

A number of laboratories have sought to describe molecules selectively expressed in human NK cells likely to be involved in determining NK phenotype and function, and to aid in the understanding of non-MHC restricted killing of tumor cell targets. We have utilized subtractive hybridization and differential screening of an NK derived cDNA library to identify mRNA transcripts for genes preferentially expressed in NK cells. Several rounds of screening for cDNA clones which did not hybridize with B cell-derived cDNA, but bound subtracted NK-specific probe narrowed our focus to 41 cDNA sequences preferentially expressed in NK cells. We next began to characterize the expression of the these transcripts in different cell types at various levels of activation. Of the eight clones examined to date, four show interesting patterns of expression by Northern analysis. These transcripts are highly expressed in NK and LAK cells, are not expressed in B cells, and show low levels or lack of expression in bulk-stimulated T cell populations. Preliminary evidence will be discussed showing expression patterns of these genes (by Northern analysis and recently developed PCR assays) in functionally characterized clones from PBLs.

DNA sequence information of these newly identified cDNA clones will be presented. Three of these genes are known to be novel (no significant homology with any known gene within Genbank), with interesting regions of local homology which may prove important in their function. Preliminary sequence information indicates that one clone, NK12, has homology to the 3' untranslated region of a zinc finger DNA binding protein, Egr-2 (early growth response gene). NK14 has been cloned into a eucaryotic expression vector and transfected into an osteosarcoma cell line to yield NK14 transcription.

These studies have led us to the elucidation of four novel transcripts preferentially expressed in NK cells, and work is proceeding to address the function of these genes.

NZ 420 *Abstract Withdrawn*

Vaccines; Novel Methods in Tumor Immunology

NZ 500VV MUC.1 IMMUNIZATION OF MICE : IMMUNE RESPONSE AND PROTECTION AGAINST GROWTH OF MURINE TUMOURS BEARING THE MUC.1 ANTIGEN.

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MUC.1 is a mucin found on the apical surfaces of some normal mammalian mucin secreting cells. It is characterized by heavy glycosylation and a 20 amino acid tandem repeat segment. In most cases of human breast adenocarcinoma this antigen is over expressed. Moreover, abnormal glycosylation exposes a novel peptide epitope within the tandem repeat, such that antibodies to this epitope can distinguish normal from malignant adenocarcinoma breast tissue. We have constructed a vaccinia virus (VV) which carries the cDNA for the MUC.1 antigen. Murine and human cells infected with this virus express the MUC.1 molecule, with 2-3 tandem repeats per molecule and with the tumour specific epitope exposed.

Mice immunized with this virus produce antibodies which recognize MUC.1 both outside the tandem repeat, within the tandem repeat and the tumour specific epitope. Some DTH type response has been observed but no MUC.1 specific CTL response has been found.

Tumourogenic P815 (DBA) and 3T3 (Balb/c) cells have been transfected with MUC.1. Thirty per cent of DBA mice immunized with VV MUC.1 are protected from growth of P815. MUC.1 tumours when implanted with 10^5 cells. Immunized Balb/c mice show a late development of transfected 3T3 tumour cells at a similar frequency. Mice transgenic for the MUC.1 antigen have been constructed and are being evaluated for immune response to MUC.1 and protection against tumour growth.

NZ 501 RECOMBINANT VACCINIA/IL-2-INFECTED TUMOR CELL VACCINE IN IMMUNOTHERAPY OF MURINE COLON ADENOCARCINOMA,

Jerry A. Bash, Department of Medical Laboratory Sciences, Florida International University, Miami, FL 33199

Vaccinia virus has been shown to augment the immunogenicity of a variety of tumors. The hypothesis that interleukin-2 (IL-2) secreted by vaccinia-specific T helper cells mediates the expansion of tumor-specific helper and cytolytic T cells is supported by the demonstration that exogenous IL-2 can replace vaccinia priming in augmentation of anti-tumor responses. To confirm that IL-2 secreted in the microenvironment of tumor cell recognition can augment host immunity, a recombinant vaccina virus (vCF13) containing and expressing the gene for human IL-2 was used to infect murine colon adenocarcinoma cell lines for use as viable tumor cell vaccines. Balb/c derived tumor cell lines CT26 and CA51 were infected with either vCF13 or the transfection control strain vTFCLZ lacking the IL-2 gene (recombinant vaccinia provided by B. Moss, NIAID) at low multiplicity of infection for 24 hours. The expression of the IL-2 gene was confirmed by ELISA and CTLL-2 bioassay of vCF13-infected tumor cell supernatants. Viable vaccina-infected tumor cells were injected subcutaneously (10^5 cells) at two weekly intervals followed a week later by a subcuaneous challenge with uninfected tumor cells (10^5). vCF13-infected CA51 immunized mice were refractory to uninfected CA51 challenge and demonstrated CA51 specific CTL activity in peripheral blood. vCF13-CT26 vaccines failed to protect against CT26 challenge, however, due to rapid and severe lymphopenia and cachexia induced by CT26 challenge. Intralesional infection of CT26 tumors induced rapid regression and reversal of disease but with no lasting immunity. These data provide both support for the potential utility of recombinant vaccinia/IL-2 in tumor immunotherapy as well as the possible limitations of this approach in immunosuppressed hosts.

This work was partially supported by a grant from the American Cancer Society, Florida Division, Inc.

NZ 502 IMMUNIZATION WITH GP96 HEAT SHOCK PROTEINS ISOLATED FROM TUMORS OR INFLUENZA VIRUS INFECTED CELLS ELICITS MHC - RESTRICTED, ANTIGEN - SPECIFIC CYTOTOXIC T LYMPHOCYTES AGAINST THE CORRESPONDING CELLS / ANTIGENS,

Nathalie E Blachere and Pramod K Srivastava, Department of Pharmacology, Box 1215, Mount Sinai School of Medicine, New York, NY 10029

Immunization of mice with gp96 elicits protection against a challenge with the tumor from which gp96 is isolated but not another tumor (1). Gp96 preparations isolated from normal tissues do not elicit protective immunity against any tumors tested (2). However, this specificity of immunogenicity of gp96 does not reside in the DNA sequence or glycosylation of gp96. In light of our demonstration that gp96 is a heat shock protein (hsp) and that some hsps are known to bind peptides we have proposed (3) that gp96 acts as a chaperonin for peptides, which confer on it the specificity of its immunogenicity. Further, as gp96 molecules reside largely in the endoplasmic reticulum (ER), we suggested (3) that it acts as a peptide-acceptor in the ER lumen, and that it may be accessory to charging of MHC class I with peptides.

We show here that immunization of mice with gp96 isolated from the UV-induced C3HHeN sarcoma 6138 elicits class I - restricted, tumor-specific CTLs against the 6138 sarcoma. Further, as we suggested a general peptide-chaperoning role for gp96, we have tested our hypothesis in a non-tumor model. Influenza-specific CTLs can be generated from spleens of mice immunized with gp96 obtained from influenza (PR8) - infected cells or cells transfected with the NP gene of the influenza virus. These observations support our hypothesis and suggest a general method for eliciting T cell immunity against tumors and infectious diseases.

1. Srivastava et al. 1986 PNAS 83, 3407. 2. Udono et al. see abstract this meeting. 3. Srivastava and Maki 1990 Curr Top Microbiol & Immunol. 167, 109

NZ 503 MECHANISMS OF INDUCTION OF PRIMARY CYTOTOXIC T LYMPHOCYTE RESPONSES,

Marloes L.H. De Bruijn, John D. Nieland, Ton N.M. Schumacher, Hidde L. Ploegh, Cornelis J.M. Melief, Michael Jackson and Per A. Peterson, Department of Immunology, The Scripps Research Institute, La Jolla, CA 92037

Dendritic cells and RMA-S cells are good inducers of CTL responses by single *in vitro* stimulation. The ability to obtain primary CTL responses makes it possible to test MHC-binding peptides functionally and entirely *in vitro* to identify potential vaccine candidates. Both dendritic cells and RMA-S cells can present antigenic peptides to generate primary specific CTL responses without the need for T helper cells or the addition of exogenous IL-2. Rapid antigen-independent aggregation of dendritic cells with unprimed T cells and high expression of relevant MHC/peptide complexes on RMA-S are characteristics apparently important in the initial contact with unprimed T lymphocytes. CD8 and LFA-1 molecules are involved in the activation of specific CTL in these primary responses induced by dendritic cells or RMA-S cells.

Integration of multiple signals influences differentiation and effector function of T lymphocytes. The B7 molecule is considered to be an important costimulator in the activation of T lymphocytes. Next to activated B cells, only dendritic cells express considerable amounts of B7. Possibly, there is a role for B7 in the efficient antigen-presenting function of dendritic cells. Work is in progress to investigate the contribution of B7 in the induction of primary CTL responses.

NZ 504 STRUCTURE AND IMMUNOGENICITY OF THE HUMAN MUCIN MUC-1 TANDEM REPEAT DOMAIN,

J. D. Fontenot[1], Nieves Domenech[1], Dawen Bu[1], Nico Tjandra[2], Chien Ho[2] and Olivera J. Finn[1]. Department of Molecular Genetics and Biochemistry, University of Pittsburgh, School of Medicine, and the Department of Biological Sciences, Carnegie Mellon University, Pittsburgh PA, 15216

The human breast and pancreatic tumor antigen encoded by the human mucin muc-1 gene contains a highly glycosylated tandem repeat domain. In tumor cells, aberrant glycosylation results in the exposure of the tandem repeat protein core throughout the cell surface. The repetitive domain consists of a 20 amino acid sequence rich in proline, threonine, serine and glycine. The protein core was thought to be random coil and to derive its structure from the addition of carbohydrates to threonine and serine residues. However, mucin specific, murine and human antibodies, and T-cell clones have been identified which react with mucin in a manner that implies that a specific 3-dimensional structure is being recognized.

We prepared synthetic peptides corresponding to one, two, three, four, and five tandem repeats of the muc-1 repetitive domain. One and two-dimensional ^{1}H-NMR correlation spectroscopy (COSY) studies indicate that the muc-1 protein core is not in a random-coil secondary structure, and that long-lived amide protons are protected in D_2O. Increasing spectral complexity in the region of the β-protons of D2 and H15 reveals that structural changes are occurring as the number of repeats increases. The greatest changes occur when the number of repeats increases from one to two. The circular dichroism spectra of the 20, 60 and 105 amino acid peptides is dominated by proline in the *trans* conformation. Intrinsic viscosity measurements indicate that a folded structure present in solution is rod-shaped. These results are all consistent with formation a poly-proline β-turn helix form of secondary structure by the muc-1 tandem repeat domain.

Immunogenicity studies in mice with peptides corresponding to one or five tandem repeats reveal a dependence on the structure of the immunizing peptide for antibody production, T-cell proliferation, and CTL production. The information gained from these studies should be important for designing therapeutic vaccines for breast and pancreatic cancer.

NZ 505 IDIOTYPIC VACCINATION AGAINST B-CELL LYMPHOMA - A GENETIC APPROACH.

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Treatment of cancer with vaccines is an attractive prospect and the idiotypic immunoglobulin of B-cell lymphomas is a suitable target. Animal models suggest this approach can be effective but making vaccines on an individual basis is time consuming and difficult.

Here we show that the V-genes of the idiotypic immunoglobulin can be identified direct from biopsies of human lymphomas by PCR amplification, cloning and sequencing. In three patients heterohybridomas prepared from the tumour were available and sequencing these confirmed the genes identified comprised the tumour idiotype. For vaccination we used an expression vector, with a retroviral promoter, that expresses the functional immunoglobulin, in the form of a single chain Fv (scFv), in fusion with a viral envelope protein to inoculate mice. Using a mouse antibody as a model for future human therapy we immunised mice directly with the recombinant DNA to generate an anti-idiotypic response. Subsequent inoculation with a vector which expresses the soluble scFv alone enables the response to be boosted and resulted in high antibody titres. The use of direct injection of DNA to express the idiotypic antibody, as an immunogenic fusion protein, *in vivo* , has obvious practical attractions and suggests a simple means by which idiotypic vaccines could be made for lymphoma patients. In addition, it should allow the presentation of appropriate epitopes in association with self MHC and thus may be effective at stimulating T-cell, as well as antibody responses. Genetic immunisation may also be valuable to immunise against other self- or mutated self-proteins including tumour antigens.

NZ 506 DETECTION OF ANTI-MUC-1 ANTIBODY IN SERA FROM PATIENTS WITH BREAST CANCER.

Yasuo Kotera, J. Darrell Fontenot, and Olivera J. Finn, the Department of Molecular Genetics and Biochemistry, and the Pittsburgh Cancer Institute, University of Pittsburgh, School of Medicine, Pittsburgh, PA, 15261

Tumor reactive CTLs have been isolated from patients with breast and pancreatic cancer. The CTLs have been shown to be specific for the tandem repeat core of the human mucin muc-1. Mucin-specific antibodies have not been detected in patients with these tumors. One explanation is that they may form Ag/Ab complexes with circulating mucin and thus would not be detectable. Another explanation is that the target antigens used to detect them, purified whole mucin or short synthetic peptides, were sub-optimal. Therefore we thought that there should be B-cell response to mucin.

We prepared synthetic antigens corresponding to 3, 4, and 5 tandem repeats of muc-1 protein core. The 60, 80, and 105 amino acid peptides were purified by HPLC and the molecular weights were verified by electrospray mass spectroscopy. Using these peptides as antigens in a solid-phase ELISA, we screened sera from 24 breast cancer patients. Using the 105 amino acid peptide we were able to detect high levels of anti-mucin antibodies in 2 out of 24 sera. Lower levels of antibodies were detected with the 80 amino acid peptide, and no antibodies were detected with the 60 amino acid peptide. Either conformational differences between the peptides or antibody avidity is responsible for the differential reactivities to the different peptides. Chimpanzee immunized with muc-1 protein also produced antibodies that were detected with 105 amino acid and not with the 60 amino acid peptide.

Interestingly, the human antibodies in "naturally" immunized patients were primarily IgM isotype. In contrast, the antibody produced by vaccinated chimpanzee was IgG isotype. The ability to change antibody isotype by vaccination may have potential therapeutic value.

NZ 507 IMMUNOTHERAPY FOR BREAST CANCER WITH SYNTHETIC PEPTIDES, Ian F C McKenzie, Vasso Apostolopoulos and Pei-Xiang Xing, The Austin Research Institute, The Austin Hospital, Heidelberg, Victoria, 3084, Australia.

Mucin1 (MUC1) is highly expressed in breast cancer (CaB), and is highly immunogenic; most murine monoclonal antibodies made to CaB react with MUC1. The cloning of the cDNA for MUC1 showed the presence of VNTRs (repeated 20 amino acid peptide domain in the extracellular portion of the molecule), and most of the antibodies which react with MUC1 protein, react with the amino acids APDTR within the repeat. Humans also react with this peptide as cytotoxic T cell lines can be developed from the lymph nodes of patients with CaB (O. Finn et al); thus, murine studies are of relevance for the design of immunization programs for the therapy of human CaB.

