MONOCLONAL ANTIBODIES

Organizers: Ellen Vitetta, Ronald Levy and Thomas Merigan

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Plenary Sessions Page

Poster Sessions

Monoclonal Antibodies

Opportunities for Using Monoclonal Antibodies (Session Sponsored by Sandoz Research Institute)

N 001 ANTIBODY TARGETING IN CARDIOVASCULAR DISEASE

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Antibodies directed to three components of the thrombus, fibrin, the platelet GP IIb/IIIa receptor, and α_2-antiplasmin, have been used in the experimental targeting of plasminogen activators. The strategy with the antibodies to fibrin and GP IIb/IIIa is to increase the concentration of plasminogen activator at the thrombus and produce more rapid and more selective lysis. With the antibody to α_2-antiplasmin, the strategy is to selectively inactivate a component of the thrombus and enhance the ability of plasmin to attack it. Fibrin was targeted because it has antigenic epitopes that differentiate it from fibrinogen, its precursor in circulating plasma. With a fusion protein comprising the combining site of an antibody that binds fibrin but not fibrinogen (59D8) and the catalytic unit of single-chain urokinase plasminogen activator (scuPA), fibrinolytic activity (in comparison with that of scuPA) increased 6 fold in an in vitro plasma clot assay and 20 fold in an in vivo rabbit jugular vein assay. Recombinant scuPA-59D8 bound fibrin with an association constant indistinguishable from that of the parent antibody, exhibited a K_m and a K_{cat} indistinguishable from those of urokinase, and was fully activated by plasmin. GP IIb/IIIa was targeted because platelets bind to one another and to fibrin through fibrinogen or von Willebrand's factor linked to the GP IIb/IIIa receptor. Urokinase conjugated to antibody 7E3, which inhibits platelet aggregation by preventing fibrinogen binding to the GP IIb/IIIa receptor, was far more effective in lysing platelet-rich thrombi and preventing platelet aggregation than was 7E3 or urokinase, or an equimolar mixture of the two. For example, ADP-induced platelet aggregation could be inhibited fully by 200 nM of urokinase, 12 nM of 7E3, or 1.6 nM of the urokinase-7E3 conjugate. An alternative to the targeting approach exemplified by scuPA-59D8 and urokinase-7E3, hybrids containing a plasminogen activator catalytic site and an antibody combining site, is the bispecific antibody, which contains two antibody combining sites. A cell fusion product that recognizes epitopes on both fibrin and tissue plasminogen activator (tPA) significantly enhanced the activity of tPA in the rabbit jugular vein model. The attractiveness of the bispecific antibody approach is in the potential to concentrate endogenous plasma tPA or urokinase at the thrombus. Alternatively, with a bispecific antibody exogenous plasminogen activators could be administered at much lower doses. Finally, the concentration of the principal inhibitor of plasmin, α_2-antiplasmin, is a major determinant of the effectiveness of plasminogen activators. α_2-Antiplasmin is present in plasma both as a soluble protein and as a protein covalently linked to fibrin by amide bonds (mediated by factor XIIIa). An antibody that selectively inhibits α_2-antiplasmin enhanced the activity of streptokinase, urokinase, and tPA 20 to 80 fold in vitro without increasing fibrinogen depletion. With a human thrombus in a rabbit model, this antibody enhanced the efficacy of tPA by approximately 3 fold. Because the covalent linkage between α_2-antiplasmin and fibrin defines an immunologic epitope, it can be used to differentiate circulating α_2-antiplasmin from fibrin-bound α_2-antiplasmin. An antibody specific for this epitope could enhance plasmin activity at the thrombus without promoting a systemic lytic state.

N 002 "FROM THE BENCH TO THE CLINIC", Thomas C. Merigan, M.D., Center for AIDS Research at Stanford University, Stanford, CA 94305.

Although there are many incentives to move a diagnostic or therapeutic approach from the bench to the clinic, there are obvious and frequently encountered problems. This presentation will concentrate on this authors experience in technology transfer from animal model and tissue culture systems to human applications. Useful background information has been provided with the experience of doing clinical trials with antivirals and cancer over the last three decades as well as working in an academic setting which allows affiliations with both traditional pharmaceutical manufacturers and new biotechnology companies. Selection of clinically important targets, utilizing sound pharmacologic principles and giving major roles to statisticians as well as clinicians in designing trials are part of the todays approaches to successful technology transfer. The regulatory atmosphere is also clearly changing as well as the role of various National Institutes of Health. Today, the climate is encouraging effective technology transfer to allow appropriate clinical testing and development and all government agencies are proud to be associated with such work when it is successful. Patients are increasingly sophisticated and are becoming partners in the clinical investigation. Hence, protocols must utilize cutting edge clinical care as a background on which new modalities are being tested. Hopefully, an awareness of these trends will insure monoclonal antibodies reach the clinic at the earliest time and have the most successful clinical results in the next few years.

N 003 **HOW DO ANTIBODIES ACT IN VIVO TO COMBAT VIRAL INFECTION?**
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The mechanism(s) by which antibodies act in vivo are poorly characterized. Understanding these mechanisms is important for designing effective subunit vaccines or therapeutic antibodies. We have approached this issue by studying the role of antibody in combatting infection with the mammalian reoviruses whose pathogenesis, genetics, and structure has been extensively characterized. The properties of a panel of monoclonal antibodies (MAbs) specific for reovirus capsid proteins (sigma 3, mu 1, lambda 2, and sigma 1) were studied in vivo and in vitro. In vivo studies showed that 1) MAbs binding several outer capsid proteins were protective against viruses utilizing a variety of pathogenetic strategies, 2) protective Mabs act by different mechanisms at defined steps in the pathogenesis of viral infection, 3) Mabs to sigma 1 (the cell attachment protein), as well as sigma 3 and mu 1 (adjacent capsid proteins) prevented neural spread from muscle or intestine to the CNS, 4) the capacity to protect was not solely dependent on the avidity of MAb binding to virus, isotype, aggregation induction, neutralization, or hemagglutination inhibition (HI). In vitro studies showed that Mabs to sigma 3 and mu 1 can inhibit functions (HI, cell attachment) previously mapped to sigma 1. In addition, Mabs to outer capsid proteins inhibit critical intracellular events in the virus life cycle. Mabs inhibited both proteolytic processing of virus in cells and chymotryptic digestion of virus in vitro. It is notable that inhibition of viral processing correlated well with the capacity to protect from lethal infection. We conclude that antibodies have different actions at different sites in vivo, that antibody to one protein can inhibit the function of an adjacent protein, and that inhibition of intracellular steps in the virus life cycle may be an important determinant of the effectiveness of antibody in vivo. Information from this model system may be relevant to understanding how to properly design anti-viral subunit vaccines and therapeutic antibody preparations.

N 004 GRAM-NEGATIVE BACTEREMIA AND ENDOTOXEMIA. Elizabeth J. Ziegler, Department of Medicine, University of California San Diego, UCSD Medical Center, San Diego, CA 92103.

Bacterial sepsis is a growing problem among hospitalized patients, and gram-negative infections account for more than half of these cases. Among septic patients with gram-negative bacteremia (GNB) the death rate remains high despite modern methods of treatment. There is increasing evidence that endotoxin in the circulation is an important trigger for the cascade of events leading to organ failure and death in GNB. For that reason efforts have been directed toward developing antibodies against endotoxin to try to improve the outcome in GNB. Human polyclonal antibody against common core determinants of endotoxin protects experimental animals against lethality from endotoxin and gram-negative infection and was shown in a randomized trial to prevent death in patients with GNB (New Engl. J. Med. 307:1225, 1982). However, vaccination of donors and administration of human serum to patients is not practical for many reasons. Therefore, a human monoclonal antibody (mAb) against endotoxin was sought, which would be protective and could be standardized easily and produced in quantity. A human IgM with such properties was developed (PNAS 82:1790, 1985) and found to be protective in animals. This mAb, called HA-1A, is directed to an epitope on lipid A, the toxic moiety of endotoxin. HA-1A has been subjected to a randomized, double-blind, placebo-controlled trial in septic patients (Clin. Res. 38:304A, 1990). Of 543 patients treated, 200 had GNB. In patients with GNB, HA-1A reduced mortality by 39% (from 49%-placebo to 30%-HA-1A, $p = 0.014$). Protection from HA-1A was evident in patients with shock ($p = 0.017$). HA-1A recipients experienced more rapid resolution of septic complications ($p = 0.020$), and a higher percentage were discharged alive (51%-HA-1A vs. 29% - placebo, $p = 0.002$). In a substudy (Wortel *et al.*, abstract 495 Proc. Int'l. Cong. for Infect. Dis., Montreal, 1990) there were two interesting observations: 1) protection with HA-1A was particularly striking in patients who had endotoxemia at base line; and 2) HA-1A treatment cause a significant decrease in serum levels of tumor necrosis factor at 24 hours ($p < 0.05$). All patients tolerated HA-1A well, and no anti-HA-1A antibodies were detected. Thus, human mAb HA-1A appears to be safe and effective for immunotherapy of gram-negative bacteremia and shock and its beneficial effects seem to be due to interference with endotoxin-induced activation of host defense cells.

Basic and Clinical Considerations of Antibody Therapy

N 005 MONOCLONAL ANTIBODIES FOR THERAPY AND IN VIVO DIAGNOSIS: REGULATORY ISSUES. Thomas Hoffman, Laboratory of Cell Biology, Division of Hematology, Center for Biologics Evaluation and Research, U. S. Food and Drug Administration, Bethesda, MD 20892

Monoclonal antibodies represent a group of biotechnology-derived products with vast potential benefits for diagnosis and therapy of many conditions. The widespread experimental use of monoclonal antibodies gives testimony to this fact. Practical use in situations where effectiveness of these agents is proven is still limited. Problems remaining to be solved as monoclonal products come to licensure are clear understanding of dose ranging and pharmacokinetics, approaches to minimizing anti-antibody responses, and careful selection of target antigens relevant to the disease or condition being addressed. The intended use of the antibody and the basis for determining endpoints for effectiveness become paramount issues for proposed licensing. For biologicals, including monoclonals, impact on patient care is a key factor in determining appropriateness for general use. This policy is applicable to in vivo diagnostic monoclonals. The FDA document, "Points to consider in the manufacture and testing of monoclonal antibody products for human use," has served adequately for manufacturing issues encountered in the IND phases of development. Even now, this reference remains the basis of evaluating products for licensure. However, as use of monoclonals becomes more widespread and new technologies for their manufacture become routine (human, chimeric, recombinant-derived antibodies), the FDA must keep pace scientifically. Proper regulation also involves judicious resource allocation in order to promulgate up-to-date guidance and to ensure fair and expeditious approval of effective products.

N 006 Biologic Properties of Genetically Engineered Antibody Molecules

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The ability to produce antibodies by gene transfection and expression makes it possible to synthesize antibodies of predefined structure. One advantage of this approach is that genetically engineered antibodies can now be used to investigate structure-function relationships within the antibody molecule. We have used genetically engineered antibodies made by either exon-shuffling or by site directed mutagenesis to define the residues determining binding to the high affinity Fc receptor (FcγRI). We have verified that the C_H2 domain is the primary site for FcγRI. Within C_H2, two regions separated in primary structure by nearly 100 residues determine binding; these regions are in close proximity in the folded domain. The first of these regions is in the lower hinge in which Leu 234 and Leu 235 are critical for high affinity binding. The second region which appears to contribute to receptor binding is a hinge proximal bind between the two beta strands within C_H2; specifically Pro331 contributes to high affinity binding. The hinge region has been shown to also modulate Fc receptor binding. Using this same approach we have demonstrated that it is the carboxy terminal half of C_H2 that determines isotype specific failure of human IgG to activate complement.

By introducing site-directed mutations in chimeric mouse-human IgG, we identified individual residues contributing to the epitope at the C_H2-C_H3 interface of IgG bound by monoclonal IgM rheumatoid factors (RFs) from patients with Waldenstrom's macroglobulinemia. We showed that replacing His with Arg at position 435 in the C_H3 domain destroys or reduced RF binding to IgG4, but that substitution of His for Arg at 435 is insufficient to enable RFs to bind IgG3. Furthermore, we showed that residues 252-254 and 309-311 in two amino acid loops in C_H2 contribute to the RF binding site, indicating that at least three loops, two from C_H2 and one from C_H3 are involved in RF binding to IgG. Although this epitope includes many of the same residues as the Staph protein A (SPA) binding site on IgG, the binding specificities of SPA and monoclonal RFs are not identical.

In addition to the amino acid sequence, the carbohydrate composition of an antibody molecule has been shown to contribute to its biologic properties. We have shown that the invariant carbohydrate present in the C_H2 domain of all IgG is necessary for many of the effector functions and presence of this carbohydrate influences the serum half-life of some isotypes. In addition, carbohydrate present in complementary determining region 2 of the variable region has been shown to influence the ability of the antibody to bind antigen. The exact position of this variable region carbohydrate is critically important for its function.

New Targeting Molecules

N 007 FUNCTIONAL EXPRESSION OF CHIMERIC T CELL RECEPTOR WITH ANTIBODY-TYPE ("T-BODY") SPECIFICITY. Zelig Eshhar, Guy Gorochov, Tova Waks and Gideon Gross, Department of Chemical Immunology, The Weizmann Institute of Science, Rehovot 76100, Israel.By replacing the varible region of the T cell receptor α and β chain genes with gene segments encoding the V_H and V_L of a specific antibody we have constructed chimeric T cell receptors (cTCR) genes which upon transfection could confer on the recipient T cells non-MHC restricted antibody-type specificity. To clone and express Fv from any monoclonal antibody as cTCR, we designed oligonucleotide primers and vectors which enable us to directly amplify and clone into expression cassettes V_H and V_L genes starting from crude hybridoma DNA or RNA. Using these protocols we have prepared several cTCR genes encoding Fv of anti-hapten, anti-id and anti-human tumor antibodies. By transfection these genes could be expressed as functional surface receptors in murine cytotoxic T cell hybridomas and the human Jurkat T cell tumor. The optimal conditions for the cTCR expression were studied in an anti-TNP specific system in which we could demonstrate that the cTCR rendered the effector T cells with MHC non-restricted and independent antigen recognition and target cell killing. Taking advantage of such "T bodies" with different affinities we studied the interrelationships between the TCR and T cell's accessory molecules. Thus we found that the CD4/CD8 molecules exert their accessory function through interactions with MHC molecules which are not associated with the TCR:Ag:MHC complex. In the same system we could show that the role of the LFA-1 cell adhesion molecule is mainly by increasing the affinity of interaction between the T cells and its antigen presenting cell. Hence, when the T cells are equipped with high affinity receptor, their dependence on LFA-1 becomes marginal. We found that soluble antigen (even multi-valent) could not stimulate T cell activation and was rather inhibitory to TCR mediated triggering. Moreover, for effective stimulation the antigen needed to be immobilized. We conclude, that for the application of the "T-body" approach for immunotherapy, cTCR should be constructed using selected antibodies directed at surface antigens which are not present in soluble form in the circulation.

