IMMUNE REGULATION BY CHARACTERIZED POLYPEPTIDES

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January 25 — February 1, 1986

Keynote Address

B0 Keynote Address : "Activation and Regulatory Mechanisms Among B and T Lymphocytes" - G.J.V. Nossal, Walter and Eliza Hall Institute.

One key operational constraint has limited our capacity to understand activation and regulatory mechanisms amongst immunocytes. It derives from the very essence of the immune system's design. Specific immunocytes are created through unique translocation events which differ amongst individual cells. The responsive immunocyte to any antigenic stimulus is therefore always in a tiny minority, making precise biochemical and cellular analysis extremely difficult.

Most laboratories have adopted two strategies to beat the heterogeneity problem. The first is to use immortalized or frankly malignant immunocytes as objects of study. This strategem is suboptimal for any analysis of immunoregulation, because the key event is the activation of a resting (not a malignantly proliferating) cell. The second strategem is to use mitogens rather than antigens, which of course bypasses the physiological receptors for triggering.

Our group has approached the problem a different way. We use antigen affinity fractionation techniques to prepare antigen-specific immunocytes; single cell cloning methods to avoid inadvertent cellular interactions or lymphokine cascades; and, where appropriate, cloned or highly purified growth factors to synergise with antigens. We also endeavour to recall that antigen can signal cells positively or negatively.

The overview lecture will seek to distill recent work on B and T lymphocytes infused with this philosophy.

Thymopoietin and Splenin

B1 CLINICAL OVERVIEW OF THERAPY WITH THYMOPENTIN, Even Sundal, Clinical Research and Development, CILAG AG, CH-8201 Schaffhausen, Switzerland.

Immune disease is believed to result from a dysbalance of T-cell subpopulations. Such dysbalance may be either in the direction of hyper- or hypo-responsiveness. Several experiments have shown that thymopentin can influence T-cell subsets, depending on the dose, route and speed of administration. Basically, it appeared that subcutaneous administration of the drug stimulates T-helper cells whereas intravenous application (particularly as i.v. prolonged injection) enhances the function of T-suppressor cells.

The above working hypothesis was tested in a series of double-blind, placebo-controlled clinical trials in the European development program of CILAG AG. The s.c. mode of administration was found to be effective in diseases characterized by low T-helper/suppressor cell ratio, e.g., 1) herpes simplex infections, 2) immune status of elderly people, 3) non-responders to hepatitis B-vaccination, and 4) further, positive responses have been documented in several cases with primary immune deficiencies (PID), granulomatous diseases (lepromatous leprosy, trichophyton rubrum) and patients with pre-AIDS.

The most frequent model disease used to study the ev. correlation between clinical course and lowering of an increased T-helper/suppressor cell ratio is rheumatoid arthritis (RA). Patients with active RA infact responded very favorably to the i.v. route of administration (particularly when thymopentin was applied as prolonged injection over 10 minutes). The unique property of thymopentin to have the capability of both stimulating and suppressing the immune system is characteristic of a so-called immunomodulatory drug.

Safety data collected from more than 2000 patients in the clinical program indicate that thymopentin is an extremely well tolerated drug.

Cyclosporine

B2 EFFECTS OF CYCLOSPORIN A ON LYMPHOKINE EXPRESSION. Verner Paetkau, Jennifer Shaw, Karen Meerovitch, John F. Elliott and R. Chris Bleackley, Department of Biochemistry, University of Alberta, Edmonton, Alberta, Canada T6G 2H7.

The effects of Cyclosporin A (CsA) on the immune system are limited and specific. Its immunosuppressive activity can probably be largely explained by its ability to block the synthesis of mRNA for IL2, GM-CSF, IFN gamma, and other lymphokines (1 - 3). This effect occurs in both T lymphocyte cell lines and primary cultures of T lymphocytes, and can be seen whether CsA is added at the time of induction or well after lymphokine mRNA synthesis has been initiated. In the latter case, inhibition is also observed in the presence of the protein synthesis inhibitor cycloheximide (CHX), indicating that sunpression does not depend on the synthesis of a PMA-induced protein. Because of its selective inhibition of lymphokine mRNA synthesis (4), CsA is a useful tool for studying its turnover without effects on bulk cellular RNA synthesis. The half-life of IL2 mRNA is between 1.5 and 3 hours in the mouse T lymphoma line EL4, and of similar magnitude in the human Jurkat cell line. The intracellular level of IL2 mRNA falls dramatically a few hours after induction of Jurkat cells, despite relatively little change in its rate of transcription. Addition of CHX leads to the rapid accumulation of this mRNA by stabilizing it. This can occur even long after induction is initiated, at times when lymphokine synthesis has effectively stopped. These results could be explained by the induction of the labile "latent RNase" system. Such a response insures that lymphokine mRNA is rapidly degraded, and expression terminated, when the inductive signal is removed. The selective induction (by PMA) and suppression (by CsA) of lymphokine mRNAs can be used to screen cloned cDNA libraries for potential new lymphokines. A library generated from the cDNA of PMA-activated EL4 cells and subtracted with mRNA from non-activated cells was first screened with a probe made in the same way. Candidate clones were then used in Northern analysis of mRNA derived from either non-activated, PMA-activated, or PMA-activated, CsA-suppressed EL4 cells to identify sequences of mRNAs which were both inducible and suppressible, a property expected of lymphokine-like molecules.