MUC1+ and MUC1- 3T3 mouse lines were developed (after transfection) and while the parent 3T3 lines grew progressively in BALB/c mice, MUC1+ 3T3 grew until ~14 days when they were rejected, due to the recognition of human MUC1. These mice were resistant to a subsequent challenge and are therefore appropriately immunized. Further studies demonstrated that: a) little antibody production to MUC1+ occurred; b) by contrast, significant cellular responses were engendered - shown by the presence of DTH, Tc and proliferative T cells; c) treatment with CD3, CD4 and CD8 antibodies showed that rejection was due entirely to CD3+ and CD8+ cells - CD4+ cells having little or no effect; d) mice immunosuppressed with CD3 antibody and with progressively growing tumors, were given large amounts of either murine γ1, or chimaeric γ1 anti-MUC1 antibody and additional complement - there was no effect on the tumors which appear to be resistant to antibody dependent destruction.

To examine whether synthetic or natural MUC1 could immunize against the tumor, BALB/c mice were immunised with human milk fat globules, synthetic peptides from the repeat C-p13-32 (CpAHGVTSAPDTRAPGSTAP), a fusion protein (containing 5 repeats), N and C terminal peptides of the mucin and an unrelated peptide, and challenged with MUC1+ 3T3 cells. There was an initial difference in tumor size with all the immunised mice having tumors slightly smaller than in the untreated recipients, but then all tumors were rejected at the same time. The mice receiving peptide immunisation had high levels of antibody before and after rejection, but little cellular immunity. We conclude that humoral immunity is not effective or desirable and that efforts should be towards inducing cellular immunity.

NZ 508 AN LHRH PILI BASED VACCINE ADJUVANTED WITH A NOVEL FATTY ACID ADJUVANT: A POSSIBLE ALTERNATE IMMUNOLOGICAL APPROACH FOR THE TREATMENT OF PROSTATE AND BREAST CANCERS. W. Reilly, R.G. Whittaker, K.G. Finney, and P.A. Jennings, CSIRO, Division of Biomolecular Engineering, Laboratory for Molecular Biology, PO Box 184, North Ryde, 2113

GnRH agonists, which are D-amino acid substituted analogues of the naturally occurring peptide hormone, luteinizing hormone releasing hormone (LHRH), have proven to have great efficacy in the treatment of metastatic prostatic cancer, endometriosis and are currently being trialed for certain types of breast cancer. Although widely accepted as the therapy of choice GnRH agonists do have some associated problems. These are in relation to the compliance-compatible long term depot injectable formulations, hormonal flare after administration and the cost of treatment per patient. While there has been an experimental interest in the development of an LHRH vaccine for prostate cancer, in recent years, these approaches have required chemical synthesis of LHRH and conjugation to a suitable carrier molecule to produce the immunogen.

We report here the use of a novel polymeric peptide expression system which upon vaccination with a novel synthetic adjuvant induces both physical and antibody responses in an outbred mouse strain. An LHRH related peptide was engineered into the C-terminus of the pilus subunit gene of *Dichelobacter nodosus* and expressed exogenously using the vector, pMF 2/1 in another type 4 piliated bacterium *Pseudomonas aeruginosa* K2. The vector is under the control of the lambda cI857 temperature suppressor gene with homogeneous expression of the engineered LHRH-pili occurring at 42°C. The expressed polymeric pili were isolated using magnesium chloride precipitation and can be stored at -20°C for subsequent vaccination. The isolated LHRH-pili were formulated with a novel synthetic fatty acid adjuvant (FAA) which after two vaccinations induced marked gonadal atrophy in outbred mice with corresponding high levels of LHRH antibodies observed in their sera.

This engineered pili protein in admixture with the novel FAA has a self adjuvanting property and may represent an inexpensive alternate method of immunotherapy for the treatment of prostatic and breast cancers. This immunotherapy would be compatible with current agonist therapies and reversible immunoprotection could be achieved for 6 months or longer with subsequent re-vaccination.

NZ 509 IDENTIFICATION OF MCF1233 MURINE LEUKEMIA VIRUS ENCODED T CELL EPITOPES, INVOLVED IN IMMUNOPROTECTION AGAINST MCF1233 INDUCED TUMORS. Elisabeth J.A.M. Sijts[1], Ferry A. Ossendorp[2], Erica A.M. Mengedé[1] and Cornelis J.M. Melief[1]. [1]Dept. of Immunohematology, University Hospital Leiden, Leiden, [2]Division of Immunology, The Netherlands Cancer Institute, Amsterdam, The Netherlands.

The MCF1233 Murine Leukemia Virus (MuLV) is a retrovirus, isolated from a C57BL (B10A) mouse lymphoma, that induces lymphomas upon neonatal inoculation into C57BL mice of various H-2 haplotypes. Tumor development occurs late in life and has been shown to be MHC-associated. In H-2 congenic C57BL mouse strains, expresssion of the b-allele at the H-2 class II I-A locus protects against lymphomagenesis. Tumor resistance is probably T-cell mediated. In this study we aimed to identify the CTL epitope involved in resistance against MCF1233 induced tumors in I-A^b mice. H-2^b mice were immunized with a syngeneic MCF1233 transformed B lymphoma. From spleen cell cultures a CD8 expressing T cell line was isolated, responsive against MCF1233 transformed tumor cells. On basis of class I K^b and D^b motifs we selected 8- and 9-mer MuLV encoded peptides and tested them for binding to H-2 class I. This strategy allowed rapid identification of we the T cell epitope recognized by the CTL line. This epitope is encoded by the env p15E transmembrane segment of MCF1233 MuLV and seems an immunodominant epitope. Preliminary results indicate that the isolated CTL line is effective in eradication of MCF1233 expressing tumors, transplanted into T cell deficient (nu/nu) mice. The therapeutic value of peptide vaccination for protection against tumor development is currently investigated.

NZ 510 ANTIGEN-SPECIFIC HUMORAL AND CELLULAR IMMUNE RESPONSES IN COLORECTAL CARCINOMA PATIENTS IMMUNIZED WITH ANTI-IDIOTYPIC ANTIBODY GA733. Rajasekharan Somasundaram, Andrea Benden, Jan Zaloudik, Michael Kane, Melinda Sperlagh, Ellen Hart*, Gerald Marks*, Michael Mastrangelo*, and Dorothee Herlyn. The Wistar Institute, and *Jefferson Medical College, Philadelphia, PA.

Anti-idiotypic antibodies (Ab2) that bind to the antigen (Ag) combining site of anti-tumor antibodies (Ab1) may functionally mimic the tumor associated Ag. Ab2 immunizations induced humoral, cellular and protective immunity in experimental animals. In a recent trial, 16 patients with resected colorectal carcinoma (CRC) were immunized with alum precipitated polyclonal goat Ab2 against anti-gastrointestinal carcinoma Ab1 GA733. Seven of the 16 patients produced anti-anti idiotypes (Ab3) which shared idiotopes with the Ab1 (5 patients) and specifically bound to CRC cells (7 patients) and isolated tumor Ag (4 patients). Two of the seven patients who demonstrated specific humoral responses after Ab2 therapy also developed T cells which proliferated *in vitro* after stimulation with either Ab2 or tumor Ag, but were unresponsive to stimulation with control proteins. Although it is too early to relate immune responses of the patients to clinical responses, five of the seven patients who responded to therapy with the production of anti-tumor antibodies are at present without evidence of disease. (Supported by NIH grants CA43735 and CA10815).

NZ 511 NOVEL APPROACH FOR ANALYSING THE HUMAN Vβ REPERTOIRE IN HEALTHY DONOR T CELLS AND MELANOMA TIL. J.Even[1,3], I.Puisieux[1], L.Ferradini[2], Ch. Pannetier[3], M.Cochet [3], A.Makensen[2], Th.Hercend[2] and Ph.Kourilsky[3]. [1]U152 INSERM-I.C.G.M., [2]U332 INSERM-I.G.R., [3]U277 INSERM- Institut Pasteur,75724 PARIS CEDEX15, France.

The disparate results obtained through semi-quantitative PCR analysis of the human T-cell receptor repertoire in pathological situations like cancer, rheumatoid arthritis and AIDS, prompted us to develop an alternative approach based on a method used to analyse the murine T cell receptor repertoire (Cochet *et al.* Eur.J.Immunol. 1992.*22*:2639-2647). This method uses mRNA and RT-PCR, but unlike the more classical techniques it is also quantitative. The use of fluorescent primers and appropriately labeled size markers allows the determination of the sizes and the relative amounts of the amplification products with the help of an automated sequencer and specially devised soft-ware. The approximately 70 different human Vβ, and 13 Jβ regions were analysed with 24 Vβ and 13 Jβ specific primers. In PBL from healthy donors, the average size distribution for any of the 24 V-C or the 13 V-J specific bands consisted of 7 to 8 peaks with a roughly gaussian intensity distribution and the deduced CDR3 sizes varied between 5 and 15 with major peaks at 9, 10 or 11 amino acids. This method was found to be sensitive enough to demonstrate the clonality of TIL from a spontaneously regressive melanoma as well as the Vβ repertoire differences *in situ* and after *in vitro* expansion.

NZ 512 HIGH TEMPERATURE RT-PCR AND EXON JUNCTION PRIMERS FOR CYTOKINE mRNAs. Jing-Zhou Hou, W. Frank Lawrence, Maria F.K.L. Leung and Wai-Choi Leung, Division of Molecular Pathology, Department of Pathology and Laboratory Medicine, Tulane University School of Medicine, New Orleans, LA 70112

The current protocols of RT-PCR for mRNA suffer from nonspecificity. We hereby reported two improvements which resulted in amplication of a single PCR product with high specificity to mRNA. First, we utilized the thermostable *rTth* polymerase to catalyze both RT and PCR reactions at elevated temperature to avoid folding of template RNA. Increased specificity in priming was also observed with primers bearing Tm set at high temperature. With this approach, the RT-PCR reaction was able to yield target specific products, even in conditions which promote nonspecificity, e.g. high concentrations of templates & primers, etc. Regions of template RNA which previously was unable to be transcribed by the Moloney reverse transcriptase was able to yield DNA product by RT-PCR using this protocol. We were able to detect and quantitate a number of cytokines from total cellular RNA, including TNFα, TNFβ, interleukin 6, etc.

Secondly, we developed an approach using exon junction primers to amplify only mRNA, but not the corresponding genomic DNA nor hnRNA. Human interleukin 6 was utilized as a model system since it has 5 exons and 4 introns. With the primers spanning the exon junctions, six possible combinations of primer sets were investigated. Primer sets from all 6 models were able to amplify PCR products specific for IL-6 mRNA from total cellular RNA. However, all primer sets were unable to amplify genomic DNA or hnRNA. Several sets of primers yielded higher amounts of PCR products. A differential yield was also observed at different temperature for thermocycling. The quantitative differences can be correlated with differences in predicted secondary structures on various regions of IL-6 RNA. Using a combination of these two approaches, we were able to detect the presence of cytokine mRNAs in conditions which they have been reported to be absent. This approach represents a significant improvement in RT-PCR for cytokine mRNAs and paves the way for the development of *in situ* PCR.

NZ 513 ACTIVATION AND EXPANSION OF BISPECIFIC ANTIBODY-TRIGGERED CYTOTOXIC T LYMPHOCYTES IN HOLLOW FIBER BIOREACTORS, CHJ Lamers, RJ Van De Griend, and RLH Bolhuis. Department of Immunology, Daniel den Hoed Cancer Center, Rotterdam, The Netherlands.

We compared the growthkinetics and cytolytic activities of peripheral blood lymphocytes, expanded using either ACUSYST hollow fiber bioreactors (HFBR) or culture bags. In contrast to bag cultures, IL2 and serum need only to be supplied to the small, cell containing compartment, i.e. the extracapillary space (ECS) of the HFBR. Both systems yielded a fifty fold increase in viable lymphocytes within 14-16 days of culture. The amount of cells required for a 'therapeutic' dose of $5x10^{10}$ lymphocytes can be harvested from a single HFBR. Addition of PHA or anti-CD3 MAb to the EC-space induced rapid activation and proliferation of lymphocytes. Harvesting of lymphocytes was achieved by simple flushing of the ECS compartment of the HFBR with medium. The harvested lymphocytes were predominantly of the $CD3^+8^-$ phenotype. Generally, the expanded lymphocytes display strong cytolytic activity directly after harvesting, and could readily be triggered for cytolysis by bispecific antibodies. For about 40% of the donors a 4 hr resting period in fresh medium was required to restore optimal Ab-triggered lytic activity of the lymphocytes. The properties of the lymphocytes circulating in the ECS, i.e. lymphocyte number, viability, phenotype as well as the cytolytic potential, appeared predictive for these features of the lymphocytes harvested from the HFBR. Thus, the large scale and rapid expansion of BsAb-triggered cytotoxic lymphocytes for clinical applications is efficient in HFBRs with reduced costs and under conditions that fulfill GLP requirements.

NZ 514 IDENTIFICATION OF CYTOLYTIC LYMPHOCYTE TARGET STRUCTURES BY CELLULAR ADSORPTION OF TUMOR BINDING PROTEINS, Mary Mondragon, Ballabh Das, Victor Fuentes and Allen Norin, Departments of Medicine and Anatomy & Cell Biology, SUNY Health Science Center Brooklyn, New York 11203

Cellular interactions involving engagement of specific receptors and counter receptors on opposing cells are important in inductive and effector phases of cellular immune responses. In order to identify specific interactive molecules we developed a novel approach that utilizes the direct binding properties of surface proteins on target cells. The validity of this approach was established by characterizing LFA-3 on sheep erythrocyte (sE) as a target structure for lymphocytes. Membrane proteins of sE were labelled with biotin and allowed to adsorb to freshly isolated human lymphocytes, extracted, separated by sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and detected on western blots. Additionally, lymphocyte-binding sE membrane proteins were detected on western blots by reacting biotin labelled with blotted sE (lymphocyte membrane protein) membrane proteins. Both methods revealed a number of lymphocyte binding proteins in addition to a 60KD band consistent with the known molecular weight of LFA-3 and verified with a specific monoclonal antibody. Biotinylated surface proteins of the K562 (NK-sensitive) and A549 (NK-resistant) cell lines were adsorbed to freshly isolated lymphocytes and adsorbed proteins were detected using above approach. Approximately 20 tumor binding proteins were revealed. Although most of the proteins were simialr in molecular weight, a 51.5 KD protein was present on K562 blot but not on A549 blot. In contrast, A549 blots contained 47KD, 50KD and 53KD proteins which were absent on K562 blots. A protein of 51.5 KD on the K562 cell lines was therefore considered to be an important molecule for NK cell mediated cytolytic process. This protein was purified to apparent homogeneity by preparative PAGE. The purified protein bound to the lymphocyte surface and exhibited dose dependent competitive inhibition of NK cell mediated cytotoxicity. These studies demonstrate that the cellular adsorption technique could be a valuable tool in identifying molecules involved in cell to cell interactions and that a 51.5 KD K562 protein is an important NK cell recognition structure. (Supported by USPHS grant CA47548).