Immunoconjugates and Radiolabeled Antibodies

N 008 THE DEVELOPMENT OF CHEMOIMMUNOCONJUGATES FOR CANCER THERAPY, T.F Bumol, L.D. Apelgren, S.V. DeHerdt, D.L. Zimmerman, Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46285

In the past seven years, several preclinical strategies of chemoimmunoconjugates have been extensively developed based on the KS1/4 monoclonal antibody and the vinca alkaloid oncolytics which resulted in two chemoimmunoconjugates; LY256787 (KS1/4-desacetylvinblastine) and LY203725 (KS1/4-desacetylvinblastine-3-carboxhydrazide), proceeding to Phase 1 clinical trials for the treatment of human adenocarcinomas. Further studies on the preclinical pharmacology and toxicology of LY203725 are presented including antitumor efficacy experiments in combination with cyclosporin A utilizing human tumor xenografts expressing high levels of the mdr-1 gene product. The preclinical pharmacology/toxicology profiles of LY203725 will be discussed with respect to toxicities observed in Phase 1 clinical trials by Dr. Schneck and colleagues. Issues surrounding antibody choice, oncolytic/linker choice and immunogenicity will be presented. Preclinical studies in the OVCAR3 human ovarian carcinoma xenograft system with chemoimmunoconjugates employing novel linker technology and non-KS1/4 monoclonal antibodies will be presented as alternative strategies for future clinical development. This presentation would not be possible without the collaborations of the laboratories of Drs. Baker, Koppel, Lindstrom, Pohland, Scott, and Zimmermann of Lilly Research Laboratories.

N 009 RADIOLABELED ANTIBODIES. Order, SE, Department of Radiation Oncology
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Radiolabeled antibodies in cancer therapy may presently be divided into two classes. In the first class there is sufficient radiation dose achieved to remit the cancer as in Hodgkin's disease and non-Hodgkin's lymphoma. The second class is in solid tumors where the variable low dose rate irradiation may lead to tumor cell redistribution into G2 and M, and/or loss of sublethal repair mechanisms making the cells more susceptible to conventional chemotherapy or radiation, as in hepatocellular cancer. Our experience in Hodgkin's disease (chemotherapy failures) demonstrates that with 131-I antiferritin, a 40% PR in 37 patients. Whereas with the more powerful isotope, 90-Y, a 60% response with 30% PR and 30% CR. Toxicity was limited to hematologic depression. In non-resectable hepatocellular cancer using 131-I antiferritin, 7% of patients receiving low dose doxorubicin and 131-I antiferritin could be converted to surgical resectability. A 5 year disease free remission has been achieved in a select group of patients. Although initially integration with doxorubicin either before or after 131-I antiferritin, amplified tumor cytotoxicity. A newer program using intra-arterial cisplatin, 50mg/m^2 +/- radiolabeled antibody has to date led to a median survival of 16 months (three times the anticipated result). Ultimately, a new Phase 3 trial will be initiated to determine the value of the radiolabeled antibody.

The issues in radiolabeled antibody in non-Hodgkin's lymphoma also include the total body effects (TBI) of systemic administered radiolabeled antibody which ordinarily contributes 1-1.5 cGy per administered millicurie and could lead to a response of antibody specificity.

In closed space applications, i.e. peritoneal administration (ovarian cancer); and intrathecal, (malignant meningitis), advantage is taken of the lack of dehalogenation due to non-access by circulating enzymes and a prolonged tumor effective half life. Therefore, greater tumor doses are achieved due to lack of dehalogenation and slow egress of the radiolabeled antibody.

New opportunities exist in the application of radiolabeled antibodies, and include chimeric antibodies, human monoclonal antibodies, new isotope linkage, and more powerful isotope radiation. The potential for further clinical progress is significant.

N 010 RADIOLABELED ANTIBODY THERAPY OF B CELL LYMPHOMAS, Oliver W. Press, Janet F. Eary, Christopher C. Badger, Paul J. Martin, Frederick R. Appelbaum, Ron Levy, Richard Miller, Wil B. Nelp, Kenneth A. Krohn, Darrell Fisher, Dana Matthews, and Irwin D. Bernstein. Departments of Medicine, Nuclear Medicine, and Pediatrics, University of Washington, Seattle, WA 98195, The Fred Hutchinson Cancer Research Center, Seattle, WA 98104, Stanford University School of Medicine, Stanford, CA 94305, The Batelle Pacific Northwest Laboratories, Richland, WA 99352, and IDEC Pharmaceuticals Corporation, Mountain View, CA 94043.

We have evaluated the biodistribution and therapeutic potential of I-131-labeled anti-B cell monoclonal antibodies (MoAbs) in 26 patients (pt) with advanced non-Hodgkin's lymphomas who failed conventional chemotherapy and/or radiation therapy. Sequential MoAb biodistribution studies were performed on successive weeks with escalating amounts of antibody (0.5, 2.5, 10 mg/kg) trace-labeled with 5-10 mCi I-131. Absorbed radiation doses to tumor sites and normal organs were estimated by the Mirdose method based on data obtained by serial whole body gamma camera imaging, serial tumor biopsies, and computed tomography. In all cases, larger antibody doses (e.g. 2.5 or 10 mg/kg) yielded better antibody biodistributions than lower MoAb doses (0.5 mg/kg). In 12 of the 26 patients, every assessable tumor site received more radiation than any critical normal organ, and these patients were considered candidates for therapeutic infusions of I-131-MoAbs. The 14 patients who did not achieve favorable MoAb biodistributions generally had large tumor burdens (>0.5 kg, 9 pt) or massive splenomegaly (10 pt). One of the 12 pt with favorable MoAb biodistributions developed human anti-mouse antibodies before radioimmunotherapy could be undertaken, and another patient was not treated since sufficient antibody was not available at the time. The other 10 patients were hospitalized for 6-8 days and given 211-1000 mg of anti-B cell antibodies (anti-CD37 MoAb MB-1, 6 pt; anti-CD20 MoAb 1F5, 1 pt; anti-CD20 MoAb B1, 2 pt; anti-idiotypic antibody, 1 pt) labeled with 232-628 mCi of I-131. Acute toxicity was limited to transient mild nausea, pruritis, and low grade fever, although all treated patients experienced significant myelosuppression 2-4 weeks following treatment. Six patients had elective reinfusion of autologous, purged bone marrow. Eight of the patients achieved complete remissions and three remain in continuous complete remission after 5-23+ months. One patient achieved a partial response and one person is too early to evaluate. We conclude that the tolerable toxicity and encouraging efficacy warrant further dose escalation in this phase 1 trial. In addition, future studies will attempt to improve the proportion of patients who achieve favorable MoAb biodistributions by performing splenectomies in patients with splenomegaly and administering cytoreductive chemotherapy to patients with tumor burdens > 0.5 kg. (Supported by NIH Grant CA 44991).

N 011 ACTIVATION OF PRODRUGS BY ANTIBODY ENZYME CONJUGATES, Peter Senter, Oncogen, 3005 First Avenue, Seattle, WA 98121

A new strategy for the delivery of cytotoxic agents to solid tumors is described in which monoclonal antibodies are used as carriers for enzymes to tumor cell surfaces. The enzymes are chosen for their abilities to convert relatively non-cytotoxic drug precursors (prodrugs) into active anticancer drugs. The drugs thus formed can then penetrate into nearby tumor resulting in cell death. A number of prodrugs have been developed that can be transformed into active anticancer drugs by enzymes of both mammalian and non-mammalian origin. The enzymes have been shown to localize into tumors when linked to monoclonal antibodies that bind to tumor associated antigens. *In vivo* studies indicate that MAb-enzyme/prodrug combinations can result in antitumor activities significantly greater than those of the prodrugs or drugs given alone. This is most likely due to the generation of large amounts of active drug at the tumor site.

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Protein Engineering I; Protein Engineering II; Immunoconjugates and Linkers; Pharmacokinetics, Biodistribution and Metabolism

N 100 GENERATION OF MONOCLONAL ANTIBODIES BY REPERTOIRE CLONING, Dennis R. Burton, Department of Molecular Biology, Research Institute of Scripps Clinic, La Jolla, CA 92037 and Krebs Institute, Department of Molecular Biology, The University, Sheffield, UK.

We have previously shown that large numbers of mouse monoclonal Fab fragments against a small hapten can be generated by antigen selection from a combinatorial library of heavy and light chains expressed in E.coli using novel phage lambda vectors (Huse et al(1989) Science 246,1275-1281). The approach has since been used to generate antibodies against haemagglutinin from a mouse immunised with influenza virus (Caton & Kaprowski (1990) Proc.Natl.Acad.Sci.USA 87,6450-6454). Recently we have shown that the method can be used to generate human antibodies. Specific diverse human Fab fragments have been prepared from the peripheral blood lymphocytes of an individual immunised with tetanus toxoid (Persson et al, submitted). We have further used the approach for the facile rescue of a human anti-rhesus D antibody from an EBV-transformed cell line (Williamson et al, submitted). The strengths, weaknesses and likely applications of repertoire cloning in the light of current experience will be discussed.

N 101 COMPLEMENT FIXATION OF ANTIBODIES OF HYBRID ISOTYPE ENGINEERED BY A NOVEL PCR-BASED APPROACH.

Tim Clackson and Greg Winter
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All antibody isotypes contain a core C1q binding motif which is required for complement (C) lytic activity; however some isotypes are still poor or non-binders and do not activate C. We have engineered domain-swapped anti-NP antibodies of hybrid isotype, between mouse IgG1 (inactive) and mouse IgG2b (active), to define the additional regions leading to these differences. The chimaeric genes were constructed by a novel strategy termed 'sticky feet' -directed mutagenesis, which allows any large segment of DNA to be replaced with any other, without using restriction sites (Nucl Acids Res. 17, 10163-10170). The technique resembles oligo-directed mutagenesis: a long primer directing the entire substitution is made by PCR and annealed to a single stranded DNA template. Hybrids so constructed reveal that features of the IgG2b C_H2 domain determine complement lytic ability, rather than the upper hinge length and concommitant segmental flexibility previously thought to be critical. We have used higher resolution mutagenesis of regions varying between the isotypes, including the lower hinge, to pinpoint the critical amino acid residues. We have also generalised our findings to other isotypes using further 'sticky feet' mutants.

N 102 THE ISOTYPE OF AN ANTI-CORE LPS MAb AS A DETERMINANT OF LIPID A REACTIVITY, Hermann Gram, Reet Tees, Robin Barclay*, Franco Di Padova

Preclinical Research, Sandoz Pharma, Basel, Switzerland, *SNBTS, Edinburgh, Scotland

The isotype of a MAb has an important effect on its biological activity. In this study we demonstrate that the isotype may influence its reactivity as well. Among several anti-core LPS MAbs, a murine IgM anti-LPS MAb crossreacts in ELISA with lipid A and EcR3, but not with EcR1, EcR2 or EcR4. In western blots of smooth and rough LPS's the MAb reacts with deep core structures. Due to the unusual pattern of reactivity we wanted to elucidate the role of the IgM isotype on the binding pattern. The heavy chain murine IgM constant part was replaced by the human IgG1 isotype by genetic engineering. To this purpose the variable regions of heavy and light chain were cloned by PCR, combined with the human IgG1 and kappa constant regions and transfected into Sp2/0Ag14-10 cells. Once chimerized, the MAb retained its pattern of reactivity with the EcR3 core region, but lost its reactivity with lipid A.

N 103 UNIVERSAL OLIGONUCLEOTIDE PRIMERS FOR PCR AMPLIFICATION OF HUMAN HEAVY AND LIGHT CHAIN VARIABLE REGIONS AND PROBES FOR CLASSIFICATION OF HUMAN HEAVY AND LIGHT CHAIN VARIABLE REGIONS. Andrew D. Griffiths†,James D. Marks†, Michael Tristem‡, Abraham Karpas‡ and Greg Winter†. † Medical Research Council, Laboratory of Molecular Biology, and ‡ Department of Haematology, Hills Road, Cambridge CB2 2QH, England.

We have optimized primers and conditions to permit amplification and cloning of all human immunoglobulin variable region families. Conserved sequences at the 5' end of framework 1 of the variable region gene and in the joining segment were used to design primers to amplify rearranged variable region genes using PCR according to their family. The primers were used to amplify total RNA from human peripheral blood lymphocytes. Sequence analysis of 83 cloned variable region genes indicated the presence of a diverse repertoire. These 83 sequences and previously available heavy and kappa light chain variable region sequences were aligned, classified by family and a conserved region in framework 1 was identified. This region was used to design family specific oligonucleotide probes which were validated by probing the cloned variable region genes. We conclude that the primers and probes can be used to amplify and classify all human heavy and kappa light chain variable regions.

N 104 AMINO ACID SUBSTITUTIONS IN THE LIGHT CHAIN CDR3 ALTER THE BINDING OF AN ANTI-DIGOXIN SINGLE-CHAIN ANTIBODY. Paul T. Hamilton and Douglas P. Malinowski. Cell Biology Department, Becton Dickinson Research Center, RTP, NC 27709.

An anti-digoxin, single-chain antibody was constructed by tethering the Vh and Vl domains together with a 15 amino acid linker and incorporating the complementary determining region (CDR) sequences of the anti-digoxin antibody 26-10. The anti-digoxin, single-chain antibody was expressed in E. coli, secreted into the periplasm using the beta-lactamase signal sequence and shown to bind digoxin. Replacement of CDR3 of the light chain with the CDR3 sequence from another anti-digoxin light chain destroyed digoxin binding. This construction was used for cassette mutagenesis of CDR3 of Vl. Bacterial colonies expressing the mutagenized CDR3 sequences were screened for their ability to restore digoxin binding. DNA sequence analysis of the mutants indicated that for the first six amino acids of CDR3, Ser in the first position, Gln in the second position and Thr in the third position were important for digoxin binding. The remaining 3 positions were more tolerant of changes than the first 3 positions.

N 105 LIGHT CHAIN V-REGION DUPLICATION RESULTS IN FORMATION OF OLIGOMERIC IgG, Linda J. Harris, J. William Finley, James Esselstyn, Walter Shuford, and Howard V. Raff, Departments of Molecular Immunology and Immune Sciences, Oncogen, Bristol-Myers Squibb Pharmaceutical Research Institute, Seattle, WA 98121

A human IgG1 antibody oligomer was isolated from a transfectoma producing monoclonal antibody (MAb) targeted against group B Streptococci (GBS). As discussed in the accompanying abstract (Raff, *et al*) the oligomer had significantly enhanced biological activity compared with the monomer. Immunochemical and DNA sequence analyses revealed that the transfectoma produced two distinct kappa light (L) chains. In addition to a normal molecular weight species (L_n; 25 kD) a 37 kD species (L_{37}) of composition variable-variable-constant was also present. Cotransfection of vectors encoding the heavy chain (H) and L_{37} resulted in production of oligomeric IgG. We will discuss models for the oligomerization and vectors we have constructed to produce oligomeric IgG of other specificities and IgG subclasses.