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2. Kronke, M., W.J. Leonard, J.M. Depper, S.K. Arya, F. Wong-Staal, R.C. Gallo, T.A. Waldmann and W.C. Greene, Proc. Natl. Acad. Sci. USA 81:5214 (1984).
3. Granelli-Piperno, A., K. Inaba and R.M. Steinman, J. Exp. Med. 160:1792 (1984).
4. Paetkau, V., Can. J. Biochem. Cell Biol. 63:691 (1985).

B3 SPARING OF SUPPRESSOR CELLS BY CYCLOSPORINE IN ALLOGRAFT RECIPIENTS, Jerzy W. Kupiec-Weglinski, Nicholas L. Tilney, Surgical Research Laboratory, Harvard Medical School, Boston, MA 02115.

Cyclosporine (CsA) is a potent immunosuppressant with proven efficacy in clinical organ transplantation. Although its mechanisms of action have not been fully defined, one of its most critical effects at least in animal models, involves a relative sparing and/or amplification of T suppressor cell (Ts) activity. Heterotopic LBN cardiac allografts are rejected acutely within 8d in unmodified LEW rats, yet survived indefinitely following transient CsA treatment (15 mg/kd/d IM for 7 days only). Immunoperoxidase stains of lymphoid tissues of treated, grafted hosts showed strikingly reduced cellularity of putative T helper (Th) and T cytotoxic (Tc) cell areas (thymic medulla, splenic marginal zone), whereas Ts areas (thymic cortex, splenic red pulp) were unaffected Adoptive transfer of splenocytes (SL) or thymocytes from CsA-treated graft recipients bearing well functioning grafts significantly prolonged test cardiac allografts placed in immunologically virgin syngeneic rats. This response became increasingly antigen-specific in time. Using monoclonal antibody immunoaffinity columns, relatively pure and non-overlapping subsets of splenic Th (W3/25+) and Tc/s(OX8+) were isolated. Administration of as few as $5x10^5$ Tc/s improved donor-origin (but not third party) test allograft survival to c.15-21d; Th were ineffectual. Immunocompetence could not be restored to CsA treated recipients of long-term allografts by transfer of large numbers of specifically sensitized lymphocytes (even when supplemented with a course of exogenous interleukin (IL-2) or combined with sublethal total-body x-radiation). However, acute rejection could be reproducibly re-created when similar animals were challenged with alloimmune cells plus cyclophosphamide (CY), an agent reputed to destroy Ts in vitro. Thus, a CY-sensitive population of Ts, at least in part, governs allograft acceptance in CsA treated hosts. Levels of endogenous IL-1, IL-2 and IL-3 production were greatly curtailed in graft recipients during the 7 day period of drug administration. Two-three weeks after CsA withdrawal, however, levels of IL-1 and IL-2 returned to normal, whereas IL-3 rose remarkably high, suggesting that this lymphokine may act as a growth factor for Ts and may be associated with Ts production and generation.

What is the interdependence between helper and suppressor activities during the maintenance phase of CsA induced unresponsiveness? Th separated from spleens of treated recipients and adoptively transferred into immunologically inert T-cell deprived "B" recipients of cardiac allografts promoted rejection of otherwise indefinitely surviving heart grafts in a tempo similar to that achieved by transfer of specifically sensitized lymphocytes. However, transfer of the T cell population from CsA treated animals containing both Th and Tc/s subsets never induced graft rejection following transfer into B hosts. These results emphasize exceptional efficacy of Ts in sustaining prolonged CsA mediated allograft survival, as well as suggesting that the drug does not effect Th function per se.

Immunoregulation with Monoclonal Antibodies

B4 HUMAN ANTIBODY RESPONSE TO MURINE OKT3.