NZ 515 MAKING SENSE OF THE COMBINED EFFECT OF IL-4 AND IL-2 - A MATHEMATICAL MODEL. Benoît Morel, Meghan Burke, Jayant Kalagnanam and Penelope A Morel. Dept. of Engineering and Public Policy, Carnegie Mellon University, Dept. of Medicine, University of Pittsburgh, Pittsburgh Cancer Institute, Pittsburgh, PA 15213

The development of an effector T helper response involves two important lymphokines, IL-2 and IL-4. IL-2 and IL-4 are pleiotropic and many cells in the immunological system simultaneously express high affinity receptors for IL-2 and IL-4.

Most of the evidence suggests that IL-4-binding down-regulates expression of IL-2 high affinity receptors in both B and T cells. It is assumed that the interaction between the receptors at the surface plays an important role in this as the down regulation occurs at 4°C as well as at 37°C. The exact nature of this interaction is still very mysterious. Furthermore, it is possible that IL-4 binding affects the dynamics of IL-2 binding and IL-2 receptor expression inside the cell too.

It has also been shown that, in some circumstances, IL-2 and IL-4 binding to the same cell have a synergistic effect, as in T cell proliferation. In view of the previous evidence this seems confusing. It is possible, however, to make sense of this apparent contradiction and reconcile all the experimental evidences existing today, if one takes a "dynamical" perspective. More precisely, it is possible to show (using a simple dynamical model) that IL-2/IL-4 binding and their interactions could result in what can look like either synergistic or antagonistic effects, while always down regulating the number of IL-2 receptors. This depends on the properties of the individual cells involved (such as the densities of IL-2 and IL-4 receptors).

A mathematical model will be presented that addresses all of these questions. This model has been rigorously tested against experimental data and can reproduce all of the published data as well as experiments specifically designed to test the model. Dynamical effects like the one suggested in this system probably play a fundamental role in the regulation of the immunological response.

NZ 516 CONSTRUCTION OF A BREAST CANCER CELL SPECIFIC CHIMERIC T CELL RECEPTOR TO ENHANCE THE TUMOR SPECIFICITY OF TUMOR INFILTRATING LYMPHOCYTES, K. Motmans[1,2], C. Vandevyver[1] and J. Raus[1,2], [1]Department of Biotechnology/Immunology, Dr. L. Willems-Instituut, B-3590 Diepenbeek, Belgium, [2]Limburgs Universitair Centrum, B-3590 Diepenbeek, Belgium

Adoptive breast cancer immunotherapy by the use of tumor infiltrating lymphocytes (TIL) is often limited by the lack of specificity of these TIL. Moreover breast TIL are predominantly of the helper type.

Recently it became possible to design and direct at will the specificity of T cells in a non major histocompatibility complex (MHC) restricted manner by generating a chimeric T cell receptor (TCR) in which the antigen binding domain is replaced by an analogous domain of a monoclonal antibody (MoAB). In this approach the availability of anti tumor MoABs and the cytotoxic capabilities of T cells are combined in a potential tumor adoptive immunotherapy (Gross *et al.* 1989, PNAS **86**, 10024). For the construction of the chimeric TCR genes, cDNA encoding the variable antigen binding domains of breast cancer cell specific MoABS (Plessers *et al.* 1986, Anticancer Res. **6**, 885) is combined with the cDNA encoding the constant regions of TCR α- and β-chains by a single tube polymerase chain reaction (PCR). The chimeric genes are cloned in mammalian Epstein Barr virus (EBV)-based episomal expression vectors (INVITROGEN).

For the enhancement of cytoxicity of breast cancer TIL, the tumor necrosis factor (TNF)- and interferon γ (IFNγ)-gene are cloned in these expression vectors. By this we hope to circumvent the fact that most TIL are CD4+.

This work is supported by a fellowship of the "Nationaal Fonds voor Wetenschappelijk onderzoek", the "Vereniging voor Kankerbestrijding", the "Limburgs Universitair Centrum" and the "Sociale Investeringsmaatschappij Limburg".

NZ 517 GENERATION OF MICE DEFECTIVE FOR TUMOR NECROSIS FACTOR RECEPTOR p55,

Klaus Pfeffer, Toshifumi Matsuyama, Andrew Wakeham, Kenji Kishihara, Julia Potter, Arda Shahinian, Katja Wiegmann[&], Martin Krönke[&], and Tak W. Mak, Ontario Cancer Institute, Departments of Medical Biophysics and Immunology, University of Toronto, Princess Margaret Hospital, 500 Sherbourne Street, Toronto, Canada M4X 1K9, [&] and Institut für Medizinische Mikrobiologie und Hygiene, Technische Universität München, Trogerstr. 4a, 8000 München 80, Federal Republic of Germany.

TNFα and TNFβ are polypeptides with multifunctional properties, including hemorrhagic necrosis of transplanted tumors and cytotoxicity. TNFα and TNFβ play a crucial role in endotoxin and superantigen shock syndromes, as well as in inflammatory, immunoregulatory and antiviral responses. We now begin to understand that the pleiotropism of TNF actions results from the complexity of the intracellular signal transduction pathways triggered by the binding of TNFα and TNFβ to their common cell surface receptor. The receptor consists of two chains with molecular weights of 55 and 75 kDa (TNFRp55 and TNFRp75). To explore the various physiological and pathological functions of TNF, a mouse strain lacking a functional TNFRp55 has been generated by gene targeting in ES cells. Spleen cells of homozygous TNFRp55$^{-/-}$ mice are not able to bind human TNF and NF-$_\kappa$B induction is absent. These mice are resistant against challenge with LPS even at 1000-fold the dosage which is lethal for TNFRp55$^{+/+}$ littermates. This mouse strain hopefully will prove to be useful to investigate the biology of TNF actions *in vivo*.

NZ 518 Retroviral vectors for secretable TNF and hypersecretable TNF: Construction, characterization of expression in TIL and tumor cells, and large scale GMP production of clinical lots. R. Ralston, M. Kriegler[1], C. Perez[1], K. DeFay, D. Lowe and B. Maiorella. Chiron Corporation, Emeryville, CA 94608.

Intralesional administration of tumor necrosis factor (TNF) has been shown to promote tumor regression in human subjects (Bartsch et al., 1988; Kahn, et al., 1989). Expression of TNF by transduced tumor infiltrating lymphocytes (TIL) could mediate or evoke a significant anti-tumor response. Retroviral vectors for expression of secretable TNF were constructed in the LXSN backbone (Miller & Rosman, 1989), using cDNA clones for either full length TNF or a hypersecretable 17 kD form containing the γIFN signal sequence (Perez *et al.*, 1990). (Master cell banks were prepared from single cell clones of PA317 cells (Miller & Buttimore, 1986) containing a single provirus (LTNFSN or LTγsigSN). Production cells consistently generated titers of $>10^6$ cfu/ml. Clinical lots of 9 L each were prepared under GMP, including release testing for absence of adventitious viruses (assays for MLV, BVDV, PPV, LCMV, HIV-1, HTLV-1, MAP and CPE), adherence to post-thaw specifications for recombinant virus titer ($>10^6$), and for TNF expression on NIH 3T3 cells. Clinical lots of TNF retrovirus were provided to NCI for transduction of human TIL and tumor cells from study subjects (S.A. Rosenberg, M.D., principal investigator). Transduction efficiency in TIL ranged from 4-16% without selection and up to 86% after 5 days of selection with G418 ([2]P. Hwu, M.D., personal communication). Expression of TNF by LTγsigSN-transduced TILs was approximately 5-fold higher than for LTNFSN-transduced TILs. Establishment of transduced carcinoma cells required extended culturing (>60 d) but the resulting lines expressed TNF very efficiently. Clinical evaluation of the transduced TIL and tumor cells is in progress.

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NZ 519 PHOTOAFFINITY LABELING OF THE T CELL RECEPTOR ON LIVING CYTOLYTIC T LYMPHOCYTES. A NOVEL APPROACH TO STUDY TCR-LIGAND INTERACTIONS, Pedro Romero, Jean-Laurent Casanova, Janet L. Maryanski and Immanuel F. Luescher, Ludwig Institute for Cancer Research, Lausanne Branch, 1066 Epalinges, Switzerland

The antigenic octapeptide PbCS 253-260, recognized by cytolytic T lymphocytes (CTL) in the context of the class I MHC molecule H-2K^d (K^d), was modified by conjugation of Lys 259, a functionally defined TCR contact residue, with photoreactive iodo, 4-azidosalicylic acid (IASA). The resulting peptide derivative binding to K^d as efficiently as the parental peptide was used to immunize mice. Eight independent CTL clones were analyzed for fine specificity of recognition, TCR sequence and photoaffinity labeling of TCR. The IASA group formed an essential part of the epitope for all the clones. As predicted, recognition of the peptide derivative by CTL was K^d restricted. However, each clone revealed a distinct pattern of recognition of peptide variants lacking substituents from the IASA group. Incubation of living cloned CTL cells with the radiolabeled photoreactive peptide derivative followed by UV irradiation resulted in photoaffinity labeling of the TCR in 4 out of 8 clones. TCR labeling was dependent on the peptide derivative binding to the K^d molecule and on intercellular interaction. Two CTL clones were labeled selectively at the TCRα-chain, one at the β-chain, while one was labeled at both α- and β-chains. Four of eight TCRs analyzed were composed of α-chains incorporating the JαTA28 segment that were associated with Vβ1-expressing β-chains. Interestingly, 3 out of 4 clones tested expressing these two dominant TCR gene elements were labeled at their TCR α-chains. These results indicate that a residue at a defined position near the COOH-terminus of the antigenic peptide may interact with either TCR α- or β-chain, or both chains, in a clone-specific manner. This may be the first direct biochemical demonstration of the interaction between an antigenic peptide-MHC complex and specific TCR. This approach opens new ways to the molecular analysis of antigen recognition by specific T cells.

NZ 520 CYTOKINE GENE TRANSCRIPTION IN RENAL CELL CARCINOMAS : A POLYMERASE CHAIN REACTION STUDY IN TUMOUR BIOPSIES.

G.C. Spagnoli, M. Zuber, A. Merlo, V. Caetano, E. Schultz, F. Harder, L. Filgueira, M.Heberer. Departments of Surgery and Research, University of Basel, Switzerland.

Tumour infiltrating lymphocytes (TIL) are mainly T-cells, expressing surface activation markers such as HLA-DR, CD69 and CD45RO, but the functional nature of the interaction between TIL and neoplastic cells remains unclear to date.

Activation of T-lymphocytes following antigen recognition, "in vitro" and "in vivo", is accompanied by transcription of cytokine genes. Furthermore, production of cytokines by neoplastic cells in clinical oncology and in experimental tumours has also been reported. Since cytokines are powerful modulators of immune responses, local production, irrespective of the cell type(s) responsible for their secretion, could be relevant in the interaction between tumour and immune system.

In this work we investigated the transcription of genes encoding for interleukin-2 (IL-2), interleukin-4 (IL-4), interleukin-10 (IL-10) and interferon-gamma (IFN-gamma) in lymphocyte infiltrated renal cell carcinoma (RCC) biopsies from ten patients using the reverse polymerase chain reaction (PCR) technique. Autologous peripheral blood mononuclear cells (PBMC) and healthy renal parenchyma were tested in parallel. The beta-actin gene, used as positive control, was found to be transcribed in all samples. In contrast, transcription of cytokine genes was confined to tumour biopsies : IL-2 gene transcripts were detectable in five biopsies, and IL-10 transcripts in seven. The IL-4 and IFN-gamma genes were found to be transcribed in one biopsy each.

These data underline that, despite histological similarities, heterogeneous patterns of cytokine gene transcription can be observed in different RCC biopsies. In two cases, in spite of the use of a highly sensitive technique, no cytokine gene transcripts could be identified. Furthermore, transcription of IL-4 and IFN-gamma, usually observed upon antigen receptor triggering of T-lymphocytes, was only rarely detectable in lymphocyte infiltrated renal cell carcinoma biopsies. Although transcription of an immunostimulatory lymphokine, such as IL-2, could be observed in 50% of biopsies, the most frequently transcribed cytokine gene encoded for an inhibitory factor, IL-10.

Local production of these factors might have a relevant impact on the generation of anti-tumor immune response.

NZ 521 SUPPRESSION OF RETROVIRUS-INDUCED AUTOIMMUNITY AND LEUKEMOGENESIS BY VACCINATION WITH FIXED MITOGEN-ACTIVATED LYMPHOCYTES.

Victor Ter-Grigorov, Eugenia Liubashevsky, Hanna Ungar-Waron, Ze'ev Trainin. Department of Immunology, Kimron Veterinary Institute, Bet-Dagan, 50250, Israel.

Hybrid (BALB/cxC57BL/6)F_1 mice were immunized with glutaraldehyde fixed Con-A activated spleen cells and thymocytes of non-infected syngeneic or semi-allogenic (parental) animals prior to Rauscher leukemia retrovirus (RV) injection. Part of the mice were immunized subsequently with fixed Rauscher leukemic cells of BALB/c origin. All control mice, whether non-immunized at all or immunized with leukemic cells only, developed leukemia 2-3 weeks after RV injection. Leukemogenesis was prevented in 90% of animals immunized with syngeneic cells and in 60-70% of mice immunized with parental cells. Complete protection of all the mice against RV leukemia was achieved when immunization with lymphocytes, whether syngeneic or parental, was followed by immunization with leukemic cells. When administered 24h after RV injection, immunization with parental lymphocytes was also effective (suppressing leukemia development in 50% of mice) whereas immunization with syngeneic cells was completely ineffective. The protective effect of vaccination was found to be based upon: i) Suppression of autoimmune reactions which RV induces in non-vaccinated mice (anti-self MHC class II antigens, CD4 etc.); ii) Production of appropriate anti-idiotypic antibodies (anti-anti-self MHC class II, CD4, etc.); iii) Production of antibodies to MHC class I antigens. Additional immunization with leukemic cells enhances the latter type of reaction as well as antiviral responses.

NZ 522 RECEPTOR-MEDIATED CYTOKINE GENE DELIVERY TO TUMOR CELLS FOR GENERATION OF CANCER VACCINES.