N 106 **ANTIBODY ENGINEERING: COMPARISON OF VARIABLE(V) DOMAIN AFFINITY**

Peter Hudson, R.Webster*, P.Colman, D.Hewish, V.Harley, N.Ivancic, R.Irving, A.Kortt, G.Lilley, R.Malby, B.Power and W.Tulip. CSIRO Division of Biomolecular Engineering, 343 Royal Pde, Parkville, Victoria, Australia 3052. Fax (03)3475481 *St Jude Children's Research Hospital, Tennessee.

We have developed novel expression systems for high-level secretion of immunoglobulin domains into the bacterial periplasm. As a unique model system we have expressed domains of NC10 and NC41 monoclonal antibodies to ascertain the relative contribution to binding made by the separate VH and VL domains. Our advantage is that the binding interface of NC10 and NC41 antibodies to influenza neuraminidase has been resolved by X-ray crystallography enabling a detailed comparison to be made of the relevant contact residues. VH domains and single-chain Fvs were engineered with extended C-terminal peptide tails for improved diagnostics and purification strategies. Isolated VH domains were found to exhibit a substantial binding affinity to neuraminidase. We envisage a number of specific applications using small immunoglobulin-like domains as targeted binding reagents. Site-directed mutagenesis and crystallisation experiments are in progress to ascertain the exact conformation of VH and scFv domains with respect to their binding interface with neuraminidase.

N 107 EXPOSITION OF A SINGLE CHAIN FV FRAGMENT ON THE CAPSID OF A FILAMENTOUS VIRION, Roberto Jappelli, Maurizio Sollazzo and Gianni Cesareni.
Department of Biology, II University of Rome, Torvergata, Rome Italy.

We have designed a procedure that should allow rapid selection of antibodies of a given specificity from a large repertoire(10^7-10^8) of antigen binding sites expressed in E.coli. The method is based on the exposition of the Fv fragment on the capsid of an E.coli virion by fusion of the scFv fragment to virion structural proteins. Upon selection of phages able to bind specific antigens, via the capsid exposed Fv fragment, it should be possible to elute the bound phage and automatically clone the phage(s) encoding the desired antigen binding site.
We have synthesized a gene encoding a human reshaped scFv, optimizing the nucleotide sequence for E.coli codon usage and for the presence of unique restriction enzyme recognition sites in strategical positions in the gene. The amino acid sequence is composed of the VH framework of NEW, a human antibody whose Fab structure is known at high resolution and, the VL framework of REI, also of known structure, linked by a 15 aminoacids oligopeptide linker, GlyGlyGlyGlySer repeated three times. The antigen binding site is constructed by grafting the CDRs of a rat anti CAMPATH monoclonal antibody according to Riechman et al. (Nature 332, 323-327, 1988) We have then fused this gene to the genes encoding the aminoterminus of two structural proteins of the E.coli filamentous virion f1. These two proteins are the product of gene VIII that coats most of the virion road (approximately 3000 copies) and the product of gene III that is present in 5 copies at one end of the virion. This latter protein has already been shown to tolerate relatively large peptide insertions (Parmley and Smith. Gene 73, 305-318, 1988). We are currently testing whether the hybrid proteins can be accomodated into functional phage particles and whether the antibody maintain antigen specificity. We will then proceed constructing libraries of "synthetic antigen binding sites".

N 108 DEVELOPMENT OF HUMANIZED MONOCLONAL ANTIBODIES WHICH NEUTRALIZE RESPIRATORY SYNCYTIAL VIRUS, Syd Johnson, Frances Dunn, Jackie Hatfield and Geetha Bansal, MedImmune, Inc., Gaithersburg, MD 20878

Respiratory syncytial virus (RSV) is the most common cause of lower respiratory tract infection in young children. In the United Sates alone, approximately 90,000 children are hospitalized and 4,500 children die from RSV infection each year. Murine monoclonal antibodies against the F glycoprotein of RSV have been shown to neutralize RSV *in vitro* and protect cotton rats from lower respiratory tract infection *in vivo*. These results suggest that these antibodies could be used for passive immunization against RSV infection. Previous studies with other rodent antibodies suggest that the development of human anti-mouse antibody responses would preclude the use of these murine antibodies directly in children. Therefore, we have generated humanized versions of several of these antibodies. We have utilized a vaccinia/T7 expression system to express recombinant single-chain versions of the parental and humanized forms. Once we have established that the humanized single-chain version retains specificity and affinity for the RSV F glycoprotein it will be expressed as a full length dimer with human constant domains.

N 109 CHIMAERIC (MOUSE-HUMAN) AND CDR-GRAFTED ANTIBODIES TO HUMAN IL2 RECEPTOR, Brigitte Kaluza, Eberhard Russmann, Helmut Lenz and Ulrich H. Weidle, Boehringer Mannheim GmbH, Nonnenwald 2, D-8122 Penzberg, FRG.

Murine antibodies to human IL2 receptor α-chain and ß-chain were made chimeric by transferring V-regions to human constant γ1- and -regions. In addition, the CDR-regions of a murine antibody to human IL2 receptor α chain were grafted onto the framework of a human antibody ("humanized antibody").

Both chimeric and humanized antibodies were expressed in transfectoma clones and tested for their biological activity. Upon administering a combination of anti-α- and anti-ß-chain antibodies, a synergistic inhibitory effect on T lymphocyte proliferation was observed.

N 110 HUMANISATION OF A MOUSE MONOCLONAL ANTIBODY BY CDR-GRAFTING: THE IMPORTANCE OF FRAMEWORK RESIDUES ON LOOP CONFORMATION, C.A. Kettleborough, J. Saldanha, V.J. Heath, C.J. Morrison and M.M. Bendig, MRC Collaborative Centre, 1-3 Burtonhole Lane, Mill Hill, London, NW7 1AD.

Mouse monoclonal antibody (MAb 425) binds to epidermal growth factor receptor (EGFR) and hence has potential as an anti-tumour agent. However, since murine MAbs are antigenic in humans, two approaches to humanise mouse MAb 425 were undertaken. The first involved joining the mouse variable regions to human constant regions to create chimaeric 425 antibody. In the second, the mouse complementarity determining regions (CDRs) from MAb 425 were grafted into the framework regions (FRs) of human variable regions. These reshaped human variable regions were joined to human constant regions to create reshaped human 425 antibodies. To assist in the design of the reshaped human variable regions, a molecular model of the mouse MAb 425 variable regions was built. FR residues at certain positions may affect antigen binding and, therefore, must be conserved in the human FRs. Nine versions of the reshaped human heavy chain and two versions of the reshaped human light chain were designed, constructed and expressed. The avidities of the resulting antibodies for EGFR were assayed and compared to murine MAb 425 and chimaeric 425 antibody. These results will be presented and the possible role of FR residues discussed. One version of reshaped human 425 antibody bound to EGFR with an avidity approaching that of the mouse MAb 425.

N 111 **CONSTRUCTION AND HIGH LEVEL EXPRESSION OF A MOUSE/HUMAN CHIMERIC CD4 ANTIBODY WITH THERAPEUTIC POTENTIAL**, D.M. Knight[1], J. Looney[1], M. Moore[1], H. Trinh[1], M. Dalesandro[2], K.Y. Pak[2], E.P. Rieber[3], G. Riethmuller[3], P. Daddona[2], and J. Ghrayeb[1], Departments of Molecular Biology[1] and Immunobiology[2], Centocor Inc., 244 Great Valley Pkwy, Malvern, PA 19355, and Institute for Immunology[3], University of Munich, D-8000 Munich 2, FRG.

The use of murine anti-CD4 monoclonal antibodies has shown considerable promise for the treatment of allograft rejection and rheumatoid arthritis. We have constructed a mouse/human anti-CD4 antibody with the goal of increasing its therapeutic potential by decreasing immunogenicity, increasing circulating half-life, and optimizing interaction with the human immune system. The chimeric antibody was constructed by cloning the H and L chain variable regions of M-T412, a murine IgG2a antibody raised against the human CD4 antigen, and joining them to the human γ1, γ4, or K constant regions in mammalian expression vectors. After transfection into mouse myeloma cells, stable cell lines were isolated that secrete up to 140 μg/ml chimeric antibody in static culture, and 200 mg/l with serum-free medium in 50 liter bioreactors.

The chimeric antibodies were equivalent to the murine antibody in their binding characteristics and relative affinity constants. The chimeric and murine antibodies were also similar in their ability to inhibit the increased Ig production resulting from the pokeweed mitogen stimulation of T cells. Experiments that measure inhibition of T cell activation by the antibodies suggest, however, that there are functional differences mediated by the Fc region. In summary, we have achieved high-level expression of a chimeric antibody engineered to optimize therapeutic effectiveness. The chimeric γ1 antibody is currently in clinical trials.

N 112 ISOLATION OF ANTIBODY SPECIFICITIES FROM E.COLI: EXPRESSION ON THE SURFACE OF BACTERIOPHAGE.

John McCafferty[1], Andrew D. Griffiths[2], Greg Winter[2]and David J. Chiswell[1]
[1]Cambridge Antibody Technology Ltd, Daly Research Labs., Babraham Hall, Cambridge, CB2 4AT, and [2] MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH

In recent years the expression of soluble antibody fragments retaining binding activity has been demonstrated in E.coli. In addition it has been shown that large repertoires of antibody encoding fragments could be generated using PCR. One problem remaining has been the development of sufficiently powerful methods for screening for antigen binding.

We have expressed functional antibody fragments on the surface of filamentous bacteriophage by introducing variable region genes into a gene encoding a viral coat protein. We have demonstrated that these phage antibodies have the same binding pattern as the original antibody. Moreover, purification of rare antigen binding phage (one in forty million) from populations has been achieved by antigen-affinity chromatography.

The phage antibody contains both a surface antigen binding domain and the DNA encoding it in a form which can readily be re-introduced into bacteria. Thus isolation of antigen binding molecules and their subsequent manipulation is greatly facilitated.

N 113 EFFICIENT EXPRESSION OF RECOMBINANT ANTIBODIES IN MAMMALIAN CELLS.

G Roberts C.R.Bebbington, G.Renner, C.M.Gofton, S.Thomson, M.McCormack, D.King and G.T.Yarranton. Celltech Limited, 216 Bath Road, Slough, Berkshire, England.

The clinical use of humanised monoclonal antibodies will necessitate the efficient manufacture of recombinant proteins from mammalian cells is frequently achieved by repeated gene amplification of vector sequences integrated into the host cell chromosomes e.g. DHFR gene amplification selected using methotrexate in dhfr$^-$ CHO cells. Glutamine synthetase (GS) is an alternative amplifiable gene which can be used as a dominant selectable marker in a variety of cell types. We have shown that using the GS system allows the efficient expression of immunoglobulin cDNA's under the control of strong viral promoters after only a single round of gene amplification. The expression of a mouse:human chimeric monoclonal antibody in either CHO or myeloma cells results in product titres of approximately 250mg/litre in suspension culture containing serum free medium.

The GS system therefore allows recombinant monoclonal antibodies to be made economically in a variety of cell types at large scale.

N 114 ImmunoZAP LIBRARIES: A BACTERIOPHAGE TECHNOLOGY FOR THE IDENTIFICATION OF MONOCLONAL ANTIBODIES

Bob Shopes*, Michelle Alting-Mees, Jeff R. Amberg, Daniel Ardourel, Marie Callahan, Jennifer Detrick, Beverly N. Hay*, Holly H. Hogrefe, Alan Greener, Eleanore A. Gross, Marjorie M. Kubitz, Rebecca L. Mullinax, Carl Wilson, Jay M. Short and Joseph A. Sorge
Stratagene Cloning Systems, Suite 200, La Jolla, CA 92037 and *Stratacyte Corp., 11099 North Torrey Pines Road, Suite 400, La Jolla, CA 92037

We have applied the techniques of molecular biology to the identification and expression of monoclonal antibodies. This process utilizes the polymerase chain reaction with primers specific for immunoglobulin genes. These primers allow the cloning of the PCR products into ImmunoZAP™ bacteriophage vectors to produce cDNA libraries consisting of up to 10^7 members. These vectors also allow for for the expression and secretion of active Fab fragments in *E. coli*. This technology may lead to a dramatic improvement in monoclonal antibody screening over the hybridoma based methods currently being used. Advances in cloning human antibodies, screening techniques, vector construction, *in vitro* maturation of clonal affinity and expression of a new types of fragments will be presented.

N 115 MURINE MONOCLONAL ANTIBODIES AND A RECOMBINANT SINGLE CHAIN Fv FRAGMENT SPECIFIC FOR THE EXTRACELLULAR DOMAIN OF THE HUMAN c-erbB-2 PROTEIN: POTENTIAL REAGENTS FOR TARGETED DRUG THERAPY, Winfried Wels, Ina-Maria Harwerth, Bernd Groner and Nancy E. Hynes, Friedrich Miescher-Institut, P.O.Box 2543, CH-4002 Basel, Switzerland

The c-erbB-2 proto-oncogene encodes a transmembrane glycoprotein of 185 kd. This protein has structural homology with the growth factor receptor-tyrosine kinases and is most likely important for the growth or differentiation of specific epithelial cells. Enhanced expression of the gp185 c-erbB-2 protein is found in about 30% of human primary breast and ovarian cancers making this protein an interesting target for directing cytotoxic reagents to these tumors. We have prepared a panel of monoclonal antibodies (MAbs) which specifically react with the extracellular domain of the c-erbB-2 protein. The MAbs are being tested for their effects upon c-erbB-2 receptor internalization and upon anchorage dependent and independent growth of cells expressing elevated c-erbB-2 levels. By reverse transcription of poly-A RNA from hybridoma cells and amplification of cDNA by polymerase chain reaction (PCR) the variable domains of FRP5, one of the MAbs were cloned. A fusion gene coding for a single chain Fv fragment was constructed and used for the expression of a 26 kd Fv in E.coli. The recombinant single chain Fv like the original Mab shows specific binding to the c-erbB-2 protein and to cells expressing high c-erbB-2 levels. Strategies for the construction of chimeric Fv-linked enzymes derived by gene fusion will be discussed.