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The murine monoclonal antibody OKT3(BALB/c IgG2a)was administered prophylactically to 25 human renal allograft recipients(5mg/day,i.v. for 14-30 days)either alone or in association with corticosteroids(0.25mg/kg/day)and azathioprine(3mg/kg/day). The IgM and IgG anti-OKT3 response was monitored by means of 4 different assays(ELISA and immunofluorescence tests). All patients treated with OKT3 alone showed a rapid and strong sensitization, that completely neutralized the therapeutic effectiveness of the monoclonal antibody. Anti-OKT3 sensitization was manifested by accelerated OKT3 clearance, abrupt reappearance of circulating OKT3+ cells and clinical signs of rejection before the end of treatment. The sensitization was significantly reduced in its incidence and intensity in patients treated with OKT3 associated with low dose corticoids and azathioprine:i.e.,mainly IgM anti-OKT3 antibodies were produced that were of low affinity. Human anti-OKT3 antibodies presented a particular specificity pattern as they did not recognize all mouse immunoglobulins.The analysis performed on whole patients' sera as well as mouse IgG2a(OKT8,OKT3,UPC-10) affinity chromatography purified fractions has shown that two categories of antibodies were detected: 1/ Anti-idiotypic antibodies, directed against OKT3 determinants located on the variable region of the molecule and totally absent from other murine IgG2a monoclonals. These antibodies inhibited OKT3 binding to T cells and abrogated its therapeutic activity. 2/ Anti-mouse IgG2a(anti-isotypic) antibodies. These antibodies did not accelerate OKT3 clearance and thus were not detrimental to its immunosuppressive capacity. Moreover, spectrotype analysis performed by isoelectrofocusing, clearly showed that the anti-monoclonal response is oligoclonal.
Finally it should be noted that these results are not unique to OKT3, since similar data have been obtained for both isotype/idiotype restriction and oligoclonality with sera collected from monkeys(maccacus rhesus) treated with anti-T cell monoclonals carrying different isotypes and showing various specificities (anti-pan T cell , anti- helper-inducer and anti-cytotoxic/suppressor T cells).
These data are interesting from the theoretical view point since they illustrate in man the notion that low dose intravenous protein immunization leads to the production of a restricted immune response. They are also important clinically as they suggest that OKT3-immunized patients could still be sensitive to the immunosuppressive effect of other anti-T cell monoclonals that do not share the OKT3 idiotype and possibly isotype.

B5 TREATMENT OF ACUTE ALLOGRAFT REJECTION WITH OKT3, A. Benedict Cosimi, Department of Surgery, Massachusetts General Hospital, Boston, MA 02114

OKT3, a murine monoclonal antibody reactive with a surface glycoprotein on post-thymic T-cells, blocks in vitro killing by cytotoxic cells as well as the generation of a number of other T-cell functions. An initial clinical study in 10 renal allograft recipients indicated OKT3 was remarkably effective in reversing acute rejection episodes. These observations were confirmed in a subsequent prospective randomized multicenter trial. Ninety-four percent of acute rejection episodes were reversed by OKT3 as opposed to 75% in the conventionally treated group. This translated into an improvement in 1-year allograft survival of 17% for the OKT3-treated patients (1).

Several limitations of OKT3 therapy were identified in the preliminary trials. These included a sometimes severe febrile or bronchospastic response following the 1st injection; the not infrequent development of recurrent rejection episodes following withdrawal of OKT3 therapy; and the production of antibodies by the host to the murine immunoglobulin. These have been addressed in subsequent patients. With the modifications and precautions employed, reactions to initial doses of OKT3 have been controlled and the incidence of antibody response to the murine globulin has been decreased from 86% to 39%. Although recurrent rejection episodes have continued to occur in Azathioprine-prednisone (Aza-Pred) treated recipients following withdrawal of OKT3, these have been reversible in most instances. An 80% one-year allograft survival has, therefore, been achieved in these subsequent patients.

Most recently, OKT3 treatment has been evaluated in allograft recipients whose baseline immunosuppression includes Cyclosporine (CsA). Initial observations have suggested approximately 15-20% of renal allograft recipients treated primarily with CsA can benefit from OKT3 treatment added at the time of rejection. Effective reversal of rejection has been achieved, essentially as observed in patients receiving Aza-Pred. Whether the incidence of subsequent rejection episodes is reduced by this combination remains to be evaluated.