Kurt Zatloukal, Ernst Wagner, Matthew Cotten, Walter Schmidt, Christian Plank, Manfred Berger, Mediyha Saltik, Georg Stingl*, Max L. Birnstiel, Research Institute of Molecular Pathology, Vienna, A-1030, Austria,* 2nd Clinic of Dermatology, University of Vienna School of Medicine, Vienna, A-1090, Austria

We have developed a new gene transfer system which uses the receptor-mediated endocytosis pathway and the endosome-disruption activity of adenovirus to introduce gene constructs into target cells. The combination of these two mechanisms is achieved by attaching the DNA binding moiety polylysine to the exterior of adenovirus particles. The gene to be delivered is complexed to the modified virus and further addition of polylysine-transferrin or other polylysine-ligands (LDL, EGF) condenses the DNA. This generates a ligand-polylysine/DNA donut linked to the exterior of a defective adenovirus particle. The ligands (and the adenovirus) mediate the binding of the complex to the tumour cell. Upon endocytosis, the adenovirus triggers an endosome disruption which allows cytoplasmic entry of the DNA.

These gene-transfer complexes allow highly efficient gene transfer to primary human melanoma cells (up to 50% transfected cells without selection) and to M-3 mouse melanoma cells with almost 100% transfection efficiency. We have tested the gene delivery activity of various ligands in different primary human melanoma cell. In general, best delivery occurs via the transferrin and adenovirus-receptors, however, in some tumor isolates, LDL-receptor ligands and EGF-receptor ligands are more efficient. In primary human melanoma cells, the E1a-defect of the adenovirus is not sufficient to prevent virus-related cytotoxicity. To achieve long-term gene expression in these cells it is, therefore, essential to further inactivate the virus by psoralen/UVA-treatment or to use other replication-defective adenovirus strains.

Murine M-3 melanoma cells transfected with IFN-γ or IL-2 gene-constructs produce high amounts of cytokines (24000 Units IL-2/ $1x10^6$ cells/24hrs; 1200 ng IFN-γ/ $1x10^6$ cells/24hrs). Transplantation of $1x10^5$ IL-2 transfected M-3 cells into syngeneic DBA/2 mice does not lead to tumor formation in 6/6 animals, whereas same amount of non transfected M-3 cells developed tumors in all animals.

Immunotherapy

NZ 600 MEASUREMENT OF THE POTENCY OF EX VIVO ACTIVATED MEMORY T-CELLS USED IN AUTO-LYMPHOCYTE THERAPY FOR THE TREATMENT OF METASTATIC RENAL CELL CARCINOMA. Bruce Babbitt, Joe Goodwin, Barry Caplan, and Michael Osband, Boston, MA.

Autolymphocyte therapy (ALT) is outpatient adoptive immunotherapy based on the infusion of memory T-cells (ALT cells) that have been activated ex vivo by a combination of OKT3, a mitogenic monoclonal antibody directed against the CD3 portion of the T-cell receptor, together with a previously-prepared mixture of autologous cytokines. In order to determine the potency of ALT cells prepared from patients, we stimulated ALT cells, as well as the peripheral blood mononuclear cells from which they were derived, with phorbol myristate acetate (PMA), which has been shown to be a second signal for the full activation of primed T cells. The PMA induced proliferation and cytokine secretion is as follows:

	Proliferation (CPM)	IFNg (pg/ml)	GMCSF (pg/ml)
PBM (N=11)	320	8	26
ALT (N=20)	43457	464	718

The proliferation and cytokine production were highly correlated for the ALT cells from each patient. In addition, in some patients, ALT cell subpopulations were stimulated to proliferate by PMA after depletion of either CD4(+), CD8(+), naive (2H4(+)), or memory (UCHL1(+)) T cells. Results indicate that each of these cell types was primed by the autokines + OKT3. Removal of memory T cells from ALT cells resulted in a large decrease in PMA-induced IFNg production. These data indicate that the ex vivo process used to prepare ALT cells results in the priming of patient PBM, especially memory T-cells. These primed T-cells should be able to respond better in vivo to a variety of biologically relevant second signals, from either immune cells, or tumor cells, and become fully activated as functional helper/effector cells. We intend to utilize this assay to determine if PMA-stimulated proliferation and/or cytokine production correlates with clinical outcome following ALT treatment.

NZ 601 ADOPTIVE IMMUNOTHERAPY. R.L.H. Bolhuis[1], G. Stoter[2], S.H. Goey[2], A.M.M. Eggermont[3] 1) Dept. Immunol., 2) Dept. Med. Oncol., 3) Dept. Oncol. Surg., Daniel den Hoed Cancer Center, Rotterdam, The Netherlands. We have performed a single institution phase II study of the combination of IL-2 and α-interferon (αIFN) and lymphokine activated lymphocytes (LAK). Treatment schedule: IL-2 18 x 10^6 IU/m^2/day, CIV, days 1-5, together with αIFN, 5 x 10^6 U/m^2 i.m. Leukapheresis at days 7-9. LAK was reinfused on days 12-14, with IL-2 on day 12-16. αIFN was given i.m. on days 12-15, 5 x 10^6 U/m^2. The cycle was repeated on day 36. Patients with stable disease or response went on to maintenance treatment with 4 monthly cycles on IL-2 18 x 10^6 IU/m^2/day on days 1-4 and αIFN 5 x 10^6 U/m^2/day, days 1-4. At this point 30 patients are evaluable: 3 have reached a CR (10%), 10 a PR (33%), the median duration of response: 11 months; median time to progression: 6 months; median survival: 28 months. In 75% of the patients dose reductions and or delays in the administration of the IL-2 and αIFN are warranted as a results of hypotension, anuria and severe intractable diarrhea accompanied with metabolic acidosis. The treatment schedule used represents therapy at the maximum tolerated level. The study is ongoing.

We also focussed on a new strategy to circumvent the difficulties in generating T cell specific antitumor responses. We combine a) the tumor and CTL selectivity of bs-mAb, b) the cytolytic capacity of in vitro-activated and expanded T cells and c) the proliferation and T cell activity enhancing capacity of IL-2 for the purpose of immunotherapy. As a result the CTL are bridged to the tumor target cell. We started a phase II clinical trial for the i.p. treatment of ovarian cancer patients (FIGO stage III) where tumor dissemination is restricted to the abdomen. Lymphocytes of patients are expanded at least 50-fold, in vitro. A treatment cycle consists of an escalating dose of bs-mAb labelled T lymphocytes (10^6 - 10^9) followed by 5 successive days of administration of 10^9 bs-mAb labelled T lymphocytes. Clinical results: all patients developed anti-mouse Ig antibodies (HAMA). Nine patients are evaluable for toxicity and response. Toxicity is minor, 3 patients showed extracavitary progressive disease and did not undergo exploratory laparotomy. Three patients who had tumor-positive ascites became negative, 6 patients were subjected to exploratory laparotomy. Two received a complete intracavitary response, but in 1 retroperitoneal lymphnode metastasis had progressed. Two patients showed partial regression of intracavitary tumor, but tumor lesions remained at inaccessible adherent peritoneal sites in 1, and new submucosal intestinal lesions had developed in the other. One patient had stable disease (until 5 months post-surgical debulking and immunotherapy), one had i.p. progressive disease.

NZ 602 DIFFERENTIAL IMMUNE RESPONSES IN TUMOUR DRAINING LYMPH NODES CORRELATING WITH ONCOGENE EXPRESSION IN THE PRIMARY TUMOUR OF BREAST CANCER PATIENTS.

James S. Clark, W.D. George and A.M. Campbell

Dept of Biochemistry and Surgery, Glasgow University, Glasgow, Scotland G12 8QQ.

The axillary tumour draining lymph nodes in breast cancer patients are usually the first major component of the immune system to come in contact with either metastatic tumour cells or tumour breakdown products. The oncoproteins c-erbB-2 and mutant p53 which could be possible immune stimulating factors were studied simultaneously with the immune response in the proximal and distal tumour draining axillary lymph nodes from 18 breast cancer patients.

The immune response in the axilla shows relevant differential changes when comparing proximal and distal nodes in relation to the tumour site. Proximal nodes contain on average more T cells expressing the activation markers HLA DR ($p=0.03$) and Tac (Interleukin 2 receptor) ($p<0.001$).

p53 and c-erbB-2 oncoproteins were quantified by flow cytometry (1) and compared with the nodal immune response.

There was no trend between c-erbB-2 expression and T and B cell activation. A trend was noted for mutant p53 expression and CD8 positive cytotoxic T cells expressing the activation marker HLA DR ($p=0.04$). This suggests that mutant p53 expression could be a possible stimulatory factor of the immune system in the axillary tumour draining lymph nodes of breast cancer patients.

The data indicates that immunotherapeutic regimes may be improved by the selection of defined immune tissues from patients with specific tumour phenotypes.

(1) Clark J.S., George W.D., Campbell A.M. 1992. Dual colour flow cytometry of p53 and c-erbB-2 expression related to DNA aneuploidy in primary and metastatic breast cancer. Cancer Letters (in press)

NZ 603 NK CELL ACTIVATION BY RETINOIDS IN BREAST CANCER CHEMOPREVENTION, Enrico Clerici, Giuseppe De Palo, Franca Formelli, Enrica Ferrario, Daria Trabattoni and Maria Luisa Villa, Istituto Nazionale Tumori and Cattedra di Immunologia of the University, Milan, Italy.

Because of its long retention in the mammary gland and low toxicity, N-(4-hydroxyphenyl) retinamide (4-HPR) is being at present used in a large scale randomized phase III trial which was started in march 1987 at the Istitute Nazionale Tumori of Milan, for the prevention of controlateral disease in breast cancer patients with no axillary lymphnode metastases who previously underwent radical surgery. Although the precise mechanism of the anticarcinogenic effect of 4-HPR is not yet understood, the present report, which deals with its influence on cellular immunity, shows that: 1) the NK activity of mastectomized women treated for six months with 4-HPR 200 mg p.o. dayly was significantly higher ($p<0.001$) than that of controls, i.e., mastectomized women receiving a placebo. 2) such activity could not be augmented by incubation with either rIL-2 or alfa-rIFN in vitro, thus suggesting that the NK activity of PBMC from 4-HPR treated women is maximized, 3) IL-2 production by PBMC cultures from patients given 4-HPR or placebo was unvaried prior and after 180 days of treatment, 4) immunophenotyping with anti Leu 11 MoAb indicates that the functional activity, but not the number, of NK cells is increased in 4-HPR treated women, 5) the NK activity of PBMC from blood donors incubated with different amounts of 4-HPR, and its major metabolite 4-MPR is never statistically different from that of controls except for 10^{-5}M 4-HPR final concentration. These findings suggest that the enhancement of NK activity may be a component of the antineoplastic activity of 4-HPR.

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NZ 604 EFFECT OF UV-B RADIATION ON THE PRESENCE OF TUMOR INFILTRATING LYMPHOID CELLS IN TRANSPLANTED MURINE MELANOMAS. Cherrie K. Donawho, Margaret L. Kripke, Corazon Bucana, Pat Cox, and H. Konrad Muller*. Department of Immunology, University of Texas M.D. Anderson Cancer Center, Houston, TX. and *Department of Pathology, Royal Hobart Hospital, Hobart, Tasmania.

We are using a syngeneic murine model to investigate the possible contributions of UV radiation to the pathogenesis of melanoma. We demonstrated that the incidence of murine melanomas was significantly increased when melanoma cells were injected into the skin of mice exposed locally to UV-B (280-320 nm) radiation. This effect of UV-B on tumor development is immunologically mediated, and tumor immunity is impaired within the UV-irradiated site. To test the hypothesis that UV inhibits the infiltration of immunological effector cells into UV-irradiated skin, we examined histologically the mononuclear lymphoid cells present within these tumors. C3H mice were exposed to 4.8 kJ/m^2 UV-B from FS40 sunlamps twice a week for 3 weeks before injection with 2 X 10^5 K1735 melanoma cells into the exposed pinna. Tumors were removed for histological examination at weekly intervals. The tumors were frozen in liquid N_2, sectioned, and incubated with anti-Lyt2, -L3T4, and -CD3 to detect T cell subsets and anti-F480 and -MOMA to detect macrophages. The positive cells were visualized with a second gold-labeled antibody. During the first 3 weeks of tumor development there was a marked increase in the number of $Ly2^+$, $L3T4^+$, and $CD3^+$ cells in the tumors from the unirradiated-control group; however, these increases were considerably less or not observed in the tumors from the UV-irradiated group. In contrast, there was a gradual increase in the number of $F480^+$ macrophages and activated ($MOMA^+$) macrophages during the 5 weeks of tumor growth in both groups. These findings demonstrate that fewer T cells infiltrate tumors growing in the UV-irradiated site, and thus supports the hypothesis that UV irradiation may increase the incidence of melanomas by inhibiting the influx of anti-tumor T effector cells.

NZ 605 GENERATION OF LEUKEMIA-REACTIVE CYTOTOXIC T LYMPHOCYTE (CTL) LINES FOR TREATMENT OF RELAPSE LEUKEMIA AFTER HLA-IDENTICAL BONE MARROW TRANSPLANTATION, J.H.Frederik Falkenburg, Laura M. Faber, Paul J. Voogt, Simone A.P. Van Luxemburg-Heijs, Annelies L. Hooftman-Den Otter, Marion M. van de Elshout and Roel Willemze, Laboratory of Experimental Hematology, Department of Hematology, University Medical Center Leiden, the Netherlands.

Previously, we have demonstrated that leukemia-reactive CTL lines and clones could be generated from HLA-identical siblings of patients with acute myeloid leukemia (AML) or chronic myeloid leukemia (CML). Both HLA-class I and II restricted CTL clones could be isolated that showed specific reactivity with the leukemic cells. Recently, it has been shown that administration of donor peripheral blood leukocytes (Pbl) to patients with a relapse CML after BMT may result in a complete remission, although a high incidence of graft versus host disease (GVHD) has been observed. We investigated whether donor-derived CTL-lines recognizing the leukemic cells from patients who relapsed following BMT could be generated in-vitro from Pbl from their HLA-identical sibling donors. Eight HLA-identical sibling combinations were tested, 5 patients with AML, 2 patients with CML chronic phase, and 1 patient with Ph' chromosome positive ALL. Donor Pbl were stimulated with irradiated leukemic cells from the patients. After 6 days, T-cell growth medium containing IL-2 was added, and every week the responder cells were restimulated with irradiated leukemic cells from the patient. In 7 out of 8 combinations, antileukemic CTL-lines could be established. In 5 combinations, the CTL-lines reacted with the leukemic cells, but not with the PHA-stimulated T lymphocytes from the same patient or from the donor in a ^{51}Cr release assay. In 2 combinations, the CTL recognized both leukemic cells and PHA blasts from the patient, but not from the donor. α-Interferon appeared to facilitate the generation of CTL responses against leukemic cells from the patient with CML, although proliferation of the CTL lines clearly decreased in the presence of α-Interferon. These results illustrate that leukemia-reactive CTL lines can be generated in-vitro from HLA-identical donors of patients with leukemia, and therefore may be used as immunotherapeutic agents in the treatment of leukemic relapses following allogeneic BMT.