N 116 SINGLE-CHAIN ANTIGEN BINDING PROTEINS, Marc Whitlow, Genex Corporation, Gaithersburg, Maryland, 20877

Single-Chain Antigen-Binding (SCA™) proteins consist of the V_L and V_H regions of an antibody tethered together by a polypeptide linker. The resulting protein retains the binding specificity and affinity of the original monoclonal antibody (MAb) in a much smaller form.

We have used an anti-fluorescein model system, based on the 4-4-20 MAb, to optimize the production and recovery of SCA proteins. A number of linkers have been designed, based initially on the crystal structure of the MCPC603 Fab found in the Protein Data Bank and more recently on the crystal structure of the 4-4-20 Fab solved in our lab. The affinity and stability in denaturants such as urea and guanidine-HCl has been tested for SCA proteins with different linkers.

Based on the results with anti-fluorescein SCA proteins, we have produced a number of anti-tumor SCA proteins. The *in vitro* affinity and specificity of these anti-tumor SCA proteins is equivalent to the corresponding Fab fragment. In *in vivo* studies, the serum and whole body clearance of the SCA protein in mice was faster than the Fab. However, in spite of the faster clearance rate, the SCA protein showed equivalent localization to a LS-174 human colon carcinoma xenograft grown in athymic mice. Moreover, the kidney showed less non-specific uptake of the SCA proteins than the Fabs.

Recently, we have constructed an SCA protein from a catalytic antibody. In this construction we demonstrated the ease of converting an Fab fragment identified in a combinatorial library in phage lambda as described by Huse *et al.* (Science, 246:1275, 1990) into an SCA protein. The catalytic SCA protein showed the same specificity and turnover number as the Fab fragment.

N 117 ANALYSIS OF SPECIFICITY IN ANTIHUMAN LDH-C4 MONOCLONAL ANTIBODIES AND THE GENETIC EXPRESSION IN ESCHERICHIA COLI, Zhou X.H., Zhao S.Y. and Steven Li. Department of Histology & Embryology, Shanghai Medical University, Shanghai 200032, China, Genetic Research Institute, Fu Dan Univ., Shanghai 200454 China, Laboratory of Genetics, NIEHS, NIH, NC 27709 USA

In the development of contraceptive vaccine we found that it is necessary to screen lines of hybridoma cells secreting antihuman LDH-C4(lactate dehydrogenase isozyme) monoclonal antibodies(McAb). Using N6-5'-AMP-sepharose and DEAE-cellulose column to purify human LDH-C4, fused by conventional method, 3 lines of hybridoma cells secreting antihuman LDH-C4 McAb were obtained. Their characteristics are: 1. The McAbs belong to mice IgG1, 2. Bidimensional immuno-diffusion test and ELISA showed the McAb reacted only specifically with human LDH-C4, 3. Immuno-hemagglutination test can inhibited specifically the agglutination of the McAb with human LDH-C4, 4. By immuno-fluorescent and ABC, PAP technique the McAb produced specific fluorescent or HRP staining on the spermhead and tail. By the application of BA-ELISA test to the LDH-C4 content of 72 human semen samples, it is confirmed that 19% of sterility patients lack LDH-C4, suggesting a Ldh-c gene defect. The human Ldh-c cDNA 300bp and 1.05kb gene fragment were ligased, transformed into E.Coli DH 5.1 strain and the process was repeated in vector PGEM-1, PGP1, then expressed in E.Coli. After breaking the membrane, separation and purification the required LDH-C4 was obtained(as tested by antihuman LDH-C4 McAb).

N 118 IN VITRO CHARACTERIZATION OF HUMANIZED ORTHOCLONE@ OKT3

R. Zivin, A. Collins, V. Pulito, P. Rao, E. Meyer, L. Jolliffe, J. Bluestone+, S. Woodle+, D. Athwal*, M. Bodmer* and J. Adair*.
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ORTHOCLONE@ OKT3, a murine monoclonal antibody (mAb) which recognizes an epitope on human CD-3 (part of the T-Cell receptor complex), has proven effective in the suppression of acute rejection of human renal allografts. Further, as suggested by analogy in a murine model, smaller amounts of this mAb may prove useful as an "immuno-activator", with potential uses as an adjuvant in anti-tumor or anti-viral therapy. To allow the exploitation of these properties while avoiding the human anti-mouse (HAMA) response, we have genetically engrafted the murine CDR-regions onto human frameworks. One of these antibodies, huOKT3-185, has been tested for "activation" ability (IL-2^R induction, release of TNF), "mitogenicity" (induction of DNA synthesis) and the ability to "modulate" CD3 (disappearance of detectable antigen). Interestingly, while the mouse and human antibodies were similar in activation, differences were observed in mitogenicity and modulation. We are currently investigating the role of the Fc region in these effects.

Surface Molecules for Therapeutics; Animal Models; Clinical Trials; Production, Purification, Scale-Up, and Hybridoma Physiology

N 200 PRODUCTION AND CHARACTERIZATION OF A MOUSE/HUMAN CHIMERIC ANTIBODY DIRECTED AGAINST HUMAN NEUROBLASTOMA

H. Amstutz[+], Ch. Rytz[+], I. Novack[°], M. Spycher[+], K. Blaser[*], J.-J. Morgenthaler[+].
[+]Central Laboratory of the Blood Transfusion Service of the Swiss Red Cross, Bern, Switzerland; [°]Paul Scherrer Institute, Villigen, Switzerland, [*]Swiss Institute of Allergy and Asthma Research, Davos, Switzerland

The hybridoma CE7 produces an antibody (γ1/κ) which binds to a 180 kd cell-surface glycoprotein of human neuroblastoma. We have constructed a mouse/human chimeric antibody (chCE7) with cloned CE7 variable and human constant gene segments expressed in SP2/0 cells. We show with a cell binding inhibition assay that the specificity of the chimeric antibody is identical to that of the original CE7 and with a C1q binding assay that chCE7 is functional in activating human complement. We have determined that chCE7 binds to 40'000 sites per neuroblastoma cell with a $K_A = 10^{10}$ M^{-1}. Tissue distribution measurements of ^{125}I-labeled chCE7 injected into nude mice carrying human neuroblastomas show an accumulation of more than 30 % of the injected dose in the tumor after 48 h. Results of tumor imaging experiments of ^{123}I and ^{131} I labeled chCE7 in nude mice are shown.

N 201 LARGE SCALE PURIFICATION OF A THERAPEUTIC MONOCLONAL ANTIBODY.

Gregory S. Blank and David Vetterlein, Recovery Process R & D, Genentech Inc., South San Francisco, CA. 94080.

A murine monoclonal antibody against breast and ovarian cancer was purified from cell culture fluid containing 3% fetal bovine serum at the 2400 L scale. The cell culture fluid was concentrated ~10 fold by ultrafiltration using 100 kDa membranes. The concentrated fluid was adjusted to pH 4.0 and applied to a S-Sepharose column. The MAb was eluted with 1.5 M Glycine, 0.4 M NaCl, pH 8.5. The eluate was brought to 3 M NaCl and applied to a Protein A column. MAb was eluted with 0.01 M Citrate, pH 3.0. Protein A column size was minimized to reduce cost by performing multiple cycles using an automated system. The Protein A pool was brought to 2 M urea and 1.25 M Ammonium sulfate and loaded onto a Phenyl Toyopearl column. Following elution with 0.6 M Ammonium sulfate, the MAb pool was concentrated and buffer exchanged by ultrafiltration/diafiltration. The dialysate was applied to a DEAE Sepharose FF column and step eluted with 0.025 M Tris, 0.1 M NaCl, pH 7.8. Formulation into PBS was done on Sephadex G-25. Total recovery for the process was ~70%. MAb purity by SDS-PAGE was > 99% with < 300 ppm bovine IgG and < 50 ppm Protein A. Over 15 logs viral clearance were achieved throughout the process. Endotoxin levels were less than 0.1 EU/mg.

N 202 MONOCLONAL ANTIBODIES AGAINST Staphylococcus aureus LAMININ RECEPTOR,

Célia Carneiro, Edilberto Postol, Camila Boilesen and Ricardo Brentani, Ludwig Institute for Cancer Research, São Paulo, Brasil.

Bacteria adhesion is the first necessary step for tissue invasion and colonization. This phenomenon is highly selective and is determined by the presence of complementary molecules that promote cell-cell and cell-extracellular matrix (EM) interactions. During the last twelve years, the importance of specific interactions between microorganisms and EM-components has been associated to pathogenicity. Adhesion to laminin, a major component of basement membranes, has been associated to bacterial virulence in some species. Our group has described a 50 kD laminin-receptor on the highly invasive bacteria Staphylococcus aureus, that was not found on S.epidermidis (Science, 229:275, 1985). In order to better address the importance of this receptor on bacteria invasive behavior, we have produced monoclonal antibodies (MAbs). Seventeen hybridomas were obtained from two cell fusion experiments using P3.653 myeloma cells and BALB/c immunized esplenocytes. Among these, twelve clones secrete IgG and five secrete IgM MAbs. The ability of these MAbs to inhibit S.aureus adhesion to laminin is presently being verified. Afterwards, the best MAbs will be evaluated for their biological activity "in vivo". The blockade of S.aureus invasion with a MAb could represent a therapeutic approach for septicemia in the future.

N 203 CONSTRUCTION OF A MONOCLONAL ANTIBODY TO THE OPIATE PEPTIDE DYNORPHIN A.
Caron, D., Goldstein, N., Waksal, S., Department of Immunology, ImClone Systems, Inc., New York, NY 10014.

Dynorphin A (Dyn-A) is a 17 amino acid peptide that binds to the kappa opiate receptor. This molecule has an N-terminal tetrapeptide (YGGF) common to many other opioids including beta-endorphin, beta-lipotrophin, and met-enkephalin.

Recent evidence has implicated Dyn-A as a factor in secondary spinal cord injury (SCI) in animal models. In addition, polyclonal antisera to Dyn-A has been shown to improve the outcome of SCI in rats. In order to study the basis of dynorphin-induced SCI as well as developing potential agents for therapeutic intervention, we have generated monoclonal antibodies which appear to recognize an epitope specific to Dyn-A.

Mice were immunized with a mixture of commercially available Dyn-A peptides which selected for the production of antibodies not recognizing the N-terminal tetrapeptide common to all opioid peptides. Spleens from hyperimmune mice were fused to the murine myeloma NS1. Hybridomas were screened by an indirect solid phase ELISA. The antibody A10.6 has a high avidity for Dyn-A and did not cross-react with Dyn-B, leumorphin, beta-lipotrophin, methionine-enkephalin, leucine-enkephalin, or irrelevant peptides.

Garnier-Osguthorpe-Robson analysis of our library of opioid peptides shows that A10.6 may bind to a "conformational" region distinct to Dyn-A. This epitope appears to be mimicked in alpha-neo-endorphin and beta endorphin.

N 204 CYTOTOXIC LYMPHOCYTE MATURATION FACTOR (CLMF): MONOCLONAL ANTIBODIES SPECIFIC FOR THE 40 kDa SUBUNIT, Richard Chizzonite, Terri Truitt, F. Podlaski, A. Wolitzky, P. Quinn, P. Nunes, A. Stern, and M. Gately, Roche Research Center, Hoffmann-LaRoche Inc., Nutley, N.J. 07110

CLMF is a novel cytokine which synergizes with interleukin-2 to stimulate proliferation of human PHA-activated lymphoblasts and in causing the induction of lymphokine-activated killer cells. CLMF is a 75 kDa heterodimer composed of disulfide bonded 40 kDa and 35 kDa subunits. Monoclonal antibodies prepared against partially purified CLMF immunoprecipitate ^{125}I-labelled CLMF and immunodeplete CLMF bioactivity as determined by the lymphoblast proliferation and the LAK cell induction assays. Western blot analysis demonstrated that each antibody binds to the 75 kDa heterodimer and to the 40 kDa subunit. A CLMF receptor binding assay has been used to evalulate the ability of individual antibodies to inhibit radiolabelled CLMF binding to its cellular receptor on PHA-activated lymphoblasts. Antibodies which inhibit ^{125}I-CLMF binding to its cellular receptor also neutralize CLMF bioactivity. The ability of antibodies specific for the 40 kDa subunit of CLMF to neutralize CLMF bioactivity suggests that determinants on the 40 kDa subunit are necessary for binding to the CLMF receptor on PHA-activated lymphoblasts.

N 205 HIGHLY SPECIFIC ANTI-CATALYTIC MONOCLONAL ANTIBODIES TO THE HUMAN 92kDa GELATINASE ISOLATED FROM THE HT1080 FIBROSARCOMA CELL LINE, Deborah L. French, Ute M. Moll, Karen B. Rosinski, Martha B. Furie, and James P. Quigley, Department of Pathology, SUNY at Stony Brook, Stony Brook, NY 11794-8691.

The catalytic degradation of extracellular matrix and basement membrane is required for the invasive and metastatic ability of neoplastic cells. Metalloproteases, serine proteases, and cathepsins mediate this process with members of the metalloprotease family providing the final catalytic functions of extracellular proteolysis. The 72kDa gelatinase/type IV collagenase has long been implicated in the invasive phenotype of a large number of tumor cells. A 92kDa gelatinase, originally observed in neutrophils, is also elevated in highly aggressive human tumor cells and has recently been implicated in this process. This metalloprotease is secreted as an inactive zymogen and can be activated by the organomercurial, *p*-aminophenylmercuric acetate (APMA), but its natural activator is at present unknown. A method was established to separate the 92kDa gelatinase from the 72kDa enzyme, also secreted from HT1080 cells. This purified preparation of 92kDa gelatinase was used to immunize mice and 1500 hybridomas were screened by ELISA. Fifteen monoclonal antibodies were identified and those that reacted specifically on Western blots with the 92kDa enzyme were purified. Two IgG1 monoclonal antibodies, designated 6-6B and 7-11C, were tested in an enzyme assay in which radiolabeled denatured type I collagen (gelatin) was cleaved by purified 92kDa gelatinase, pre-activated with APMA. The 6-6B monoclonal antibody specifically inhibited the catalytic activity of the activated 92kDa gelatinase while the 7-11C monoclonal antibody had no inhibitory activity. These antibodies have been selectively added to cell culture and organ culture systems and unique effects on the tumorigenic phenotype have been observed.

N 206 PRODUCTION OF A BISPECIFIC ANTIBODY BY LINKAGE OF TWO RECOMBINANT SINGLE CHAIN Fv MOLECULES, Andrew J. T. George, Sarah M. Andrew, Pilar Perez, Peter J. Nicholls[1], James S. Huston[2], and David M. Segal, Experimental Immunology Branch, NCI, and [1]Surgical Neurology Branch, NINDS, National Institutes of Health, Bethesda MD 20892, [2]Creative Biomolecules Inc., 35 South Street, Hopkinton, MA 01748.