In conclusion, OKT3 has been found to be a highly effective agent for reversing acute allograft rejection in both Aza-Pred and CsA-treated recipients. Addition of this agent to the therapeutic protocol when rejection has been diagnosed has almost universally reversed the rejection episode which then allows adjustment of maintenance immunosuppression for improved long-term allograft function.

(1) Ortho Multicenter Transplant Study Group: A randomized clinical trial of OKT3 monoclonal antibody for acute rejection of cadaveric renal transplants. N Engl J Med., 313: 337-342, 1985.

B6 IN VIVO EFFECTS OF MONOCLONAL ANTIBODY TO L3T4, William E. Seaman and David Wofsy, Department of Medicine, VA Medical Center and University of California, San Francisco, CA 94121

In mice "helper/inducer" T cells are distinguished from "suppressor/cytotoxic" T cells by the surface antigen, L3T4, homologous to the human antigen T4 (CD4). In normal mice (C57BL/6), 1 mg of monoclonal antibody to L3T4 (hybridoma GK1.5, rat IgG2b) depletes $\geq$95% of target cells from the blood, spleen and lymph nodes within 72 hrs. L3T4+ cells cannot be fully depleted from the thymus. Extrathymic L3T4+ cells gradually recover over 60 days following treatment. Mice given anti-L3T4 make little or no antibody against rat immunoglobulin and can be treated repeatedly without anaphylaxis or loss of efficacy. Recovery ensues even after weekly treatment for 8 months. Depletion of L3T4+ cells is accompanied by a profound suppression of humoral immunity; there is no specific antibody formation in response to protein in Freund's adjuvant. For antigens in Freund's adjuvant, immunization at the time of treatment with anti-L3T4 does not induce immune tolerance; specific antibodies are formed as L3T4+ cells recover. However, treatment with anti-L3T4 alone appears to induce tolerance to cross-reacting antigens on other Ig2b rat antibodies. Depletion of Lyt-2+ cells from mice does not reduce humoral immunity, nor does it restore immunity in mice that have been immunosuppressed by partial depletion of L3T4+ cells. Thus, deficiencies in "helper" T cells cannot be overcome by concomitantly depleting "suppressor/cytotoxic" T cells.

Depletion of L3T4+ cells prolongs skin graft survival from 9 days to 18 days, but allo-specific cytotoxic T cells develop. Similarly, proliferation in mixed lymphocyte culture (MLC) by spleen cells from mice treated with anti-L3T4 is reduced, but not absent, and the in vitro generation of cytotoxic T cells is reduced but not absent. The MLC by L3T4-depleted spleen cells is inhibited 85% ($p<0.005$) by the presence of anti-Lyt-2 in the culture and only 35% (N.S.) by incubation in the presence of anti-L3T4. This indicates that when L3T4 cells are depleted, T cell activation may be primarily dependent on $L3T4^-$,Lyt-2+ cells.

Mice treated weekly with 1 mg anti-L3T4 have a 2-4 fold increase in natural killer cell activity after 2 days and this is sustained for at least 30 days.

In autoimmune mice (NZB/NZW, BXSB, MRL/lpr), anti-L3T4 does not efficiently clear target cells, a defect that increases as disease increases. Nonetheless, weekly treatment of NZB/NZW mice beginning either at the onset of disease (4 months) or after disease is active (7 months) retards autoimmunity and prolongs survival. Autoimmunity in BXSB mice can also be retarded. Similar results have been found by Steinman and his colleagues in mice with experimental autoimmune encephalitis.

Antibody to human T helper cells may be useful in treating autoimmunity, but it will also suppress normal immunity. Humoral immunity is most profoundly suppressed, cellular immunity is partially suppressed, and natural killer activity is increased.

B7 THE T CELL RECEPTOR/T3 COMPLEX, Cox Terhorst, Department of Molecular Immunology, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA 02115

Of fundamental importance in understanding the events involved in T cell activation is the identification and characterization of the cell surface molecules involved. Antigen induced stimulation and susequent activation of the T cell are initiated through interactions with the T cell antigen receptor. Several lines of evidence have demonstrated the intimate association between the T cell antigen receptor and T3, thus forming the so-called T3/T cell receptor complex. First, in immunoprecipitates with either anti-T3 monoclonal antibodies, or with anti-T cell receptor antibodies five polypeptide chains have been detected. These are two disulfide bridged variable glycoproteins (α and β chains) and three invariable structures the T3-γ, δ and ϵ chains with mw 25kD, 20kD and 20kD, respectively. Second, mutants of a T leukemic cell line which were selected for the loss of the T3 complex from their surface by treatment with an anti-T3 antibody and complement concomitantly lost expression of the clonotypic heterodimer. Third, monoclonal antibodies directed at either the T cell receptor α and β chains or at the T3 chains affect T cell functions in an identical fashion. Thus, we see that the complex formed between the T cell receptor and the T3 molecules is functionally as well as structurally central to the immune response.