NZ 606 MONOCLONAL ANTIBODIES AGAINST REGULATORY T CELL FACTORS ALTER THE INDUCTION AND PROGRESSIVE GROWTH OF MALIGNANT CELLS. Patrick M. Flood and Branka Horvat. Departments of Microbiology and Immunology and Periodontics, University of North Carolina, Chapel Hill, NC 27514, and the Howard Hughes Medical Institute, Department of Pathology, Yale University, New Haven, CT, 06510.

Immunotherapeutic treatments of animals with monoclonal antibodies to regulatory T cell factors leads to profound resistance to tumor induction in animals chronically exposed to Ultraviolet (UV) light, as well as to the outgrowth of transplanted UV- or chemically-induced tumors. The efficacy of the treatment was found to be dependent on both the route of injection of the monoclonal antibody, as well as the doses of antibody injected. Therapeutic treatment of animals with monoclonal antibodies to regulatory T cell factors throughout the 40 week period of induction of UV tumors by chronic exposure to ultraviolet light leads to the total elimination of tumorogenesis in mice. In addition, initiation of therapeutic treatments of animals concurrent with transplantation of lethal doses of UV-or chemically induced tumors prevented the outgrowth of these tumors. The therapeutic uses of these antibodies were even more effective when used in synergy with rIL-2. Simultaneous injections of monoclonal antibodies and 10,000 units of rIL-2 initiated 2 weeks after the transplantation of UV- or chemically-induced tumors leads to total regression of the progressively growing tumor cells. Animals "cured" of their progressively growing malignancy exhibit antigen-specific CTL activity against the transplanted tumor, as well as transplantation resistance to the injection of homologous tumor cells. Characterization of the factor recognized by the monoclonal antibodies shows that this factor appears to be a secretory form of the T cell antigen receptor α chain, and indeed therapeutic intervention using a monoclonal antibody against the TcR α chain shows similar results. This work is supported by grant DE-09426 from the National Institutes of Health.

NZ 607 TREATMENT OF ESTABLISHED MURINE RENAL CELL CARCINOMA WITH FLAVONE-8-ACETIC ACID (FAA) AND rhIL-2 CORRELATES WITH REVERSAL OF TUMOR-INDUCED T-CELL SIGNAL TRANSDUCTION DEFECTS, Jose L. Franco[1], Robert H. Wiltrout[1], Hiromoto Mizoguchi[3], Augusto C. Ochoa[2], John J. O'Shea[1], Dan L. Longo[3] and Kristin L. Komschlies[4]. [1]Laboratory of Experimental Immunology, [3]Biological Response Modifiers Program; [2]Clinical Services Program, [4]Biological Carcinogenesis and Development Program, PRI/DynCorp; NCI-Frederick Cancer Research and Development Center, Frederick, MD 21702-1201.

Conventional chemotherapy for renal cell carcinoma is relatively ineffective, but some anti-tumor effects have been observed with biological response modifiers (BRM). Previous studies from our laboratory have demonstrated that FAA + rhIL-2 successfully treats about 80% of BALB/c mice bearing established murine renal cancer (Renca) with subsequent resistance to rechallenge. Using mice depleted of T-cell subsets, we have demonstrated that $CD8^+$ T cells are critical for the anti-tumor response generated with FAA and rhIL-2. Recently, Mizoguchi et al. (Science, in press) have demonstrated that T cells from mice bearing the experimental murine colon tumor MCA-38 have profound alterations in their signal transduction processes. Based on these observations, we have begun to examine how the immune system is circumvented during tumor progression and how FAA and rhIL-2 act as an effective anti-tumor therapy by determining if this combination is able to reverse these T-cell defects. Splenic T cells from Renca-bearing mice also show profound alterations in the tyrosine phosphorylation pattern, associated with reduced levels of $p56^{lck}$, $p59^{fyn}$, PLCγ1 and zeta (ζ) chain of the CD3-complex. The treatment with FAA and rhIL-2 reverses these T-cell changes in those mice that respond to therapy. This finding suggests that the FAA and rhIL-2 therapy may act by reversing negative effects that Renca may have on T cells, thus restoring the signal transduction pathways and allowing the T cells to mount an effective anti-tumor response.

NZ 608 HUMAN ANTI-MOUSE ANTIBODIES (HAMA) GENERATED BY INFUSIONS WITH OC/TR-TARGETED LYMPHOCYTES DO NOT INHIBIT OC/TR-MEDIATED CYTOLYSIS DURING THE TREATMENT PERIOD, JW Gratama, CHJ Lamers, H Van Haarlem*, SO Warnaar*, BA Luider, and RLH Bolhuis. Department of Immunology, Daniel den Hoed Cancer Center, Rotterdam, and Centocor Leiden*, The Netherlands.

The bispecific antibody (BsAb) OC/TR, specific for lymphocyte CD3 and for the ovarian carcinoma-associated antigen MOv18, is used for treatment of ovarian cancer in a Phase I/II study. Eight patients received intraperitoneal infusions with activated T lymphocytes targeted with F(ab')2 fragments of OC/TR. The Phase I (days 1-4) constited of increasing doses (10^6-10^9) of OC/TR-targeted lymphocytes only, whereas in the Phase II (days 7-14, and days 28-34) patients received daily infusions of 10^9 lymphocytes with 2mg OC/TR. We studied the human anti-mouse antibody (HAMA) responses in these patients. All patients developed HAMA (IgG) in serum and peritoneal fluid, respectively from 2 and 3 weeks of treatment onwards, with maximum titers reached 2-3 weeks after the second therapeutic cycle. The reactivity of HAMA with OC/TR was much stronger than with an irrelevant BsAb (C95), suggesting an anti-idiotypic response. This supposition is confirmed functionally in a cytoxicity assay. Following therapy, serum and peritoneal fluid of patients aquired the ability to inhibit the OC/TR-triggered cytolysis of the ovarian carcinoma cell line Igrov-1 by blocking both the MOv18 and CD3 idiotype of the OC/TR. This inhibition was detected in peritoneal fluid of only 1 of 8 patients during treatment period (up to day 35). In another patient peritoneal fluid, obtained after a third therapeutic cycle (days 48-52), completely inhibited the OC/TR-mediated cytolysis, even when free OC/TR (1000 ng/ml) was added in the assay. These results are in agreement with our published data that only relative large amounts of anti-CD3 or anti-MOv18 MAb, in relation to the amount of BsAb, inhibit BsAb-triggered CTX. Furthermore, we have detected a functional affinity maturation of HAMA in the patient receiving three therapeutic cycles. We conclude that the BsAb adoptive immunotherapy at mg levels of BsAb per day should not be given beyond 35 days of treatment. These results have important implications for the design of future studies using bsAb-mediated adoptive immunotherapy.

NZ 609 OPTIMIZATION OF *IN VIVO* INDUCTION OF IMMUNOLOGIC CONDITIONS ABLE TO MEDIATE ANTIBODY DEPENDENT CYTOTOXICITY VIA MULTIPLE EFFECTOR CELLS, Jacquelyn A. Hank, Dan Burns, Jacek Gan, Jean Surfus, Kristin Tans, Joan Schiller, Paul M. Sondel, Department of Human Oncology, University of Wisconsin, Madison, WI 53792

In Vivo IL-2 dramatically augments the ability of peripheral blood mononuclear cells (PBMC) to mediate antibody dependent cell mediated cytotoxicity (ADCC) *in vitro*. We have shown that combination of murine anti-GD-2 monoclonal antibody 14.G2a with IL-2 in neuroblastoma patients induces *in vivo* conditions simulating *in vitro* ADCC. One hour following an infusion of 14.G2a, functional antibody could be detected and quantitated by a cell binding flow cytometric method. This post infusion serum, containing functional antibody, was able to serve as an effective source of antibody for ADCC mediated by PBMC obtained from the patient following therapy. Thus, this combined regimen generates conditions within the peripheral blood of neuroblastoma patients that enable their own Fc receptor bearing lymphocytes to recognize and destroy tumor with the anti-tumor antibody found in the patient's serum. While IL-2 activates CD 16+ Fc receptor bearing lymphocytes to mediate ADCC, other effectors are also capable of mediating anti-tumor ADCC. These include the monocyte/macrophage and polymorphonuclear neutrophil (PMN). We are currently combining IL-2 with GM-CSF *in vivo* in a phase 1B study in cancer patients and examining ADCC mediated by PMNs and activation of monocytes as detected by increased expression of HLA Class II and Fc receptors, and increased binding to tumor cells *in vitro* with and without anti-tumor monoclonal antibody. Results of the immunologic monitoring will be presented, and further studies will evaluate additional patients treated with regimens of cytokines combined with antitumor monoclonal antibody to determine to what extent conditions which evoke ADCC *in vivo* with lymphocytes, macrophages and PMNs will elicit anti-tumor responses.

NZ 610 Hayashi, Robert J., and Pardoll, Drew M. Department of Oncology and Pediatrics, The Johns Hopkins School of Medicine.

LYMPHOKINE EXPRESSION OF T CELLS IN RESPONSE TO LYMPHOKINE TRANSDUCED TUMORS

T cells play a prominent role in the response in certain murine tumor systems. Various cytokines transduced into murine tumors have been shown to elicit a T cell mediated response against them. To further characterize this response, we have developed a system to measure lymphokine expression using quantitative PCR. Mice immunized with cytokine transduced tumors were analyzed at various time points in the evolving response. Using monoclonal reagents coupled to magnetic beads, specific T cell subsets from draining lymph node cells were isolated from contaminating cells with a magnetic column to greater than 90% purity. RNA isolated from these purified T cell populations are then analyzed for expression of IL-2, IL-4, and γIFN using PCR coupled to a reverse transcriptase reaction. Quantitation of this lymphokine expression is achieved by comparing the amplified message with the amplification of a synthetic RNA standard template which contains the sequences of the primers specific for the lymphokines of interest. Using this technique, the pattern of lymphokine expression of both CD 4 and CD 8 cells have been characterized, demonstrating both the evolution of the expression of lymphokines during the response to the immunizing tumor and thus the nature of lymphokine expression necessary to elicit tumor specific immunologic responses.

NZ 611 CLINICAL AND IMMUNOLOGICAL EFFECTS OF HUMAN RECOMBINANT INTERLEUKIN-2 GIVEN BY REPETITIVE WEEKLY INFUSION TO NORMAL DOGS Stuart C. Helfand and Steve A. Soergel, Department of Medical Sciences, School of Veterinary Medicine, University of Wisconsin, Madison, WI 53706

Four normal adult dogs received two consecutive weekly cycles of human recombinant interleukin-2 (IL-2) by continuous infusion for 4 days/week. The dose of IL-2 given to each dog was 3×10^6 units/m^2/day. Toxicities consisted of mild vomiting, diarrhea, and lethargy to varying degrees in all the dogs. These side effects were reversed when the treatment was discontinued. A marked lymphocytosis developed in all dogs after completion of each course of IL-2 and persisted for more than one month in some. Fresh peripheral blood lymphocytes (PBL) obtained during this lymphocytosis mediated enhanced lysis *in vitro* of a natural killer cell-sensitive canine tumor cell line (CTAC). The *in vitro* proliferative responses of these same PBL to IL-2 could be detected earlier, progressed faster, and involved more cells than PBL tested prior to IL-2 infusion. Thus, a relatively well-tolerated regime of IL-2 in dogs can induce dramatic increases in lymphocyte numbers and activation which is associated with augmentation of their *in vitro* antitumor reactivity. The clinical effectiveness of this immunotherapeutic approach remains to be tested in tumor-bearing dogs where it could serve as a relevant large animal model for immunotherapy of cancer with IL-2.

NZ 612 SPECIFIC ANTI-TUMOR IMMUNE RESPONSES GENERATED DURING THE PROGRESSION OF A RAT MALIGNANT GLIOMA, Frank P. Holladay and Gary W. Wood, Departments of Pathology and Surgery, University of Kansas Medical Center, Kansas City, KS 66160-7410

A study was conducted to determine whether an immunogenic, highly malignant avian sarcoma virus (ASV)-induced rat glioma (RT2) generates cytotoxic immune responses during its progression. RT2-specific primed lymphocytes may be produced by immunizing immunologically naive rats with tumor cells and C. parvum. Specific differentiation of primed cells may be stimulated in vitro with RT2 tumor cells and IL-2. Subcutaneous progression of RT2 produced primed precursors for ASV-induced glioma-specific cytotoxic immune cells. There was no evidence for suppression of priming as tumors increased in size. In contrast, intracerebral progression of RT2 failed to generate primed precursors. When rats bearing intracerebral gliomas were immunized subcutaneously with RT2 and C. parvum the rats also were primed, demonstrating that failure of intracerebral tumor to prime was not due to suppression. When rats bearing intracerebral glioma were treated on day five of tumor growth by intravenous injection of terminally differentiated immune lymphocyte populations and a 5 day course of systemic IL-2, rats were cured of their tumor. Immune effector cells could be generated either from immunized normal rats or from immunized progressor rats. IL-2 was essential for successful therapy. Various control treatments, including similar numbers of LAK cells that effectively killed all of the tumor target cells, had no effect on tumor growth. The results demonstrated that rapidly progressing malignant brain tumors may be treated effectively by systemic administration of tumor specific immune cells and IL-2.

NZ 613 ADOPTIVE IMMUNOTHERAPY WITH NORMAL DONOR CELLS AFTER ALLOGENEIC BONE MARROW TRANSPLANTATION PROVIDES AN ANTILEUKEMIA RESPONSE WITHOUT GRAFT-VS-HOST DISEASE, Bryon D. Johnson, Michael B. Weiler and Robert L. Truitt, Medical College of Wisconsin, Milwaukee, WI 53226

Allogeneic bone marrow transplantation (BMT) is now commonly used in the treatment of leukemia. A major problem of allogeneic BMT is graft-vs-host disease (GVHD). GVHD can be eliminated by depleting mature donor T cells from the BM inoculum. However, T cell depletion often results in an increased incidence of graft rejection and increased frequency of leukemia relapse. We have used MHC-matched, allogeneic [B10.BR into AKR] and haplotype-mismatched [SJL into $(SJLxAKR)F_1$] murine BMT models to develop strategies for avoiding GVHD while still providing an antileukemia or graft-vs-leukemia (GVL) effect, which is primarily mediated by donor T cells. AKR recipient mice preconditioned with 9 Gy total body irradiation (LD_{50}) and transplanted with B10.BR bone marrow did not develop clinical GVHD and became long-term, mixed donor-host T cell chimeras. In this transplant model, incomplete donor T cell chimerism is associated with decreased GVL reactivity. AKR hosts transplanted with 10×10^6 B10.BR bone marrow cells admixed with 30×10^6 B10.BR spleen cells (as a source of T cells) rapidly became complete donor T cell chimeras, but only 5% of the mice survived due to the development of severe GVHD. When infusion of donor spleen cells (30×10^6) was delayed until 21 days after transplantation with B10.BR bone marrow, few mice exhibited any signs of clinical GVHD and 96% of the mice became long-term survivors. Importantly, the infused spleen cells induced complete donor T cell chimerism in AKR recipients by 21 days after infusion and the cells were able to mount an *in vivo* antileukemia effect. Multiple constant doses and incremental doses of spleen cells have also been administered to AKR recipients post-BMT without any evidence of GVHD. In the haplotype-mismatced BMT model, F_1 hosts that received post-transplant SJL spleen cell infusions also did not develop GVHD. Thus, post-transplant immunotherapy with normal mononuclear cells from the transplant donor may be an effective way of eliminating residual disease or treating leukemia relapse without significant GVHD.