Bispecific antibodies may be used to redirect cellular cytotoxicity against target cells carrying the appropriate antigen. One possible approach in the construction of bispecific antibodies is to join two single chain Fv molecules (sFv), which consist of the immunoglobulin heavy and light chain variable domains (V_H and V_L) linked with a synthetic peptide spacer, into single molecule. We have used a series of oligonucleotide primers to amplify by the polymerase chain reaction the cDNA of the gene fragments encoding the Fv regions of the anti-CD3 Mab OKT3 (IgG_{2a}), and of the anti-DNP Mab U7.6 (IgG_1). Suitable restriction sites were introduced to enable forced cloning of V_L and V_H genes from both antibodies into a single cloning vector. An artificial linker of DNA was placed between the sFv constructs such that the 3' end of the anti-DNP was linked to the 5' end of the anti-CD3. This construct has been sequenced and cloned into a bacterial expression vector. A protein of the predicted MW (≈56,200D) has been isolated from the bacterial inclusion bodies, and various expression systems are being used to recover active single-chain bispecific antibodies with the ability to target human cytotoxic T cells against TNP-coated target cells.

N 207 DEVELOPMENT OF AN IMMUNOASSAY FOR EOSINIPHIL CATIONIC PROTEIN (ECP) USING MONOCLONALS CHARACTERIZED IN BY A NOVEL TECHNOLOGY, BIAcore™.

Russ Granzow†,Pirjo Lehtonen§, Karin Nygård§, Helena Butt§, Jenny Nystrand§, Christer Petterson§, †Pharmacia Biosensor NA Piscataway, NJ 08854 and §Pharmacia Diagnostics AB, Uppsala, Sweden

We report the use of a novel system to give significantly more information at an earlier stage in development of a diagnostic immunoassay. This new system uses advances in biosensors, microfluidics and a novel matrix to measure interactions as they occur in unpurified, label-free environments.

Eosinophil cells participate in inflammatory reactions in tissues by releasing several potent mediators upon stimulation with allergen or parasites. It has been suggested that eosinophil secretory products may also be harmful to the affected organs as is the case in bronchial asthma. Therefore it could be helpful to monitor eosinophil derived proteins in asthmatic patients for the optimization of treatment regimes. Firstly, fresh leucocyte buffy coats were used to purify ECP by a series of chromatographic techniques. After a final step to remove contaminating elastase by Zn-chelating Sepharose, a recovery of 2-5 mg of ECP was typically obtained from 100 blood donors.

Monoclonal antibodies were prepared by immunizing mice with 50 ug of purified antigen in adjuvants followed by boosters of 25ug of antigen during 3 consecutive days. Fusion of one spleen was made and supernatants analyzed in ELISA for activity against ECP. Positive reactivity with ECP of 79 clones was identified. Some of these clones were further characterized by BIAcore for their epitope specificity. Antibodies reactive with four different epitopes on ECP were identified. Based on these data four different clones were selected for expansion, purification and iodination. Finally, these labelled antibodies were tested in a sandwich assay together with solid phase coated unlabelled antibodies. We believe these data indicate that by using BIAcore, appropriate selection of monoclonals, suitable for development of sandwich-type immunoassays, can rapidly and efficiently be performed at a stage earlier than previously possible in monoclonal development.

N 208 HIGH AFFINITY HUMAN IgE RECEPTOR (FcεRI): ANALYSIS OF THE FUNCTIONAL DOMAINS OF THE α-SUBUNIT WITH MONOCLONAL ANTIBODIES,

Maureen Griffin, Frank Riske, Bob Pilson, Ping Lin, Waleed Danho, Jarema Kochan, John Hakimi and Richard Chizzonite, Roche Research Center, Hoffmann-LaRoche Inc., Nutley, N.J. 07110

The binding of IgE to the high affinity Fcε receptor on mast cells and basophils is mediated by the α-subunit of the postulated tetrameric receptor complex. The binding sites of antibodies specific for the human α-subunit have been separated into inhibitory and non-inhibitory epitopes. An inhibitory antibody, 15A5, blocks ^{125}I-IgE binding to target cells, immunoprecipitates ^{125}I-labelled α-subunit, induces histamine release from human basophils and binds to a synthetic pepitde corresponding to amino acids 125-140 of the α-subunit. A non-inhibitory antibody, 11B4, does not block ^{125}I-IgE binding to target cells, immunoprecipitates ^{125}I-labelled α-subunit, induces histamine release from human basophils and binds to a synthetic peptide corresponding to amino acids 43-58 of the α-subunit. Based on sequence homologies, the α-subunit is a member of the immunoglobulin superfamily of proteins and has two predicted disulfide bonded domains. These data suggest that the C-terminal domain (amino acids 110-205) of the α-protein may mediate IgE binding while a portion of the N-domain (amino acids 26-109) may not directly participate in IgE binding.

N 209 THREE-DIMENSIONAL STRUCTURES OF Fab FRAGMENTS FROM ANTI-DNA AND ANTI-FLUORESCYL ANTIBODIES: AN EXAMINATION OF HAPTEN-ANTIBODY INTERACTIONS. James N. Herron[1], Xiao-min He[2], Edward W. Voss, Jr.[3] and Allen B. Edmundson[4]; [1]Department of Pharmaceutics, University of Utah, Salt Lake City, UT 84112; George C. Marshall Flight Center, NASA, Huntsville, AL 35812; [3]Department of Microbiology, University of Illinois, Urbana, IL 61801; [4]Harrington Cancer Center, Amarillo, TX 79106.

Crystal structures were determined for the antigen binding fragments (Fabs) of two different murine monoclonal antibodies: (1) BV04-01, an autoantibody directed against single-stranded DNA; and (2) 4-4-20, a high affinity (10^{10} M^{-1}) antibody which binds the fluorescent hapten, fluorescein. The Fabs of these antibodies were remarkably similar in their overall structures, except for the truncation of the third hypervariable loop of the 4-4-20 heavy chain by three amino acids. However, the combining site of the BV04-01 Fab for single stranded DNA was a large groove, while the binding site for fluorescein in the 4-4-20 Fab was a relatively narrow slot. Crystal structures were determined for both the unliganded and liganded forms of BV04-01. Comparision of the two structures indicated that changes in both tertiary and quaternary structure accompanied hapten binding. The following residues were important for the binding of deoxythymidylic acid: tyrosine L32, serine L91, and histidines L27d & L93 from the light chain; and arginine H52, serine H52a, asparagine H53, and tryptophan H100a from the heavy chain. The most predominant interaction involved the intercalation of one thymine base between tryptophan H100a and tyrosine L32. Furthermore, this interaction allowed the formation of two hydrogen bonds between the base and the protein. In the anti-fluorescein antibody, the fluorescein hapten was accommodated by an aromatic slot formed by tyrosine L32, and tryptophans L96 & H33. Furthermore, arginine L34 formed an ion pair with one enolate oxygen atom, and histidine L27d was located within hydrogen bonding distance of the second enolic group.

N 210 TARGET CELL INDUCED T CELL ACTIVATION WITH BI- AND TRISPECIFIC ANTIBODY FRAGMENTS, Gundram Jung, Uwe Freimann, Zofia v.Marschall, Till Lorenz and Wolfgang Wilmanns, Medizinische Klinik III der Universität München, Klinikum Großhadern, D-8000 München 70, Germany

Previously we proposed a concept for tumor immunotherapy in which two different bispecific antibody conjugates, an anti-targetXanti-CD3 and an anti-targetXanti-CD28 conjugate, induce the activation of resting human T cells upon binding to the respective tumor target cells. After in vivo application of those reagents this model of a "target cell induced T cell activation" envisages the destruction of target cells by in situ activated T cells. Obviously however, for in vivo application the use of Fc free antibody fragments is mandatory to prevent binding of the conjugates to Fc receptor bearing cells. Here we report a simplification of published procedures for the generation of bispecific Fab hybrid fragments, univalent for each antigen. We demonstrate that an anti-targetXanti-CD3/anti-targetXanti-CD28 combination of these hybrids, as well as an identical combination of covalently coupled Fab2 fragments, mediate "target cell induced T cell activation" in a simple in vitro test system. Thus those reagents appear suitable for systemic in vivo application within the concept outlined above. A trispecific conjugate with anti-target-, anti-CD3- and anti-CD28 specificity appears to be unsuitable for this purpose because it activates resting T cells without requiring immobilisation through binding via its anti-target portion.

N 211 CARCINOEMBRYONIC ANTIGEN (CEA): PRODUCTION OF SYNGENEIC ANTI-IDIOTYPE MONOCLONAL ANTIBODIES AND DEVELOPMENT OF A QUANTITATIVE ASSAY, J.Daniel Lopes*, Jane Z. de Moraes and Célia R. W. Carneiro, Dept. of Internal Medicine, Faculdade de Medicina USP, Institute of Microbiology Rio de Janeiro and Ludwig Institute for Cancer Research, São Paulo branch. *Av. Dr. Arnaldo, 455 - 3º, 01246, São Paulo, Brazil.

Anti-idiotype antibodies can mimic the three-dimensional conformation of the original antigen, CEA for instance, and act as antigen surrogates for vaccination and/or serological purposes. To investigate this potential ability, BALB/c mice were immunized with the previously described anti-CEA MAb 5.D11, which showed an affinity for the antigen high enough to be used as capture antibody in a two-site quantitative assay. After cell fusion, fifteen stable cloned cell lines secreting anti-Id were established. All MAbs obtained inhibited the binding of labeled CEA to 5.D11 to some extent, up to 100%. Absence of rheumatoid factor activity was demonstrated by adsorption through a mouse IgG column, by using 5.D11 $F(ab')_2$ fragments and by western blots, where anti-Ids recognized only non-reduced 5.D11. Anti- 5.D11 MAbs did not recognize neither 10.B9, another anti-CEA MAb obtained in the same fusion that originated 5.D11, nor several anti-CEA MAbs from an international workshop besides two GOLD 4 MAbs, thus confirming their specificity. When applied to an Id-anti-Id quantitative assay, CEA levels as low as 2 ng/ml could be discriminated, a sensitivity sufficient for clinical use. Whether any or the anti-Id MAbs have the potential to be used as CEA vaccines is currently under investigation.

Supported by PADCT-FINEP and FAPES.

N 212 T-CELL ACTIVATION BY MURINE AND HUMAN-MURINE CHIMERIC ANTI-CD3 MONOCLONAL ANTIBODIES, *Paul Parren, Leonie Boeije, Marlieke Geerts and Lucien Aarden,*
Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Plesmanlaan 125, 1066 CX Amsterdam, The Netherlands.

The CD3 antigen on human T-cells is associated with the T-cell receptor (TCR). Monoclonal antibodies (mab) directed against the TCR-CD3 complex are capable to induce rapid phosphatidyl-inositol turnover, which results in T-cell responsiveness to accessory cell mediated signals. Anti-CD3-induced in vitro T-cell proliferation is isotype dependent, both in the presence or absence of accessory cells (i.e. monocytes). Monocyte-supported T-cell proliferation is dependent on the ability of the antibody to bind a Fc-receptor, resulting in crosslinking of the CD3-TCR complex, and production of cytokines (i.e. IL1). Purified T-cells only respond to anti-CD3 mab of moderate avidity, provided that an additional signal (such as IL1, IL2 or anti-CD28) is present. Antibodies to CD3 of high-avidity are non-inducing under these conditions. However, when heavy chain isotype switch variants of a high avidity anti-CD3 mab were prepared, it turned out that induction of proliferation did occur by the IgE variant. To extend these observations to other (human) isotypes we prepared recombinant anti-CD3 monoclonal antibodies. We cloned the functionally rearranged variable region of the heavy chain of this antibody producing hybridoma into the plasmid pSV_2gpt in the context of the IgH promotor/enhancer and a genomic immunoglobulin heavy chain constant gene coding for murine IgM, IgG3 or human IgM, IgG1, IgG2, IgG3, IgG4, IgE or IgA_2 respectively. These constructs were expressed in a heavy chain loss variant of the murine IgG1 anti-CD3 producing cell line. This set of antibodies was then used to study their ability to induce proliferation of T-cells. In addition to the mIgE, the hIgE and $hIgA_2$ and polymeric (but not the monomeric) mIgM anti-CD3 were able to activate purified T-cells, when combined with IL2 or anti-CD28 in contrast to the other isotypes. hIgM contains only the monomeric form which does not induce proliferation. In monocyte-dependent T-cell activation, responsiveness was found with all human subclasses exept hIgG3. Blocking of FcRgamma revealed that hIgG1 and hIgG4 acted via FcRI in contrast to hIgG2 which, at least partially, acted via FcRII.

N 213 USE OF TRUNCATED COLLAGENASES AND CHIMERIC METALLOPROTEINASE TO CHARACTERIZE MONOCLONAL ANTIBODIES AGAINST PROCOLLAGENASE AND PROSTROMELYSIN, Jui-Lan Su*, J. David Becherer, Christine Edwards* and Gerald McGeehan, Division of Biology, Glaxo Inc. Research Institute, Research Triangle Park, NC 27709

A panel of mouse monoclonal antibodies against human recombinant fibroblast procollagenase and prostromelysin has been generated. Domains containing epitopes within the enzymes have been assigned. Assignment is based on immunoreactivity of antibodies with truncated collagenase fragments, whole pro-enzymes and collysin (a chimeric molecule constructed from fragments of collagenase and stromelysin) on ELISA or Western blot. Out of eight anti-procollagenase antibodies only one crossreacts with prostromelysin, while eight out of ten anti-prostromelysin antibodies crossreact with procollagenase. Based on the immunoreactivity, antibodies may be divided into 4 groups; (1) antibodies that recognize the NH_2-terminal 19k Da collagenase fragment (2) antibodies that recognize COOH-terminal domain of collagenase and stromelysin (3) antibodies that recognize a 25k Da NH_2-terminal collagenase fragment but not the 19k Da NH_2-fragment, including a neutralizing antibody and (4) an antibody that recognizes only the 19k Da NH_2-fragment of stromelysin. With these antibodies as tools, we may be able to purify active and autocatalytic fragments of enzymes and study their pathological roles in rheumatoid arthritis and metastasis.

N 214 THE RELATIVE ADCC AND CDC ACTIVITIES OF MURINE ISOTYPE SWITCH VARIANTS AND THEIR HUMAN IgG1 CHIMERIC ANTIBODIES DEPENDS ON THE NATURE OF THE TUMOR ANTIGEN, Dale E. Yelton, Michael MacLean, Bruce Mixan, Ulrike Stevenson, Karl Erik Hellstrom, and Ingegerd Hellstrom, Bristol Myers Squibb Pharmaceutical Research Institute, Seattle, WA 98121

Antibody dependent cellular cytotoxicity (ADCC) and complement dependent cytotoxicity (CDC) are two in vitro assays commonly used to determine the potential usefulness of monoclonal antibodies (MAb) for tumor therapy. Previous work by ourselves and many others has shown that the activity of a MAb in these assays is a function of the isotype of the antibody. We have undertaken a more thorough evaluation of this issue by deriving a set of isotype switch variants from several murine anti-tumor antigen MAbs. These antibodies were chosen to represent a variety of types of human tumor antigens including glycoprotein, ganglioside and carbohydrate antigens found on both carcinomas and melanomas. In some cases we have also produced the human IgG1 chimerics (human C-region, mouse V-region) from the murine MAb. These sets of antibodies (15 total against 5 antigens) were run in quantitative ADCC and CDC assays to determine the relative specific activities of each antibody on panels of tumor cells expressing different levels of tumor antigen. These results clearly demonstrate that the relative activity of the antibodies in these assays is a function not only of the isotype of the antibody but also of the nature and density of the tumor antigen.

N 215 MONOCLONAL ANTIBODIES IDENTIFYING SURFACE ANTIGENS OF THE EQUINE LYMPHOID CELLS, Chonghui Zhang and Douglas F. Antczak, James A. Baker Institute for Animal Health, Cornell University, Ithaca, NY 14853

A panel of monoclonal antibodies (mAbs) was selected from some 2400 hybridomas raised against equine lymphocyte antigens. Six mAbs (CZ1-CZ6), which precipitate molecules of 44KDa, identify a gene product or products of class I Major Histocompatibility Complex of the horse. Five mAbs (CZ1.2-CZ1.6) are reactive with 75-85% of peripheral blood lymphocytes and recognize distinct determinants expressed mainly on T-lymphocytes, as assessed by immunohistochemical staining of lymphoid tissue sections and by single- or dual-color immunofluorescence flow cytometry. MAbs CZ2.1 and CZ2.2 identify markers (80-90KDa) on B-lymphocytes and the germinal center cells of lymphoid tissues, respectively. Two other mAbs (CZ3.1 and CZ3.2) recognize antigens with subunit molecular weights of 180 and 100KDa, similar to the human lymphocyte functional antigen (LFA-1). In addition, mAb CZ3.3 appears to identify a molecule expressed on macrophages. This panel of mAbs has been used to characterize lymphocytes from horses with immunodeficiencies or leukemias. Studies are in progress to determine if these monoclonal antibody-defined molecules in horses are homologous to the CD antigens designated in humans.

N 216 GENERATION OF MONOCLONAL ANTIBODIES DIRECTED AGAINST ORGAN-SPECIFIC ENDOTHELIAL CELL SURFACE DETERMINANTS, Duzhang Zhu, Bendicht U. Pauli, Cancer Cell Biology Laboratories, Department of Pathology, Cornell University College of Veterinary Medicine, Ithaca, NY 14853

Monoclonal antibodies (mAbs) are generated against organ-specific determinants expressed on the lumenal membranes of microvascular endothelial cells. Since these molecules are unstable in vitro, they must be induced by growing unspecific endothelial cells [e.g., bovine aortic endothelial cells (BAEC) on the extracellular matrix derived from the desired organ. Immunization is accomplished with outside-out membrane vesicles prepared by incubating monolayers of BAEC grown on lung-derived matrix with a solution of 100 mM paraformaldehyde, 2 mM dithiothreitol in serum-free medium, or after passive immunization with antiserum directed against membrane vesicles from BAEC grown on plastic. A specific mAb, termed 6D3, exemplifies the usefulness of the described culture system. MAb 6D3, recognizes a determinant on the endothelia of postcapillary venules and small veins in bovine lungs and of mouse lung capillaries. Using the assay system that served to generate the endothelial membrane vesicles as a tumor cell-endothelial cell attachment assay, the molecule that is recognize by 6D3 is identified as an adhesion molecule for lung-metastatic melanoma cells. The described system is useful in the molecular characterization of heterotypic cell interactions with organ-specific endothelium. Supported by NIH grant CA 47668.

N 217 THE PRODUCTION OF SECOND GENERATION ANTI-CANCER ANTIBODIES BY IMMUNISATION WITH SYNTHETIC PEPTIDES, Ian F.C. McKenzie and Pei-xiang Xing,Research Centre for Cancer and Transplantation, Department of Pathology, University of Melbourne, Grattan Street, Parkville, Melbourne, Victoria, 3052, Australia.

Mucins are produced in great abundance in solid tumours - particularly of breast, colon and pancreas. These are highly immunogenic and accordingly, many Mabs to these tumours - produced by immunising with solid tumours or their extracts, react with mucins and can be subdivided into a predominant reaction with either carbohydrate or protein. Recent major advances have been made with the isolation of cDNA clones encoding the protein core of MUC1 (breast) and MUC2 and 3 (colon) mucins. These share a common feature of containing VNTRs (variable number of tandem repeats) which have 60bp, 69bp and 51bp respectively giving rise to 20-23 and 17 amino acid repeats. We know that for MUC1, many antibodies react with the protein core; indeed within the amino acids APDTRPA, Mabs can react with at least 4 different epitopes (eg APDTR, PDTR, DTR, TRP). Of real interest is the finding of Dr Olivera Finn (Duke University) that these antibodies will block the cytotoxic activity of human T cell clones with human tumours. Using this information and the sequences for MUC2 and 3 we have now produced synthetic anti-peptide antibodies to MUC-1 and MUC-2 peptides. The anti-breast peptide antibody (MUC1) gives a similar tissue reaction to antibodies made against whole tumours - the antibodies are not breast specific but react with colon and pancreas (as do anti-mucin antibodies). It remains to be seen whether these second generation antibodies are more valuable as diagnostic or therapeutic reagents than other antimucin antibodies. Nonetheless, anti-peptide antibodies are now being examined as dAb and Fv antibodies and as "humanised" versions of the original antibodies. By contrast the anti-MUC2 antibodies (to colon derived peptides) appear to be highly specific for colon tissue and give strong reaction with cancers of the colon. They could well be superior reagents for the diagnosis and treatment of colon cancer.

Regulatory Toxicology and Environmental Concerns; In Vivo Diagnostics and Ligand-Isotopes; Immunogenicity; Binding Sites

N 300 T CELL ACTIVATION BY BISPECIFIC MONOCLONAL ANTIBODIES FOR IMMUNOTHERAPY OF CANCER: FUNDAMENTAL, PRACTICAL AND CLINICAL ASPECTS. Reinder L.H. Bolhuis Department of Immunology, Daniel den Hoed Cancer Center, PO Box 5201, 3008 AE Rotterdam, The Netherlands. Under physiological conditions T lymphocytes become activated when their T cell receptor (TCR) binds to antigen/MHC complex on another cell, resulting in multiple link between them. This interaction also involves costimulatory molecules on lymfocytes such as LFA-1. For cytotoxic T lymphocytes (CTL) the result is delivery of a lethel hit to its conjugated target cell. Lymphocyte activation and triggering for cytolysis can also be induced by bispecific monoclonal antibodies (bs-mAb) which recognize lymphocyte and target cell structures, respectively and bridge these cells. Such bs-mAb therefore retarget the specificity of CTLs, which is MHC-unrestricted. Our investigations revealed that 1) the combined use of bs-mAb preparations, comprising both, CD3 and LFA-1 costimulator specificities, induce effective lysis of (also ICAM$^-$) tumor cells. ICAM$^-$ tumor cells may escape immunedestruction; 2) tumor cells which do not express the tumor associated antigen (TAA) recognized by the bs-mAb may still be lysed: bystander killing; 3) bs-mAb retargeted lymphocytes become readily inactivated upon interaction with ovarian cancer (OVA-CA) tumor cells; 4) the lymfocyte inactivation process is not due to antibody binding site occupency by soluble TAA nor to shedding of the bs-mAb from the surface of lymphocytes. 5) bs-mAb retargeted lymphocytes can enter multiple lytic cycles provided and become retargeted with readded bs-mAb. The use of bs-mAb allows the biochemical analysis of lymphocyte TCR/CD3 complexes which have been engaged in signal transduction versus "naive" TCR/CD3 molecules. Our findings also have redirected the design of the immunotherapy protocol involving the treatment of ovarium carcinoma patients which in vitro expanded and activated lymphocytes retargeted with bs-mAb.

N 301 FETAL MODELS FOR HUMAN DISEASES AND IMMUNOGENECITY: Godfrey Caesar, 209 West, 137 th. Street, New York, N.Y. 10030.

Since it has been established that certain immune mechanisms can be influenced when they are not fully developed as they are in the mature animal, I published the idea in 1979 and 1980, that embryonic cells can be used to cross immunological barriers.
I confirmed this hypothesis in a 1981 publication, by conferring tolerance of embryonic rabbits upon genetically mature mice.
McCune et al who were able to convert SCID mice to SCID-hu mice with human fetal liver, human fetal thymus, and human fetal lymph node cells support this idea, 1988.
Although the preparation of fetal cells vary , they seem to be the constant in transplantation, as an alternative to immunosuppressing drugs, and also at least in attempting to correct some diseases, such as diabetes.

N 302 FUNCTIONAL EFFECTS OF MONOCLONAL ANTIBODIES ON BLOOD COAGULATION AND FIBRINOLYTIC REACTIONS, William R. Church, Kenneth G. Mann, Terri L. Messier and Laurie A. Ouellette, Department of Biochemistry, University of Vermont, Burlington, VT 05405.
The hematologic processes of blood coagulation and clot lysis require both soluble proteins and either a phospholipid or fibrin surface. Monoclonal antibodies have been shown to be specific, high affinity, reversible inhibitors of clot formation. Inhibitory antibodies to the protein components of the prothrombinase complex have been identified. These antibodies suppress formation of thrombin by 1) preventing the factor Xa-factor Va interaction on phospholipid surfaces, by 2) inhibiting the interaction between the substrate (prothrombin) and the proteolytic enzyme (factor Xa) or, by 3) interfering with the prothrombin-factor Va interaction. Antibodies have also been identified that inhibit the intrinsic and extrinsic factor X activation pathways. The effectiveness of an antibody to block fibrin formation is increased by the potential of a single antibody to inhibit both zymogen activation and the biological activity of activated coagulant protein. By labeling the activated enzyme species at the active site histidine using peptidyl chloromethylketones, monoclonal antibodies have proven useful in active site-specific immunoassays quantitating levels of activated coagulation and fibrinolytic enzymes. Several monoclonal antibodies to the protein components of fibrinolysis including plasminogen, tissue plasminogen activator, and fibrinogen also have been shown to inhibit their respective macromolecular reactions. In addition to defining functionally important protein topographical sites, monoclonal antibodies are thus capable of altering and quantitating the reaction products of the multi-component processes of coagulation and fibrinolysis.

N 303 **ANTI-CORE LPS MAbs BLOCK LPS INDUCED CYTOKINE RELEASE BY MURINE PERITONEAL MACROPHAGES**, Franco E. Di Padova, Hans P. Kocher, Robin Barclay*, Hermann Gram, Max H. Schreier
Preclinical Research, Sandoz Pharma, Basel, Switzerland, *SNBTS, Edinburgh, Scotland

LPS induces murine peritoneal cells to release IL-6 and IL-1. Different Anti-LPS MAbs recognizing public or private epitopes on the LPS core have been tested for their ability to block the release of IL-1 and IL-6 by murine peritoneal cells stimulated by smooth or rough LPS. IL-1 and IL-6 were measured by biological assays. IL-1 was evaluated by measuring IL-2 release by the LBRM-35-1A5 cell line. IL-6 was measured in a proliferation assay using the IL-6 dependent cell line B13-29. Mabs recognizing both private and public epitopes were able to inhibit IL-1 and IL-6 release. A good correlation was found between crossreactivity of the Mabs to different LPS's in ELISA and crossprotection in the cytokine release assay. These data indicate the biological role of anti-core LPS MAbs.

N 304 HUMAN MONOCLONAL ANTIBODIES REACTING WITH THE MAJOR GANGLIOSIDES OF HUMAN MELANOMAS, Josef Endl, Herbert Jungfer, Winfried H.W. Albert and Michael Brandt
Boehringer Mannheim GmbH, Werk Penzberg, Nonnenwald 2, D-8122 Penzberg
A series of human monoclonal antibodies (H-MABs) against melanoma associated antigens was produced by Epstein-Barr-Virus (EBV)-transformation of human B-cells. The antibodies were screened on melanoma tissue sections by a special immunoperoxidase assay system. Lymphoblastoid cell lines selectively reactive with melanoma were cloned on a single cell level. The H-MABs reacted with more than 90 % of nevi, primary melanomas and metastases tested but not with melanocytes. In addition the H-MABs stained about 50 % of breast carcinomas. Cross reactivity to normal human tissue was intensively investigated. The only reactivities found were to collecting ducts of liver and pancreas as well as to a minority of cells in pituitary and testis. Fine specificity analysis of the target antigens of these H-MABs revealed that they recognized gangliosides. They mainly stained GM3 and GD3 in immunoblots; weak staining was detected with GD1B. As GM3 and GD3 are the prominently expressed gangliosides in primary and metastatic melanomas these H-MABs may have potential for cancer therapy.

N 305 ENHANCEMENT OF THE ADCC OF HUMAN LYMPHOCYTES WITH rIL-2 AND rIFN-α. Annemarie Hekman, Wim M.J. Vuist, Marjan Visseren, Myrthe Otsen,
Florry Vyth- Dreese and Cornelis J.M. Melief. Division of Immunology, The Netherlands Cancer Institute, Amsterdam, The Netherlands.
Antibody dependent cellular cytotoxicity (ADCC) is regarded as an important mechanism by which monoclonal antibodies (mAb) may exert an anti-tumor effect *in vivo*. Conceivably, the therapeutic efficacy of mAb can be enhanced by cytokines that enhance the ADCC activity of human effector cells. $CD3^-$, $CD56^+$, $CD16^+$ natural killer (NK) cells probably represent the major ADCC effector cell population in man. We investigated *in vitro* the effects of recombinant interleukin 2 (rIL-2) and recombinant interferon-α (rIFNα), alone or in combination, on the antibody-dependent and antibody-independent cytotoxic activity of human peripheral blood lymphocytes (PBL). ADCC was determined using murine mAbs against human B cell lines. Both rIL-2 and rIFNα enhanced the ADCC activity of the human effector cells. However, the effect of the two cytokines on the ADCC activity differed with respect to kinetics and dose-response relation. rIL-2 and IFNα in combination enhanced the ADCC activity of the PBL to a higher level than each cytokine alone. ADCC was mediated by the Fc receptor III (CD16), as indicated by the inhibition of the reaction with anti-CD16 mAbs. However, increased ADCC activity was not correlated with increased CD16 expression or an increased number of $CD16^+$ cells. These results suggest that rIL-2 and/or IFNα may be used to enhance the effect of mAb treatment.