NZ 614 **DIETHYLCARBAMAZINE TREATMENT OF MCA-106 SARCOMA-INOCULATED MICE, Lynn W. Kitchen and James A. Ross, Department of Medicine, Marshall University School of Medicine and Veterans Administration Medical Center, Huntington, WV 25704, USA.**

A central question in cancer research is whether immunization with tumor antigens can mitigate subsequent metastatic tumor spread. The effect of diethylcarbamazine (N,N-diethyl-4-methyl-1-piperazine carboxamide [DEC]) in conjunction with gradually increasing doses of tumor cells was studied using an established tumor mouse model. We chose to work with DEC because our previous research on feline and murine leukemia virus models (J Clin Lab Immunol 25:101-3 and 27:179-81, 1988; 33:97-105, 1990) and bacterial and fungal mouse models (Int J Antimicrob Agents, in press) indicates that DEC treatment enhances host immune response to a variety of infectious agents. In the current experiments, prior intravenous injection of mice with 2 small doses of MCA-106 cells did not prevent metastatic spread to left lung subsequent to a third large injection of tumor cells, whether or not the mice received DEC treatment. Fewer DEC-treated mice given only one or two MCA-106 doses had left lung tumors than non-DEC-treated mice, but the results did not reach statistical significance. These results suggest that the anti-infective effect of DEC may be stronger than its direct antitumor effect.

NZ 615 THE IN VIVO EFFECTS OF rhIL7 ON T-CELL NUMBER AND FUNCTION IN NORMAL OR TUMOR-BEARING MICE. Kristin L. Komschlies[1], William J. Murphy[2], Dan L. Longo[2], Theresa A. Gregorio[2], and Robert H. Wiltrout[2]. [1]Biological Carcinogenesis and Development Program, PRI/DynCorp and [2]Biological Response Modifiers Program, NCI-FCRDC, Frederick, MD 21702-1201.

IL7, originally described as a B-cell lineage growth factor, has also been shown in vitro to enhance T-cell responses to various stimuli. Administration of IL7 to mice for one week increases the numbers of splenic pre-B cells, B cells and T cells by 3- to 4-fold. The increase in T cells is due primarily to a preferential expansion of $CD8^+$ T cells, resulting in an alteration of the CD4:CD8 ratio from 1.5:1 to 1:2. Further, the increase in T-cell numbers is predominantly due to an expansion of peripheral T cells rather than recruitment from the thymus. To determine whether in vivo administration of rhIL7 affects T-cell function, splenic T cells from mice treated with 10μg of rhIL7 twice a day for 7 days were tested for their responses to various in vitro stimuli. These results demonstrate that T cells from rhIL7-treated mice proliferate to Con A, PHA, allogeneic cells and anti-CD3 antibody more rapidly and to higher levels compared to controls. Intraepithelial lymphocytes (IEL) also increase 2-fold in number with in vivo treatment of rhIL7; however, their proliferative capacity to either anti-CD3 and rhIL2 or to PMA and ionomycin is not enhanced. Subsequent studies have also shown that rhIL7 has anti-metastatic effects against both murine colon and renal tumors in syngeneic mice with up to a 90% reduction in the number of pulmonary metastases. These anti-metastatic effects are accompanied by up to a 9-fold increase in splenic and lung T-cell numbers and an alteration in the CD4:CD8 ratio from 1.6:1 to 1:2.8 compared to HBSS treated tumor-bearing mice. We have also injected human PBL or T cells and rhIL7 into SCID mice bearing the HT-29 human colon tumor and demonstrated that human T cells in combination with rhIL7 delayed the appearance of HT-29, and extended survival compared to human lymphocytes or rhIL7 alone. These data support the conclusion that in vivo administration of IL7 increases the functional capacity of T cells and contributes to T cell-dependent effects against both mouse and human tumors.

NZ 616 TUMOR ASSOCIATED MAST CELLS AND MACROPHAGES AND THEIR PRODUCT TNF-α ARE INVOLVED IN ß-GLUCAN AND LPS INDUCED MODULATION OF Meth A FIBROSARCOMA GROWTH, Zbigniew Konopski, Baldur Sveinbjørnsson and Rolf Seljelid, Department of Experimental Pathology, Institute of Medical Biology, University of Tromsø, N-9037 Tromsø, Norway.

CB6F1 mice were injected s.c. with syngenic Meth A fibrosarcoma cells added low dosis of ß-glucan or LPS (ßT-tumor and LT-tumor respectively) on one side of abdomen and with Meth A cells only (cßT-tumor growing with ßT and cLT-tumor growing with LT) on the other side. Growth of ßT and LT was much faster, while growth of cßT and cLT was slower compared to control tumors. Histologic examination showed that ßT and LT contained a big central necrosis and few small focal necrotic areas. cßT and cLT contained central necrotic areas, while control tumors did not show any necrosis. Immunohistochemically, using anti-mouse macrophage antibodies (F4/80) we identified two types of tumor associated macrophages; connective tissue macrophages (CTM) surrounding tumor and tumor infiltrating macrophages (TIM) in the tumors. CTM were TNF-α positive in all examined tumors as shown by immunohistochemistry using anti-mouse TNF-α antibody. In the fast growing tumors (ßT and LT) two types of TIM were identified . One type was TNF-α positive located on the border between necrotic and non necrotic tissue and focally in non necrotic tissue. The other type was TNF-α negative located in non necrotic tissue. Both ßT and LT contained increased number of mast cells in the connective tissue surrounding tumor (CTMc) (++) and tumor infiltrating mast cells (TIMc) (++) as shown by specific staining. TIM in cßT (the slow growing tumor) expressed TNF-α positivity on the border between necrotic and non necrotic tissue only. cßT had increased number of CTMc (++) but no TIMc. TIM in cLT (the second slow growing tumor) expressed TNF-α positivity similar to cßT and additionally in some focal areas in non necrotic tissue. There was increased number of CTMc (+++) and TIMc (++) in cLT. Control tumors did not contain any TIMc and very few (+) CTMc.Their TIM were TNF-α negative. All CTMc were elongated, less spread than TIMc and were TNF-α positive. In ßT and LT,TIMc were well spread and strongly TNF-α positive. Our results so far demonstrate involvment of tumor associated mast cells, macrophages and TNF-α in ß-glucan and LPS induced modulation of tumor growth.

NZ 617 **INDUCTION OF LYMPHOMA-REACTIVE HUMAN T CELLS IN SCID MICE**, Vera Malkovska and Francine Cigel, Department of Medicine, University of Wisconsin, Madison, WI 53792

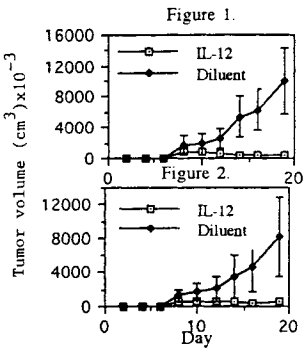
The in vitro culture of human peripheral blood lymphocytes (PBL) with Daudi (Burkitt lymphoma) cells results in selective proliferation of Vγ9/Vδ2 T cells with high cytotoxic activity against Daudi lymphoma. To test whether cytotoxic Vγ9/Vδ2 T cells can be induced in SCID mice, 10^6 human PBL and $2x10^7$ irradiated (11,000 cGy) Daudi cells were injected i.p. into each of ten SCID mice. Control animals received either PBL alone or PBL and Raji Burkitt lymphoma. After 7-14 days, flow cytometry analysis of cells recovered from PBL+Daudi treated mice showed that 13-69% cells from peritoneal cavity, 7-21% cells from pooled lymph nodes and 3-11% of nucleated blood cells double stained with BB3 (specific for Vδ2) and TiγA (specific for Vγ9) monoclonal antibodies (MoAb). In control animals, human γδ T cells represented 1-13% of peritoneal cavity cells, 0-2% lymph node cells and 0-2% blood cells. Surface antigen staining with TCR-γδ-1 combined with DNA staining of cells from PBL+Daudi treated mice showed significantly higher numbers of human γδ T cells in S phase compared to controls. Cells recovered from the peritoneal cavity of PBL+Daudi treated mice showed high cytotoxic activity against Daudi lymphoma (17-66% specific lysis at 20:1 E:T ratio) but not against control human and murine targets (<8% specific lysis at 20:1 E:T) in a 51 Cr-release assay. Depletion experiments with MoAb and magnetic beads showed that the cytotoxic activity against Daudi was exerted by human cells (Ly-5 negative) and not murine cells (HLe-1 negative). Cells recovered from control mice showed <10% specific lysis of Daudi targets at 20:1 E:T. These data demonstrate that human antigen-driven T cells are functionally active in SCID mice. This opens the possibility to develop in vivo models for studying human T cell responses.

NZ 618 IMMUNOTHERAPY OF MINIMAL RESIDUAL DISEASE (MRD) BY ALLOGENEIC PERIPHERAL BLOOD LYMPHOCYTES FOLLOWING AUTOLOGOUS BONE MARROW TRANSPLANTATION (ABMT): IMMUNOLOGICAL EVALUATION, Shoshana Morecki, Aliza Ackerstein, Arnon Nagler, Yael Puyesky, Reuven Or, Ella Naparstek, Paul Drakos, Joseph Kapelushnik, Maria Deluikina and Shimon Slavin, BMT Dept. and Cancer Immunobiology Research Laboratory, Hadassah University Hospital, Jerusalem 91120, Israel.

A clinical trial is currently underway investigating the feasibility and clinical efficacy of cell-mediated (CMI) as well as cell-mediated cytokine activated immunotherapy (CCI) using allogeneic peripheral blood lymphocytes for prevention and/or treatment of relapse following ABMT. Immunological parameters were evaluated in 9 patiens (CML 2, Acute Leukemia 5, non-Hodgkin's Lymphoma 2), age of 1.5-57 years (median 38), treated with fully matched (n=4) or mismatched (n=5) 10^6-10^7 cells/kg (CMI) 1-12 wks post ABMT followed by another dose of 10^7 cells/kg concomitantly with 1-3 days of rIL-2 ($6x10^6$ IU/m^2/day) (CCI) given one month later, pending no graft vs. host disease. Blood samples were collected prior to CMI, pre-and post CCI and 12 wks post CMI. Results showed an improved reconstitution of T cell-dependent mitogenic responses at 12 wks post CMI, while no major changes in cell surface markers characteristics of T cells, B cells, monocytes and NK cells post CMI and CCI. Soluble IL-2R was increased in 3/3 evaluated patients upon termination of rIL-2 treatment. Spontaneous non-MHC restricted cytotoxicity directed against NK-sensitive (K562) was markedly increased post CCI in 5/5 patients treated with mismatched allogeneic cells and was not affected or decreased in 4/4 patients treated with matched allogeneic cells. Cytotoxicity directed against NK resistent targets cells (Daudi) was increased in both matched (3/4) and mismatched (5/5) CCI treated patients. The results indicated that both CMI and CCI could activate the host defence immune system thus supporting the potential role of cell therapy in mediating possible in vivo anti-tumor response in patients with MRD post ABMT.

NZ 619 **Local Administration of Interleukin-12 Blocks *In Vivo* Progression of Murine Adenocarcinoma and Sarcoma** Nastala,† CL, Edington,† H, McKinney,† TG, Tahara,† H, Brunda*, M, Gately*, M, and MT Lotze†. †Departments of Surgery, Biochemistry and Molecular Genetics, University of Pittsburgh and *Hoffmann La-Roche, Nutley, NJ.

Anti-tumor properties of recombinant murine interleukin-12 (r-mIL-12) are suggested *in vitro* by potent augmentation of NK lytic activity and IFN-γ induction. Our recent observation that local (paracrine) r-mIL-12 production *in vivo* by fibroblasts transfected with the IL-12 genes delays growth of melanoma prompted this investigation of the effect of local rmIL-12 administration on tumor growth. Direct daily subcutaneous (sc) r-mIL-12 administration of 1.0 and 0.1ug on day1 following sc innoculation of $1x10^5$ MC-38 adenocarcinoma cells will abrogate progression of tumor growth in C57BL/6 mice (Fig 1). When rmIL-12 is administered on day 5 following innoculation of MCA-105 sarcoma, growth of established tumors is arrested during the treatment regimen. Tumor regression by IL-12 was apparent in not only tumors directly injected but in "sentinel" (untreated) tumors at a distant site (Fig 2). A combination of interleukin-2 (IL-2) with r-mIL-12 is equally as effective as rmIL-12 in abrogating tumor growth, but tumor progression occurs when IL-2 is used alone. No apparent toxicity was noted in the IL-12 treatment groups, although splenomegaly was consistently observed. Splenocyte total mononuclear cell counts were decreased overall and phenotypic analysis suggests a relative increase in T-cells. Tumor immunohistochemical analysis and NK/LAK activity of the groups are being investigated. These results demonstrate potent murine anti-tumor effects of IL-12 serving as a rationale both for phase I human trials and continued investigation into gene therapy with r-mIL-12.



NZ 620 INTERLEUKIN-2 GENE TRANSFER INTO A NON-IMMUNOGENIC BRONCHIAL TUMOR CELL LINE REDUCES TUMOR TAKE AND INDUCES ANTITUMOR IMMUNITY. J.L.Noteboom, D.Valerio and D.W.van Bekkum, Institute for Applied Radiobiology and Immunology, TNO Health Organization, P.O.Box 5815, 2280 HV, Rijswijk, the Netherlands.