N 306 SYNERGISTIC INHIBITION OF LYMPHOID TUMOR GROWTH IN VITRO BY COMBINED TREATMENT WITH THE IRON CHELATOR DEFEROXAMINE AND AN IMMUNOGLOBULIN G MONOCLONAL ANTIBODY AGAINST THE TRANSFERRIN RECEPTOR, John D. Kemp, Kevin M. Smith, Lawrence J. Kanner, Francisco Gomez, John A. Thorson, and Paul W. Naumann, Departments of Pathology and Microbiology, University of Iowa College of Medicine, Iowa City, IA 52242. The growth of 5 out of 5 murine lymphoid tumors can be inhibited in a synergistic fashion *in vitro* by combined treatment with the iron chelator deferoxamine (DFO) and an IgG monoclonal anti-transferrin receptor antibody (ATRA). Flow cytometric studies further support the view that IgG ATRAS impair transferrin receptor (TR) function by causing TR down-modulation and degradation, even when DFO acts to promote increased cell surface TR expression. Moreover, an IgG ATRA is nearly as effective as an IgM ATRA in inhibiting tumor cell growth when used in combination with DFO. Finally, studies with the iron chelator picolinic acid show that it produces only additive, or very slightly supra-additive, effects when used in combination with the ATRA. These studies therefore make the following new points: (1) the clinically familiar iron chelator deferoxamine, but not all iron chelators, produces synergistic inhibition of tumor growth in vitro with ATRAS; and (2) IgG ATRAS, which may be clinically more attractive reagents than IgA or IgM ATRAS because of better access to extra vascular tissue spaces, have unexpectedly been found to function as powerful growth inhibitors when used in combination with DFO. Combination chelator/ATRA therapy warrants further investigation as a tool in the therapy of hematopoietic malignancies.

N 307 AGGLUTINATING MONOCLONAL ANTIBODIES AGAINST SALMONELLA AND THEIR USE IN SEROTYPING, N.Mohan, R.Kumar, R.Thakur, S.Garg, B.D.Bright and M.K.Bhan, Deptts. of Microbiology and Pediatrics, All India Institute of Medical Sciences, New Delhi-110029, India.

The purpose of this study is to produce monoclonal antibodies (MAbs) against Salmonella and to evaluate their utility in serotyping. BALB/c mice were immunised with either purified antigens or with killed bacteria. Fusions resulted in five hybridomas secreting MAbs which are designated as MK 2, MK 11, MK 20, MK 32 and MK 44 recognizing Vi, O-9, H-d, H-b and H-a antigens of Salmonella respectively. MK 2 and MK 11 are of IgM, MK 20 and MK 44 are of IgG1, and MK 32 is of IgG2b immunogloubulin class. To study the potential application of these MAbs, they were used in slide agglutination tests with a number of commonly encountered Salmonella strains and other closely related bacteria (Table). These results show the selective

Bacteria ↓	MK2	MK11	MK 20	MK 32	MK 44
with specific antigen	21/21	40/40	37/37	9/9	17/17
without specific antigen	0/33	0/40	0/35	0/32	0/30

reactivity of MAbs. Thus, the MAbs produced in this study would be better reagents for serological identification of Salmonella than the conventional polyclonal antisera because of their specificity, low cost and availability.

N 308 MONOCLONAL ANTIBODIES DETECTING INFECTIOUS HEMATOPOIETIC NECROSIS VIRUS, A RHABDOVIRUS OF SALMONID FISH.
Sandra S. Ristow and Jeanene Arnzen de Avila, Department of Animal Sciences, Washington State University, Pullman, WA 99164-6332.

Infectious hematopoietic necrosis virus (IHNV) is a major pathogen of salmonid fish in aquaculture, causing roughly $5 million in damages per year in the Pacific Northwest alone. Currently there are no licensed vaccines for IHNV or quick diagnostic tests available to distinguish this pathogen from other viral diseases important to the industry. We have produced a number of monoclonal antibodies to the nucleoprotein and glycoprotein of the virus which may be used in simple diagnostic tests to detect the pathogen. One of the anti-nucleoprotein antibodies, when conjugated to fluorescein, can detect IHNV in tissue cultured cells 16 hours after infection with an homogenate, replacing the currently used 10 day plaque assay for the virus. An antibody has been produced which distinguishes electrophoretic type 2 IHNV from the other four types. Another monoclonal antibody detects an epitope of the nucleoprotein of Coleman virus, a virus first isolated from chinook salmon (*Oncorhynchus tshawytscha*) in California. Utilizing the 27 antibodies comprising the library, it was be shown that a number of changes have occured in the glycoproteins and nucleoproteins of the virus in the Pacific Northwest over the past ten years.

N 309 **THE HER-2 ENCODED p185 RECEPTOR TYROSINE KINASE AS A TARGET FOR ANTIBODY-MEDIATED THERAPY OF HUMAN CANCER,**

H. Michael Shepard, Dept. of Developmental Biology, Genentech, Inc., So. San Francisco, CA. 94080.

The amplification and/or overexpression of the HER2 protooncogene has been associated with agressive breast, ovarian and lung adenocarcinoma. In addition, other work has shown that the overexpression of this gene can result in morphological transformation, growth in soft agar and tumor formation in nude mice. In addition, HER2 overexpression leads to increased tumor cell resistance to macrophages and TNF-a, a cytotoxic molecule important to macrophage-induced tumor cell cytotoxicity. These results support the role of p185-HER2 in tumorigenesis. We have derived a family of monoclonal antibodies in an effort to find effective antagonists of the receptor. The 4D5 monoclonal antibody has been shown to bind specifically to p185-HER2, to inhibit the growth of overexpressing tumor cells, and to compete with ligand for binding to the receptor. These results support the importance of the p185-HER2 protooncogene in human tumorigenesis, and the potential utility of the 4D5 monoclonal antibody as a therapeutic. Further data will be presented which describe the engineering of the murine 4D5, including some of the unique properties of the engineered antibody.

N 310 *Abstract Withdrawn*

N 311 PRODUCTION AND USE OF MONOCLONAL- AND ANTI-IDIOTYPE- ANTIBODIES FOR BARLEY RESEARCH, Gert van Duijn, Wilma Donker-Koopman, Rob Derksen, Simone van der Veen, Freek Heidekamp and Betty E. Valk, Department of Molecular Plant Biotechnology, TNO, P.O. Box 360, 3700 AJ Zeist, The Netherlands.

In our research concerning lipid metabolism in germinating barley, lipoxygenase is one of the enzymes under investigation. For the discrimination of different barley lipoxygenases, various monoclonal antibodies have been generated which distinguish at least two lipoxygenase isoenzymes in an ELISA system as well as in Western blotting experiments.

In behalf of the study concerning the secretion of hydrolases from barley aleurone, three types of monoclonal antibodies towards 1-3,1-4 -beta-glucanase are available. The first two types of MAbs recognize specifically glucanase isoform 1 and 2 respectively, both in ELISA and Western blottings. The third type of antibodies is cross-reactive with both glucanases in similar tests.

Within the scope of our research on hormonal regulation of gene expression we are interested in the cellular recognition and signal transduction mediated by abscisic acid (ABA). For identifying an abscisic acid receptor in barley, anti-idiotype antibodies will be produced. As a first step, MAbs towards carrier-coupled ABA have been generated. Three different monoclonal antibodies which recognize ABA in free form are available now.

N 312 **ANTIBODIES ENHANCING IN VIVO LEVELS OF IL-3.** Amanda Jones and Hermann J. Ziltener, The Biomedical Research Centre, 2222 Health Sciences Mall, UBC, Vancouver, British Columbia, V6T 1W5, Canada

The clinical utility of cytokines in disease states is restricted by their short biological half-lives, ranging from minutes to several hours. The short half lives necessitate continuous intravenous infusion for maximum therapeutic effect, with consequent morbidity and restriction of patient activity. Antibodies to bacterial exotoxins and to the cardiac glycoside digoxin have long been used in clinical practice with apparent safety and efficacy, however these antibodies have always been used in the conventional sense,that is to antagonize the actions of their target molecule and to enhance elimination. We have attempted to derive antibodies which may specifically enhance the actions of their target cytokine, based on knowledge of the structure and receptor binding site of the cytokine. We have selected antibodies based on their ability to augment the molecular weight of a particular cytokine and hence reduce its glomerular filtration rate. In addition, antibodies selected must show little or no neutralization of cytokine bioactivity in vitro. This approach has been particularly successful using rabbit antipeptide antibodies raised against residues 1-29 of murine IL-3. This antibody has some neutralizing properties but it is not a competitive antagonist of binding to the IL-3 receptor. In addition, receptor-sandwich assays using receptor bearing R6X cells indicate that rabbit anti-1-29 (IL-3) antibody binds away from the IL-3 receptor binding site. Anti-1-29 (IL-3) antibody equilibrated for 30 minutes at room temperature with IL-3 was tested by passage over a sephadex G100 column. Anti-1-29 (IL-3) antibody caused an apparent shift in the molecular weight of IL-3 and IL-3 bioactivity co-eluted together with anti-1-29 (IL-3) antibody. Anti-1-29 (IL-3) antibody has been used in mice in some preliminary studies of IL-3 clearance in vivo. Co-administration of rabbit anti-1-29 (IL-3) antibody together with 1000 units of IL-3 resulted in substantial inhibition of IL-3 clearance. The calculated $t_{1/2}$ for IL-3 clearance in vivo was 10 minutes. The half lives for IL-3 together with antibody were approximately 10 minutes for the α phase and 3 hours for the β phase. Knowledge of the structure of cytokines allows antibodies to be developed against sequences which are not involved in receptor binding. If these antibodies are of sufficiently high affinity they are likely to have enhancing effects in vivo. Such enhancing antibodies may prove therapeutically useful and may extend our knowledge of the role of cytokines in disease.

Effector Mechanisms; Anti-ID; Cancer; Viral Disease

N 400 RADIOLABELED ANTIBODY TARGETING AND THERAPY OF THE HER-2/neu ONCOPROTEIN.
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The HER-2/neu oncogene encodes a 185 Kd transmembrane glycoprotein which is over-expressed in 25 - 30% of breast and ovarian neoplasms, and portends a poor prognosis. Monoclonal antibodies (MoAbs) 4D5 and 7C2 recognize distinct epitopes on the extracellular domain of the HER-2/neu protein. Utilizing a murine model we have studied the feasibility of targeting this oncogene product with radioiodinated anti-HER-2/neu MoAbs. Beige/nude mice bearing subcutaneous NIH3T3 HER-2/neu tumor grafts were injected with 10 mcg of I-131 labeled 4D5, along with an I-125 labeled isotype matched irrelevant control antibody (DA4-4). Specific localization of MoAb 4D5 was seen with tumor : normal organ ratios of radioactivity as high as 20 : 1. The % injected dose of radioactivity per gram of tumor peaked at 26% 24 hours post-injection and decreased to 6% at 120 hours, presumably a result of intratumoral antibody catabolism. Radioimmunotherapy was also performed by injecting tumor bearing mice with 700 uCi of I-131 4D5. Tumor growth curves were markedly delayed in these animals when compared to control groups given unconjugated 4D5, or PBS. Mice treated with an equivalent dose of I-131 conjugated to DA4-4 also demonstrated tumor growth delay, although the degree of inhibition was not as profound as the I-131 4D5 treated group. Furthermore, all mice treated with I-131 4D5 are currently surviving greater than 4 weeks while 60% of the I-131 DA4-4 treated animals have died. None of the mice treated with unconjugated 4D5 or PBS have survived. Tumors were readily visualized by radioimmunoscintigraphy in I-131 4D5 treated animals with greatest resolution occurring 3 - 6 days post-injection. We conclude that radiolabeled antibody targeting of the HER-2/neu oncoprotein is feasible in a pre-clinical model and may represent a promising approach to the diagnosis and treatment of human tumors showing high expression of this antigen.

N 401 RE-SHAPING OF HUMAN ANTIBODIES FOR THE TREATMENT OF INFECTIOUS DISEASES, William J. Harris, Philip R. Tempest, Department of Molecular and Cell Biology, University of Aberdeen, Marischal College, Aberdeen AB9 1AS, Scotland, U.K.

It is now accepted that monoclonal antibodies (MABS) for in vivo use should be preferably of human origin. Recombinant DNA technology can be applied to re-shape human MABS with a desired specificity by CDR grafting. In one product, a human IgG1 MAB was re-shaped with neutralising activity against respiratory syncytial virus (RSV). While the initial re-shaped anti-RSV had lost binding affinity an additional amino acid substitution restored binding affinity. This product shows broad cross reactivity with a range of human clinical isolates, neutralises the virus in vivo and both protects and cures mice of infection by this major childhood infection. This data will be described as well as additional research to re-shape human antibodies which neutralise human cytomegalovirus and Pseudomonas aeruginosa.
Our data demonstrates that re-shaping of human antibodies by protein engineering with murine MAB's is an effective route to provision of human therapeutics.

N 402 ANTIBODY INDUCTION AND TOLERABILITY OF A NEW INACTIVATED HEPATITIS A VACCINE - A COMPARISON OF 2 IMMUNIZATION SCHEDULES
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[1]Universitätsklinik Mainz, [2]Landesinstitut für Tropenmedizin, Berlin, FRG, [3]SmithKline Beecham, München, [4]Bernhard-Nocht-Institut, Hamburg, [5]Krankenanstalten "Bergmannsheil", Bochum, [6]Kliniken der Med. Hochschule Hannover, [7]SmithKline Beecham Biologicals, Rixensart
A new inactivated hepatitis A vaccine (HM 175 Strain, 360 El-U, absorbed onto $Al(OH)_3$; SmithKline Beecham Biologicals) was investigated in a randomized, prospective, multicenter trial in healthy adult volunteers for immunogenicity and reactogenicity. Two vaccination schedules (month 0, 1; booster month 6 and month 0, 1, 2; booster month 12) were compared. Immunogenicity was evaluated by ELISA inhibiton assay, reactogenicity by symptom assessment sheets. Laboratory tests including liver enzymes and medical follow-up were performed. Seroconversion rates (%) and GMT's (mIU/ml) were as follows:

		day 15	Mo 1	Mo 2	Mo 3	Mo 6
Group I	%	47,2	75,0	100,0	100,0	100,0
	GMTs	163,4	140,9	327,7	325,3	217,6
Group II	%	38,5	70,0	89,6	93,6	97,1
	GMTs	205,1	205,7	360,7	1029,8	511,2

The vaccine was well tolerated and no serious adverse events occured. These data suggest that already 2 vaccine injections induce adequate immune response.

N 403 HUMAN PBL RETARGETING BY BISPECIFIC ANTIBODIES: PRECLINICAL DATA AND PHASE I CLINICAL TRIAL. Delia Mezzanzanica on behalf of the International Group for Ovarian Carcinoma Therapy. Experimental Oncology E, Istituto Nazionale Tumori, Milan, Italy.