The prognosis of lung cancer patients is still very poor. A novel method to evoke host derived anti-tumor immune responses is grafting of tumor cells that produce high amounts of certain cytokines resulting in their rejection and sometimes also in the rejection of co-transplanted parental tumor cells. This has been shown in a variety of immunogenic tumors in laboratory animals. We currently investigate to which extend inoculation of cytokine producing lung tumor cells could be beneficial in lung cancer. The experiments are performed in a panel of transplantable rat bronchial cancers that have many properties in common with human lung tumors, especially non-immunogenicity. We report on the first experiments with the tumor cell lines, that have been genetically modified to produce IL-2. The tumor cells were infected with the supernatant of a psi-2 cell line producing the ecotropic retroviral vector pLJ-IL-2, encoding mouse Il-2, promoted by the viral long terminal repeat (LTR) and the neomycin resistance gene (Neo^R) under control of the SV40 promotor. Infected cells were selected for the Neo^R-gene with neomycin (1mg/ml), cloned and expanded. Supernatant of 15 clones was tested for IL-2 concentration in the CTLL-2 ^3H-thymidine incorporation assay according to Gillis et al. (1978). Subsequent in vivo experiments were performed with clones expressing high levels of Il-2. Tumor take as a function of cell dose was reduced. We then tested tumor take of in rats that had been pretreated with a non-tumorigenic dose of cells from the IL-2 producing L44 clone. The results show that L44 cells that produce IL-2 have become immunogenic. These observations implicate that it is possible to direct the immune system towards non-antigenic bronchial cancer. The strategy so far has not resulted in amajor therapeutic effect.

NZ 621 **TUMOR CELLS TRANSDUCED WITH IL-2 AND IFN-γ CARRYING RETROVIRAL VECTORS INDUCE SYNERGISTIC ANTITUMOR ACTIVITY,** **Felicia M. Rosenthal, Kathryn Cronin and Bernd Gansbacher, Department of Hematologic Oncology, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY 10021**

To investigate whether antitumor immune responses to single cytokine transduced tumor cells could be enhanced, we have developed a model in which we study the effects of vectors carrying two cytokine genes. We previously reported that either IL-2 or IFN-γ secretion by CMS-5 cells, a murine fibrosarcoma, induces a potent specific antitumor response. We now have constructed retroviral vectors carrying both the IL-2 and IFN-γ cDNA and show that they can be used to stably transfect CMS-5 cells. Both cytokines are secreted for prolonged periods of time and both cytokines are biologically active in vitro and vivo. IL-2/IFN-γ transduced CMS-5 cells showed increased levels of MHC I expression. Tumor growth of parental CMS-5 cells versus cytokine transduced CMS-5 cells was monitored. As compared to CMS-5 cells carrying only the IL-2 gene more rapid regression of tumors was observed. In vivo depletion of $CD4^+$ or $CD8^+$ effector cell subpopulations suggests that $CD8^+$ cytotoxic T cells are primarily responsible for the rejection of IL-2/IFN-γ secreting fibrosarcoma cells. Our data indicate that there is a synergistic effect on tumor growth inhibition if both IL-2 and IFN-γ are secreted by the tumor cell.

NZ 622 EXTENDED SURVIVAL OF CLINICALLY FAVORABLE PATIENTS WITH METASTATIC RENAL CELL CARCINOMA (RCC) TREATED WITH ex vivo ACTIVATED MEMORY T-CELLS (AUTOLYMPHOCYTE THERAPY). Susan D. Ross, Philip T. Lavin, Jeffrey Martin, Fred Miesowicz, and Michael E. Osband. Cellcor, Inc., and Boston Biostatistics, Inc., Newton, MA, and Boston University School of Medicine, Boston, MA.

Autolymphocyte therapy (ALT) is outpatient adoptive immunotherapy using memory T-cells activated ex vivo by a combination of OKT3, a mitogenic monoclonal antibody directed against the CD3 portion of the T-cell receptor, and a mixture of autologous cytokines. Patients also receive oral cimetidine to block suppressor T-cells. We have previously described that in a randomized clinical trial ALT prolonged survival, induced durable tumor responses, and was accompanied by minimal toxicity. We have an active clinical database that currently has comprehensive information on 543 RCC patients. Multivariate analysis describes a series of clinical factors that are associated with an unfavorable survival outcome including: performance status $\geq$ 2, hypercalcemia (Ca > 11.5), thrombocytosis (platelets > 500,000), anemia (Hct $\leq$ 35%), brain metastases, steroid use, sarcomatoid histology, and poorly differentiated pathology. Presence of 1 or more of these factors defines a clinically unfavorable group, while absence of all risk factors defines a clinically favorable group. In the literature, patients who are described as clinically favorable, by various criteria, have survival of 7-12 months (e.g., Elson, et al, Cancer Res 48:7310, 1988). In our database, clinically favorable patients who have not received ALT, have a median survival of 14 months. Since the conclusion of the randomized trial, 78 clinically favorable patients have received ALT (defined as mean cell infusion > 600 million ex vivo activated cells prepared from an appropriate autologous cytokine mixture, and patient taking > 1400 mg/day cimetidine). Median follow-up on these 78 patients is 11 months. At 1 year, 81% of these patients remain alive and the median survival has not yet been reached. The Cox proportional hazards model predicts that the median survival on these patients will be > 32 months, representing a 2-3 fold advantage over expected survival for clinically comparable patients. Toxicity in these 78 treated patients has been minimal: 98/1170 (8.9%) of pheresis/infusion procedures were accompanied by Grade 1-2 adverse reaction; no Grade 3 or 4 adverse reactions occurred. This prolonged survival and minimal toxicity suggests that ALT should be considered as a treatment alternative for clinically favorable patients.

NZ 623 LOCOREGIONAL THERAPY OF THE LINE 10 TUMOR IN THE GUINEA PIG WITH MODIFIED rIL-2 CAUSES TUMOR REGRESSION AND INDUCES T-HELPER CELL MEDIATED IMMUNITY.

PA Steerenberg[1], LTM. Balemans[1], V Mattijssen[2], FJ Koppenhagen[1], G Storm[3], PHM De Mulder[2], W Den Otter[1] *Natl. Inst. Public Health and Environm. Protection, Bilthoven[1]; Dept. Med. Oncol, Univ. Hospital, Nijmegen[2]; Dept. Farmacy, State University Utrecht[3], The Netherlands.*

R-IL-2 was modified either by the covalent attachment of polyethylene glycol (peg) or by incorporation of r-IL-2 into liposomes.

Locoregional therapy of intradermal Line 10 (L10) tumors with 200,000 IU PEG-IL-2 resulted in regression of the primary tumor and prevention of the development of lymph node metastases. Intratumoral (i.t.) administration of PEG-IL-2 3x a week for 5 weeks starting at day 7 or day 14 resulted in cure of all animals. A second tumor cell inoculum was rejected, showing induction of immunity. The i.t. route of administration is thought to be essential in tumor regression and prevention of metastases. Injection of PEG-IL-2 near the axillary lymph node or intranodular treatment did not affect the growth of metastases.

During PEG-IL-2 therapy no *in vitro* cytotoxic activity of spleen or lymph node cells could be demonstrated. Immunity to L10 cells was adoptively transferred by spleen cells of cured animals. Adoptive transfer of spleen cells depleted for T-helper cells resulted in L10 tumor growth, while administration of a suspension depleted for T-cytotoxic cells did not influence the transfer of L10 immunity, showing the role of T-helper cells in PEG-IL-2 induced tumor regression.

R-IL-2 bound to liposomes appeared to be biologically active and was gradually released from the liposomes under biological conditions. Intratumoral administration of liposomal bound r-IL-2 3x a week for 5 weeks resulted in tumor regression and prevention of metastases at a dose of 200,000 IU r-IL-2.

In summary, the i.t. route of administration of (PEG-)IL-2 is important for the induction of tumor regression and tumor immunity. Both PEG-IL-2 and liposomal bound r-IL-2 are effective therapeutic agents in this hepatocarcinoma animal model.

NZ 624 A NOVEL APPROACH FOR THE TREATMENT OF CHRONIC HEPATITIS B. Sheng Su and Ning Yao, Hepo Medical-Tech Research Institute, Beijing, China

This report summarizes the clinical studies of Ara-AMP and autologous lymphokine-activated killer (LAK) cells, given alone or in combination, for the treatment of patients with chronic hepatitis B (CHB) in China. It represents multi-center prospective randomized controlled studies conducted in 1988-1991 by Su, Yao and their associates. Diagnosis of CHB was based on standard criteria, with patients having HBV markers, abnormal aminotransferase (ALT), and characteristic liver histopathology. Patients were randomized into an experimental and a placebo control group. Sixteen of 42 (38%) of patients given Ara-AMP for 28 days (10 mg/kg IV for 5 days, then 5mg/kg IM for 23 days) became HBeAg negative, compared with 3 of 41 (7%) placebo controls ($p < 0.005$). Twenty-eight of 49 (57%) of patients treated with autologous LAK cell reinfusions (1-2 times/week for 6 weeks) became HBeAg negative, compared with 5 of 36 (11%) of controls ($p < 0.005$). Treated patients also showed improvement in ALT and increased anti-HBe seropositivity. Nineteen months follow-up of 30 LAK cell treated patients showed an increase of HBeAg seronegativity from 57% to 80%. The results indicate that treatment of CHB patients with either Ara-AMP or LAK cells is effective in eliminating HBe antigenemia, increasing anti-HBe seroconversion and improving liver function.

NZ 625 ERADICATION OF MURINE INTRACRANIAL TUMORS BY SYSTEMIC T CELL IMMUNOTHERAPY, Jeffrey J. Sussman, Wendy L. Wahl, Alfred E. Chang and Suyu Shu, Department of Surgery, University of Michigan, Ann Arbor, MI 48109. Lymph nodes (LN) draining a progressively growing tumor contain tumor sensitized but functionally deficient cells. Upon activation *in vitro* they are capable of eradicating visceral tumors when transferred systemically. In human trials, patients with brain metastases are usually excluded for adoptive immunotherapy because the blood brain barrier is thought to prohibit the proper trafficking of lymphocytes. We have recently shown that systemically transferred lymphocytes can treat intracranial tumors. We now further characterize this effect as tumor-specific, exogenous IL-2 independent, and can be augmented by sublethal irradiation. LN cells draining progressive MCA 205 tumors for 12 days were activated *in vitro* with immobilized anti-CD3 for 2 days followed by culture in 10 U/ml IL-2 for 3 days. The therapeutic effects of these cells were tested by adoptive immunotherapy of 3-day established transcranially induced brain tumors. Median survival time was extended from 19 days to 31, 55 and >75 days following i.v. transfer of $2x10^7$, $4x10^7$ and $8x10^7$ cells/mouse respectively. Administration of exogenous IL-2 (15,000 U i.p.BIDx4d) had no enhancing effect. In fact, in one experiment exogenous IL-2 reduced survival from 100% to 25%. This is in sharp contrast to the immunotherapy of pulmonary metastases using the same activated cells where IL-2 is known to increase the potency of the effector cells by four fold. On the other hand, sublethal (500R) irradiation of tumor bearing mice enhanced the cellular therapy. Irradiated mice receiving i.v. effector cells led to significantly more prolonged survival compared to non-irradiated mice. Sublethal irradiation alone did not affect the survival of tumor-bearing mice, thus, irradiation may be reducing potential tumor-induced immunosuppression or altering lymphocyte trafficking patterns. Transfer of effector cells generated from MCA 207 tumor-draining LN cured the mice with 207 intracranial tumors but they were ineffective against 205 tumors, thus demonstrating the effect is tumor specific. Mice cured of an intracranial tumor developed systemic immunity capable of rejecting challenges with the same tumor either subcutaneously or transcranially. These results suggest that properly sensitized T cells were capable of mediating the regression of brain metastases. However, the conditions and requirements for successful therapy might be different from treating visceral metastases in the lung or liver.

NZ 626 T-CELL RECONSTITUTION BY MOLECULAR, PHENOTYPIC AND FUNCTIONAL ANALYSIS IN THE THYMUS, BONE MARROW, SPLEEN AND BLOOD AND IMMUNOTHERAPY FOR BREAST CANCER IN MICE RECEIVING MULTI-DRUG CHEMOTHERAPY J. E. Talmadge, J. D. Jackson, and G. A. Perry Dept. of Path. Micro. and Cell Bio. and Anat., U. of Neb. Med. Ctr., Omaha, NE. 68198.

We examined the effect of a maximum tolerated, split dose chemotherapy protocol of cyclophosphamide, cisplatin, and BCNU on neutrophil (PMN) and lymphocyte sub populations in the blood, thymus, bone marrow (BM) and spleen. This chemotherapy protocol was modeled after that used with autologous BM transplantation (AuBMT) for breast cancer. We have obtained molecular, phenotypic, and functional data on the reconstituting cells in chemotherapy treated mice with or without BMT and/or CSF-G treatment. These studies demonstrate a rapid reconstitution of PMN (by 15 to 18 days) with a slower lymphocyte reconstitution which varies by site (blood, spleen, BM and thymus) with the normalization of all phenotypes by day 60. In contrast to the recovery of the CD-3, CD-4 and CD-8 cells the ability of the splenic lymphocytes to respond to Con-A remained depressed. The lymphocytic dysfunction was not associated with any specific phenotype including $V\beta$-5+; $V\beta$-8+; γ/δ+ or CD-4-, CD-8- α/β+ cells. Furthermore, no thymic or splenic natural suppressor (NS) cell activity was observed (BM NS activity was found but did not correlate with loss of splenic mitogenesis). These studies also revealed, on a time dependent basis, a marked thymic response to Con-A and helper cell activity. There appears to be an increase in TH_2 relative to TH_1 cell activity based on RT-PCR of the spleen lymphoid cells which may contribute to the immune dysfunction. Therapeutic studies in mice bearing a large metastatic tumor burden (mammary cancer) using this chemotherapy protocol have revealed that BMT and CSF-G prevent infectious disease associated mortality but provide no additional therapeutic efficancy. Supported in part by the Nebraska Cancer and Smoking Disease Research Program.

NZ 627 EXPRESSION OF THE T CELL COSTIMULATORY LIGAND B7 BY A MELANOMA INDUCES REJECTION MEDIATED BY DIRECT ACTIVATION OF CD8+ T CELLS, Sarah E. Townsend and James P. Allison, Department of Molecular and Cell Biology, University of California at Berkeley, 94720

A variety of tumors are potentially immunogenic but are incapable of stimulating an effective anti-tumor immune response in vivo . One possible reason for this poor immunogenicity might be that tumors may be capable of delivering antigen-specific signals to T cells, but may not deliver the costimulatory signals necessary for full activation of T cells. We show that expression of the costimulatory ligand B7 induces the rejection of a murine melanoma in vivo. This rejection is mediated by CD8+ T cells; CD4+ T cells are not required. Prior rejection of B7+ melanoma cells rendered mice capable of rejecting B7- cells. These results suggest that B7 expression renders tumor cells capable of effective antigen presentation, leading to their eradication in vivo. Manipulation of costimulation offers a novel strategy for tumor immunotherapy.

NZ 628 **ANTI-TUMOR PROTECTION FROM THE MURINE T CELL LEUKEMIA/LYMPHOMA EL4 BY THE CONTINUOUS SUBCUTANEOUS COADMINISTRATION OF RECOMBINANT MACROPHAGE-COLONY STIMULATING FACTOR (M-CSF) AND INTERLEUKIN-2 (IL2)**
Daniel A. Vallera, Patricia A. Taylor, S. Lea Aukerman, and Bruce R. Blazar. Departments of Therapeutic Radiology and Pediatrics, University of Minnesota Hospital and Clinic, Minneapolis, MN 55455 and the Chiron Corp. Emeryville, CA 94608.

We investigated the combined continuous subcutaneous coadministration of M-CSF + IL-2, to mice given intraveous injections of a lethal dose of EL4 T cell leukemia/lymphoma cells, not expressing M-CSF or IL-2 receptors. A group of 30 mice in 2 experiments given 14 day pumps on day -3 and EL4 on day 0 were protected from EL4 tumorigenicity by 20 ug/day M-CSF and and 5 ug/day IL-2 (80% actuarial survival at day 100). Treatment with a similar dose of IL-2 alone resulted in early toxic deaths (20% actuarial survival at day 10) likely related to the known toxic effects of IL-2. We have shown that several cell types are affected by this cytokine combination. 1) Macrophages are involved since M-CSF is lineage-restricted and two color immunofluorescence/flow cytometric studies on day 7 revealed that macrophages (F4/80+ cells) were higher in normal or EL4-injected mice given M-CSF+IL-2 than in mice given M-CSF alone, IL-2 alone, or PBS. 2) T cells are involved in M-CSF+IL-2 efficacy since adult thymectomy/T cell depletion significantly inhibited the ability of cytokine coadministration to protect against EL4. In other studies, mice given M-CSF+IL-2 showed higher anti-EL4 CTL activity than mice given either cytokine alone. 3) NK cells are involved in M-CSF+IL-2 efficacy since the in vivo depletion of NK cells in EL-4 injected mice inhibited the ability of M-CSF+IL-2 treatment to protect against EL-4. Splenic NK cytolytic activity was highest in M-CSF+IL-2 treated mice. Also, flow cytometric studies showed that splenic NK cells (NK1.1+CD3-) were highest in M-CSF+IL-2 treated mice. 4) Granulocytes are also involved since M-CSF +IL-2 survivors showed significant elevations in neutrophils from blood smears and in flow cytometry studies. Although this study provides ample evidence that coadministration of M-CSF+IL-2 is an effective immunoenhancing regimen, as yet, we have found no explanation for the ability of M-CSF to protect against IL-2 toxicity. This regimen deserves further investigation as a means to improve therapeutic potential of toxic IL-2.

NZ 629 CD8 DEFICIENT MICE FAIL TO REJECT VIRUS-INDUCED SYNGENEIC LYMPHOMA, BUT STILL REJECT MCA-INDUCED SYNGENEIC SARCOMA, Tao Wen, Lorenz H. Trümper, Waiping Fung-Leung, George Klein and Tak W. Mak, Ontario Cancer Institute, 500 Sherbourne Str. Toronto, Canada M4X 1K9 and Dept. of Tumor Biology, Karolinska Institute, S10401 Stockholm, Sweden

Experimentally induced tumors can be rejected by the immune system of a syngeneic host, if the host has been immunized with the same lethally treated tumor cells prior to tumor transplantation. In order to further investigate the role of each T cell subset in such transplantation rejection, we generated a novel mouse strain (C57BL/6 background) which lacks CD8+ T cell subpopulation, using homologous recombination to disrupt the CD8 gene in mouse genome. Tumor transplantation rejection was tested on those mice with syngeneic (C57BL/6 derived) Rauscher virus induced T cell lymphoma (ALC) and methylcholanthren(MCA) induced fibrosarcoma(MC57X).

CD8 deficient mice(CD8-/-), heterozygous littermates(CD8+/-) and wild type C57BL/6 mice(CD8+/+) were divided into experimental and control groups. The experimantal groups were injected s.c. with 10^6 lethally irradiated tumor cells weekly,over 5 weeks. One week after the last immunization, both groups were inoculated with 10^5 living tumor cells. Tumor incidence was followed for 8 weeks after the inoculation, and summarized as follows: (Mice with tumor / Total inoculated mice)

1. Rauscher Virus Induced Lymphoma ALC (i.p. inoculation)

Experimental Group			Control Group		
CD8-/-	CD8+/-	CD8+/+	CD8-/-	CD8+/-	CD8+/+
8/10	1/12	0/8	6/6	8/8	11/11

2. MCA Induced Fibrosarcoma MC57X (s.c. inoculation)

Experimental Group			Control Group		
CD8-/-	CD8+/-	CD8+/+	CD8-/-	CD8+/-	CD8+/+
0/8	0/9	0/8	6/6	7/7	8/8

It is shown that CD8+ T cell population is necessary for rejecting the syngeneic Rauscher virus induced lymphoma, but not for the MCA induced fibrosarcoma. We are presently testing more virus and MCA induced tumors on CD8 deficient mice to develop a comprehensive picture of the role of CD8+ T cells in tumor immunity.

Late Abstracts

TH-2 LIKE CELLS REGULATE ALLOREACTIVITY IN VIVO, **Daniel H. Fowler and Ronald E. Gress, National Institutes of Health, NCI, DCBDC, EIB, Bethesda, MD 20892**

Allogeneic BMT is primarily limited by cell-mediated GVH and rejection reactions. In vivo alloreactivity in murine models is characterized by TH1 cytokine activation, especially IFN-γ. Multiple murine disease states illustrate that TH1 predominant, cell-mediated in vivo responses can be downregulated by TH2 cells and the TH2 cytokines IL-10 and IL-4. The possibility that an in vivo alloreactive transplantation response can be regulated by cells with a TH2 cytokine profile was assessed in a murine GVHD model.

GVHD was induced by iv injection of B6 parental spleen cells into a B6C3F1 host. As shown by others, LPS endotoxin administration resulted in TNF-α release and acute lethality. In vivo cytokine activation was studied using RT/PCR. Compared to negative controls, T lymphocytes from GVHD mice had markedly increased IFN-γ mRNA (30-50 fold increase); other cytokine transcripts (IL-2, IL-4, and IL-10) were modestly increased (3.5 fold or less).

Parental cells of TH2 cytokine mRNA phenotype were generated by in vivo treatment of B6 mice with combination IL-2/IL-4. The CD4+ enriched spleen cell population from these mice had increases in IL-4 and IL-10 mRNA, with concommitant decreases in IL-2 and IFN-γ mRNA. These parental cells of TH2 mRNA phenotype were non-lethal in this GVHD model. Additionally, mice receiving a cell mix of unmanipulated parental spleen cells + parental CD4+, TH2-like cells were protected from lethality.

T lymphocytes from recipients of these parental cell mixes showed greatly decreased IFN-γ mRNA, with concommitant increases in IL-4 and IL-10 mRNA. These experiments show that T-cell mediated in vivo allogeneic responses can be regulated by CD4+ cells with a TH2-like cytokine profile and that such regulation is associated with decreased IFN-γ mRNA levels in the T cell population.

DEFINING IN VIVO REQUIREMENTS CONTROLLING ANTIGEN PRESENTATION USING LIPID MATRIX BASED SUBUNIT IMMUNOGENS. Raphael J. Mannino and Susan Gould-Fogerite, Department of Laboratory Medicine and Pathology, UMDNJ, New Jersey Medical School, Newark, N.J. 07103-2714.

Immunologically important determinants, (defined B and T cells epitopes) formulated with various lipid matrix based structures produce well characterized and highly immunogenic preparations. Minimal characteristics essential to the induction of both humoral and cell mediated immune responses in vivo have been defined through a systematic variation in the structure of theses preparations.

Using defined antigens from a variety of pathogens including HIV, SIV, Malaria, influenza and parainfluenza viruses, we have developed a number of simple, gentle and relatively rapid procedures for formulating antigens, in the form of peptides, proteins or whole pathogens, with lipid matrices to produce effective, chemically well defined and safe immunogens. Through varying the structural characteristics of the association between the antigen and the lipid matrix, one can maximize either humoral or cell mediated immunity.

Humoral Immunity - An immunogenic formulation capable of stimulating antibody production can be comprised simply of peptide epitopes representing B cell and T helper cell determinants presented to the immune system as a multivalent complex in the context of a phospholipid bilayer. Antibody production has been stimulated using synthetic peptide epitopes derived from known B and T helper cell determinants. The antibody response is boostable, shows class switching and the antibodies cross-react with the proteins from which the sequences are derived.

Cytotoxic T Lymphocytes - An immunogenic formulation capable of inducing epitope specific, CD8+ CTL in vivo can be comprised of a short synthetic peptide recognized by class I molecules encapsulated within a fusogenic proteoliposome (containing lipid bilayer-integrated Sendal virus glycoproteins) or an amphipathic peptide which can be recognized by both class I and class II molecules presented to the immune system in the context of a peptide-phospholipid structure. Immunization with phospholipid formulations containing whole proteins or synthetic peptides has induced a boostable, epitope specific, CD8+ CTL response in mice and, with collaborators, in monkeys.

Identification of murine melanoma T cell epitopes recognized by CD8+ tumor infiltrating lymphocytes in the context of H-2K^b. T. Itoh*,#, W. J. Storkus*,#, E. Gorelik#, and M. T. Lotze*,#, From the *Departments of Surgery and Molecular Genetics and Biochemistry, University of Pittsburgh School of Medicine, and the #Pittsburgh Cancer Institute, Pittsburgh, PA 15261.

Previous research examining the in vivo immunogenicity of the BL6 melanoma and BL6 melanoma variants (i.e. H-2 class I transfectants and MNNG-mutagenized BL6) in C57bl/6 (B6, H-2^b) mice has suggested a strong correlate between melanoma immunoreactivity and melanoma expression of the H-2K^b class I allele (Tanaka et al., Mol. Cell. Biol. 8:1857-1861 (88)). In these studies, one of the most immunogenic BL6 variants examined in vivo was the CL8-1 (H-2K^{b+} transfected, H-2D^{b+}) cell line. Tumor infiltrating lymphocyte (TIL) lines were derived from CL8-1 tumors removed from B6 mice and subsequently grown in vitro for 3-4 weeks in the presence of irradiated CL8-1 tumor and rhIL-2 (100 IU/ml). The CL8-1 TIL efficiently lysed the CL8-1 (H-2K^{b+}, H-2D^{b+}) and BL6-2K (H-2K^{b+}, H-2D^{b-}) melanoma as well as the MCA 105 sarcoma (H-2K^{b+}, H-2D^{b+}), but not the BL6 (H-2K^{b-}, H-2D$^{b+/-}$), EL4 (H-2K^{b+}, H-2D^{b+}), or normal B6 splenocyte targets. This strongly suggested that the CL8-1, BL6-2K, and MCA 105 target cells expressed shared or cross-reactive T cell epitopes recognized in the context of the H-2K^b class I allele. Class I bound peptides were acid eluted from the BL6, BL6-2K, and CL8-1 melanoma cell lines in addition to the MCA 105 sarcoma, the EL4 thymoma, and normal B6 splenocytes. These peptides (1-5 x 10^9 cell equivalents) were subsequently fractionated by reverse-phase HPLC, lyophilized, and individual HPLC fractions reconstituted in buffered saline. Aliquots of these fractions were coincubated with previously ^{51}Cr-labeled EL4 target cells for 1 hour. These targets were then assayed for sensitivity to CL8-1 TIL mediated lysis in standard 4h cytolytic assays. At least three HPLC fractions (F1, F2, F3) containing peptides derived from the CL8-1 melanoma were recognized by CL8-1 TIL when presented by H-2^b EL-4 target cells but not H-2^d P815 mastocytoma target cells. These same three fractions were identified by CL8-1 TIL in BL6-2K, but not BL6, EL4, or normal B6 splenocyte peptide preparations. Interestingly, CL8-1 TIL appeared to recognize at least two species corresponding to F1 and F3 derived from the MCA 105 sarcoma. These data support H-2K^b-restricted recognition of F1-F3 by the CL8-1 TIL and suggest that F1-F3, when identified at the amino acid sequence level may constitute the basis for a melanoma/sarcoma peptide vaccine in B6 mice.

Immunogenicity of activated ras oncogenes
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During initiation and development of many different human tumor types a wide variety of genes, such as the *ras* oncogenes, may become activated or inactivated. Activation of any of the three human *ras* genes occurs in approximately 20% of all human cancer syndromes. In several studies it has been shown that this activation may be indicative for the prognosis and survival of the patient. The cellular immune system constitutes the major host protection against tumor initiation and progression. The first line of defense probably consists of Natural Killer cells with broad specificity, whereas T-cells recognize distinct foreign peptide antigens as presented by MHC molecules. The *ras* oncogenes are activated through point mutations at three restricted aminoacid positions. These altered proteins may be presented to the immune effector cells as tumor antigens. We have investigated the binding of mutant and wildtype *ras*-derived peptide sequences in the context of HLA-A2.1, and the subsequent generation of peptide specific CTL from healthy donors or patients. By using a system that scores peptide sequences for the HLA-A2.1 binding motif, we have been able to select the peptides that most probably associate specifically with this molecule. These peptides where tested for actual binding, using the mutant cell line T2, which expresses unstable A2.1-molecules. Two peptides specific for wildtype and mutant sequences were found to bind strongly. These peptides have been used to induce a primary response with PBL of an HLA-A2.1 positive healthy donor.

ROLE OF LFA-1/ICAM-1 INTERACTION IN THE LYMPHOCYTE PROLIFERATION RESPONSE TO INTERLEUKIN-2,

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Several adhesion routes have been implicated in lymphoid cell-cell contacts, including those mediated by the receptor/ligand pairs LFA-1/ICAM-1 and CD2/LFA-3, the VLA antigens and the CD44 molecule. To study the role of these adhesion receptors in the proliferative response of peripheral lymphocytes to IL-2, blocking studies were performed with a panel of monoclonal antibodies (mAb). Inhibitory effects were observed only of mAb directed to LFA-1 α or β or its ligand ICAM-1. Preferentially resting lymphocytes were susceptible to the inhibition, particularly in an early phase of culture and when stimulated with relatively low rIL-2 doses. By using mab that specifically could block distinct rIL-2 activation pathways, LFA-1/ICAM-1 interaction was found to control the interaction of rIL-2 with its high affinity receptor, mediated by p55, but not with its intermediate affinity receptor, p75. Furthermore, it was shown that T lymphocytes, but not NK cells were inhibited in their rIL2-response by anti-LFA-1 mAb. This would suggest that LFA-1/ICAM-1 interaction directs a focal release of IL-2 and is required for an optimal upregulation of the expression of p55IL-2R. In this way cell-cell interactions may accelerate cytokine responsiveness. Additional experiments indicated that NKI-L16, a mab directed to the activated state of the LFA-1 α molecule, which strongly induces cell adhesion, also inhibited the p55 mediated rIL-2 response. In view of the reported partial association between p55IL-2R and ICAM-1, it is assumed that impaired mobility of the p55 IL-2 receptor may play a role.