We analyzed the ability of bispecific monoclonal antibodies (bi-MAbs), directed against the CD3 molecule on T cells and against a glycoprotein associated to human ovarian carcinomas (OvCa), to retarget human activated PBL in vivo. Highly purified bispecific $F(ab')_2$ were tested in nu/nu mice bearing an human OvCa line growing as ascite (OVCAR-3). The mice were treated with bi-MAb-coated PBL 4 days after tumor injection. The experiments were evaluated either as ability to block the ascite formation or as long term survival. After 15 days about 75% of treated mice were protected from tumor growth, whereas the protection in the control groups was below 20%. In the long term survival experiments a 2-fold increase in the mean survival time was observed: 50 days in the control groups, more than 100 days in the treated mice. The bi-Mabs were then used for a phase I clinical trial on OvCa patients who failed chemotherapy. The patients received 2 cycles of treatment, the first one with 4 escalating doses (daily) of bi-MAb-coated PBL from 10^6 cells/1μg Ab to 10^9 cells/1mg Ab in the presence of a low dose of r-IL2; the second one with 5 maximal doses (daily). At the moment 4 patients entered the trial. The treatment did not present any major side effect but gave rise to a high human anti-murine antibody response.

N 404 QUANTITATIVE ANALYSIS OF THE REDISTRIBUTION OF DESIPRAMINE IN RATS BY ADMINISTRATION OF A DESIPRAMINE-SPECIFIC MONOCLONAL ANTIBODY: EVALUATION OF THE AFFINITY CONSTANT FROM *IN VIVO* STUDIES. Susan M. Pond, Paul R. Pentel, Daniel E. Keyler and Donald J Winzor, Departments of Medicine and Biochemistry, University of Queensland, Princess Alexandra Hospital, Brisbane, Australia, 4102 and Department of Medicine, Hennepin County Medical Center, Minneapolis, MN, 55415

Although there are many *in vitro* procedures for determining the affinity of antibodies for antigen (K_{ab}), the determination of K_{ab} from *in vivo* data has been given little attention. We have derived quantitative expressions to calculate the *in vivo* K_{ab} by analyzing the redistribution of antigen resulting from administration of the antibody. The expressions were used to predict the general efficacy of immunotherapy as a treatment of drug overdose, using digoxin and the tricyclic antidepressant (TCA), desipramine (DMI), as examples. Then, K_{ab} for the interaction of subtoxic doses of DMI with a specific anti-TCA monoclonal antibody was calculated from plasma [DMI] measured in rats given a single intravenous dose of DMI (0.0004 - 4 mg/kg), before and after the administration of antibody (molar ratio of anti-TCA binding sites to DMI, 0.004 - 210). K_{ab} obtained *in vivo* (4.7 μM^{-1}) was two orders of magnitude below the values of 0.3 nM^{-1} and 0.15 nM^{-1} determined by two independent *in vitro* methods. This value for *in vivo* K_{ab} was supported by results from rats given 30 mg/kg DMI and anti-TCA (ratio of binding sites to DMI, 0.55), in which unbound plasma [DMI], total plasma [DMI], and plasma [anti-TCA] were measured and used to determine K_{ab}. The K_{ab} for this group, 0.48 ± 0.22 μM^{-1}, was even lower than for the first. The difference between the *in vivo* and *in vitro* estimates of K_{ab} probably reflect endogenous ligands which bind to anti-TCA.

N 405 CONSTRUCTION OF HUMANIZED ANTIBODIES AND TESTING IN PRIMATES, Cary Queen, Man Sung Co, Marguerite Deschamps, *Richard Whitley, †William Benjamin and †John Hakimi, Protein Design Labs Inc., 2375 Garcia Ave., Mountain View, CA 94043, *Department of Pediatrics, University of Alabama at Birmingham, Birmingham, AL 35294 & †Department of Immunopharmacology, Hoffmann-La Roche Inc., 340 Kingsland St., Nutley, NJ 07110

A humanized antibody is a genetic combination of the complementarity determining regions (CDRs) from a mouse antibody with the variable region framework and constant regions from a human antibody. Humanized antibodies are expected to be less immunogenic when used in human patients than mouse or even chimeric antibodies, but high-affinity humanized antibodies are difficult to construct. Recently, we have humanized the anti-Tac antibody, which binds to the human IL-2 receptor (Proc. Nat. Acad. Sci. USA 86: 10029, 1989). Computer modeling was used to identify several framework residues in anti-Tac that are likely to interact with the CDRs. These amino acids were retained in the humanized antibody to preserve high affinity. Now we have extended these methods to produce several other humanized antibodies, all of which retain high binding affinity. For example, we constructed two humanized, strongly neutralizing antibodies against herpes simplex virus (HSV). As the original mouse antibodies are effective against HSV in animal models, the humanized antibodies may be useful therapeutically.

In addition, we have injected both murine anti-Tac and humanized anti-Tac into cynomolgus monkeys. Even a single dose of mouse anti-Tac was strongly immunogenic, but no antibody response against a single dose of humanized anti-Tac was detectable. After multiple doses, humanized anti-Tac did elicit some antibody response, but much less than mouse anti-Tac.

N 406 BIOSYNTHETICALLY DERIVED-IgG OLIGOMER WITH ENHANCED IN VIVO EFFICACY, Howard V. Raff, Walt Shuford, James Esselstyn, William Finley, Linda J. Harris, Departments of Immune Sciences and Molecular Immunology, Oncogen, Bristol-Myers Squibb Pharmaceutical Research Institute, Seattle, WA 98121

An IgG_1 antibody homodimer (oligomer) was isolated from a transfectoma that produced human monoclonal antibody (MAb) targeted against group B Streptococci, an important neonatal bacterial pathogen. Compared to the IgG1 monomer, the oligomer had 100-fold greater antigen binding and opsonophagocytic activity, and significantly enhanced (10-fold) in vivo protective efficacy. Further, the oligomer crossed the placenta of pregnant rat dams, and remained physically intact after 3 weeks of in vivo circulation. Immunochemical analyses revealed that 1) the transfectoma produced normal (L_n) and aberrant (L_{37}) light chains of 25 and 37 kD, respectively, 2) by non-reduced SDS-PAGE, the oligomer disassocciated and separated into three bands ($H+2L_{37}$, $H+L_{37}+L_n$, and $H+2L_n$), and 3) using 0.5 M Urea-PAGE the oligomer resolved into discrete monomer, dimer, and trimer bands. In the accompanying abstract (Harris, *et al*) is described the fine structure of the IgG homodimer that may result in oligomerization. Our results suggest biosynthetically derived, human IgG oligomers possess functional advantages drawn from both IgG and IgM antibodies, and may offer the potential for improving the efficacy of other IgG MAbs.

N 407 **RAPID DIAGNOSIS OF OCCULT ABSCESSES USING ^{99m}Tc-DIRECT LABELED ANTIBODY,** Buck A. Rhodes, RhoMed Inc., 1020 Tijeras NE, Albuquerque, NM 87106

Acute infections, such as appendicitis and occult infections in AIDS patients, can be diagnosed within two hours by gamma scintigraphy after i.v. administration of ^{99m}Tc labeled antibodies reactive with human granulocytes. The antibody, murine IgM anti-SSEA-1, is partially reduced using Sn(II) to expose and protect reactive sulfide groups. The antibody is then purified, stannous tartrate and stabilizers are added, and the mixture is lyophilized. To label, sodium pertechnetate is added. After a 15 minute incubation the tracer drug is injected. The rate of accumulation and degree of concentration at the site of infection is presumptively determinative of the severity of the infection.

Acceptance criteria and tests for the ^{99m}Tc labeled antibody product have been established and validated. Greater than 93% of the ^{99m}Tc is firmly bound to the protein as determined by quantitative HPLC. Radiochemical impurities, colloidal ^{99m}Tc and free pertechnetate are together less than 4% as determined by thin layer chromatography. The immunoreactive fraction, measured by binding to solid phase antigen, and affinity, measured by ELISA, are unchanged by the ^{99m}Tc-direct labeling process. Two hour blood clearance in rats is within 90% of the value of the ^{125}I labeled analog. The immunoreactive fraction decreases less than 10% when incubated in human plasma for 24 hours. This method has been compared to other direct labeling methods, and found to give higher radiochemical yields.

N 408 **Effects Of Chimeric Cytotoxin IL2-PE40 On Adoptively Transferred Experimental Allergic Encephalomyelitis,** John W. Rose[1], Haya Lorberboum-Galski[2], David Fitzgerald[2], Richard McCarron[3], Ken Hill[1], Jeannette J. Townsend[4] and Ira Pastan[2] 1) Neurovirology Research Laboratory, VAMC, Salt Lake City Utah, 2) Laboratory of Molecular Biology, NCI, Bethesda Md. 3) LNNS, NINDS, Bethesda Md. 4) Department of Pathology University of Utah.

IL2-PE40 is a chimeric protein composed of human IL-2 genetically fused to a modified form of *Pseudomonas* exotoxin (PE40)which retains domains for translocation across cell membranes and for ADP-ribosylation but lacks a cell binding domain. This chimeric cytotoxin is directed to IL2 receptor bearing cells and produces cell death by inhibition of protein synthesis secondary to ADP-ribosylation of elongation factor 2. IL2-PE40 is cytotoxic for IL-2 receptor bearing lymphocytes in culture and can inhibit activation of T cells in vivo.

IL2-PE40 can significantly diminish antigen stimulated proliferation of lymphocytes sensitized to myelin basic protein (MBP). PE40 which is not fused to IL2 does not reduce MBP specific proliferation. Intraperitoneal administration of IL2-PE40 markedly inhibits the acute clinical manifestations of adoptively transferred experimental allergic encephalomyelitis (EAE) and prevents subsequent relapses. Treatment with IL2-PE40 significantly reduces both the inflammation and demyelination characteristic of EAE.

N 409 TWO CHIMERIC MONOCLONAL ANTIBODIES WITH IDENTICAL IgG_1 HUMAN Fc DOMAINS EXHIBITS DIFFERENT ANTI-TUMOR EFFECTS ON HUMAN COLON CANCER IN VIVO

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Chimeric monoclonal antibodies (cMAbs) have recently been used in treatment of human malignancies. However, the in vivo effector mechanisms of cMAbs are unknown and discrepancies between results in animal model systems and human clinical trials have been described. Therefore, we have investigated in vivo two different cMAbs with identical human IgG_1 Fc domains (chimeric SF-25 MAb: cSF-25 and chimeric 17-1A MAb: c17-1A) using a new animal model system of human colon cancer and examined the possible effector mechanisms involved in their anti-tumor effects. We found that single injection of cSF-25 (100 μg/mouse) significantly reduced both intrahepatic metastatic spread and local extrahepatic tumor established in nude mice. In contrast, c17-1A administration had no effect on hepatic tumor spread, though this cMAb inhibited extrahepatic tumor formation. In vitro, ADCC activity by murine splenocytes and human K cells in the presence of c17-1A was always lower than that observed with cSF-25 under the same conditions. However, we found that the expression of 17-1A antigen on LS-180 cells was even higher than that observed with the SF-25 antigen. More importantly, target cells preincubated with c17-1A at 37°C demonstrated strikingly diminished ADCC activity at 1 hour and all activity diminished at 20 hours. In contrast, ADCC activity exhibited by cSF-25 did not change under the same conditions. Flow cytometric analysis demonstrated the loss of 17-1A antigen antibody complex from the cell surface at 37°C, whereas SF-25 antigen antibody complex remained intact on the cell surface. Finally, we found that in vivo macrophage depletion completely inhibited the anti-tumor effect of cSF-25 on the extrahepatic tumor, however anti-tumor effect on hepatic metastasis was only partially inhibited. We conclude that the different anti-tumor effects exhibited by the two chimeric antibodies is due in large part to the nature of the cell surface antigen.

N 410 Mechanisms of anti-tumor activity mediated by monoclonal antibodies and interleukin-2

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Immunotherapy experiments with IgG1, IgG2b and IgG2a isotype switch variants of an anti-CD19 monoclonal antibody (mAb) showed that only the IgG2a subclass mAb is able to inhibit the growth of a Burkitt lymphoma cell line transplanted in nude mice. This anti-tumor activity is only apparent if treatment is started immediately after tumor cell injection, e.g. if treatment is delayed until day 7 no anti-tumor activity is observed. Combining this delayed mAb treatment schedule with recombinant interleukin-2 (rIL-2), however, restored the tumor growth inhibitory effect of the mAb, while rIL2 alone has no anti-tumor effect. Furthermore, the ineffective IgG1 and IgG2b subclass anti-CD19 mAb could be rendered effective if combined with rIL-2. The results of *in vitro* experiments suggest that two distinct antibody dependent effector mechanisms may be operative in effective treatment *in vivo*. One of these mechanisms is mediated by macrophages and is dependent on the presence of IgG2a subclass mAb on the target cells. This macrophage mediated, IgG2a dependent effector mechanism could be enhanced by rIL-2 activation of the effector cells. The other mechanism is mediated by lymphocytes and is only operative after activation of these effector cells with rIL-2. After appropriate rIL-2 activation the lymphocytes killed $CD19^+$ target cells very efficiently, irrespective of the anti-CD19 mAb subclass used to sensitize the target cells.

N 411 DELIVERY OF THERAPEUTIC PROTEINS TO THE BRAIN USING A MONOCLONAL ANTIBODY TO THE TRANSFERRIN RECEPTOR, Lee Walus and Phillip Friden
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The delivery of nonlipophilic therapeutic compounds, especially proteins, to the brain is severely hindered by the presence of the blood-brain barrier (BBB), which is formed by the tightly apposed brain capillary endothelial cells. However, brain endothelial cells do have specific receptor-mediated transport mechanisms so that substances required by the brain can cross the blood-brain barrier. Using a monoclonal antibody (OX-26) that binds to the rat transferrin receptor on the luminal surface of brain capillary endothelial cells, we have taken advantage of the transport system responsible for the delivery of iron to the brain to deliver therapeutic molecules across the BBB. Antibody-CD4 and antibody-NGF conjugates were synthesized using a disulfide linkage and characterized in vitro. In vivo experiments, using immunohistochemistry to localize the antibody-protein conjugates after i.v. administration into the tail vein of rats, have shown that both the carrier antibody and the protein "passenger" accumulate in brain capillaries. We have also examined the carrier-mediated delivery of radiolabeled protein across the BBB in vivo using capillary depletion. We have observed that the amount of protein passenger in the capillary fraction of the brain, which peaks within 1 hour post-injection, decreases with time while the amount in the brain parenchyma increases, suggesting that the protein is delivered across the blood-brain barrier to the brain tissue.