The structure, biosynthesis and regulation of gene expression of the T3/T cell receptor complex will be discussed. An attempt will be made to relate the structural information to the function of the T3/T cell receptor complex.

Thymulin and Thymosin

B8 THYMULIN IN RHEUMATOID ARTHRITIS: A DOUBLE BLIND PLACEBO CONTROLLED TRIAL, Bernard Amor, Maxime Dougados, Christian Méry, Annie de Géry, Nicole Simon-Lavoine, Mireille Dardenne, Jean Francois Bach, Department of Rheumatology, Cochin Hospital, Paris, France.

Synthetic thymic serum factor (FTS) now called Thymulin (Thy) is a pharmacologically active compound which can have various effects on T-lymphocytes. We previously reported a beneficial effect of Thy in rheumatoid arthritis (RA) in an open study (1). Therefore, we conducted a randomized double blind placebo controlled trial to assess the effectiveness of Thy and to compare 2 different dosages.

Forty nine RA out patients (pts) with active disease despite a symptomatic treatment including steroidal and non-steroidal anti-inflammatory agents entered the study and received one injection subcutaneously each day for 2 weeks and then 3 times a week for 6 months, but they were randomly allocated to either of 3 groups: placebo group (P:17pts), Thy 1 mg per injection (Thy 1:16pts), Thy 5 mg per injection (Thy 5:16pts). Assessment criteria were: morning stiffness, grip strength, proximal interphalangeal circumference, Ritchie index, Lee functional index, pain assessment, overall assessment by the pt and the investigator, average of daily prednisone consumption. Five pts withdrew from the study: 2 in group P: painful injections (1 case); inefficiency after 4 months (1 case); 1 in group Thy 1: inefficiency after 4 months; 2 in group Thy 5: thrombocytopenia at 3 months (1 case) vasculitis at 5 months (1 case). Statistical analysis (U test of Mann and Whitney) were performed upon the clinical and biological changes after 6 months. Comparing P and Thy 1 groups, no statistical difference excepted the overall assessment by the investigator ($p < .05$) was observed. At variance, comparing P and Thy 5 groups, a significant improvement of the grip strength ($p < .03$) and a reduction of the Lee index ($p < .05$) were observed in group Thy 5. Furthermore, in this group, a significant ($p < .05$) reduction of daily prednisone consumption was possible. Overall assessment by the patient ($p < .02$) and the investigator ($p < .01$) were also in favor of Thy 5. Interestingly, these clinical improvements were not associated with significant changes in biological parameters (sedimentation rate, rheumatoid factor, T-cell subsets).

These data suggest that (a) despite painful injections, Thy seems to be a safe treatment (b) Thy seems to be an effective treatment of RA when using a high dosage.

(1) Thymulin (FTS) in rheumatoid arthritis. B. Armor, M. Dougados, C. Méry, A. de Géry, J. Choay, M. Dardenne, J.F. Bach, Arthritis Rheum. 1984. 27:117-118.

B9 THYMIC HORMONES AND T-CELL DIFFERENTIATION, Jean-François BACH, INSERM U 25, Hôpital Necker, 161, rue de Sèvres, 75015 PARIS, France.

Thymic epithelial cells produce a number of polypeptides with immunodifferentiating activities. The apparent multiplicity of putative thymic hormones has been the matter of confusion. The critical appraisal of the characteristics of thymic peptides with reference to strict endocrinological and pharmacological criteria indicates that only a very limited number of thymic hormones probably exists. These peptides are derived from high M. W. precursors whose syntheses are subjected to the regulatory influence of a number of endogeneous and extrinsic factors. Synthesis is stimulated by contact with lymphoid stem cells, peripheral depletion of the hormone and several hormones and drugs such as thyroxin and cyclosporin. Thymic hormones induce the de novo appearance of T-cell differentiation alloantigens in T precursor cells after interacting with high affinity receptors. The effect is specific and depends on the activation of a second messenger (cyclic nucleotides, prostaglandins). They also induce or enhance various T-cell functions in conjunction with other cellular or humoral signals. They may thus represent potentially useful immunoregulatory drugs which can modulate helper or suppressor T cell function depending on the peptide considered, the dosage and the test. Some peptides such as thymopoietin and thymic humoral factors seem to be best active on helper T cells whereas others, such as thymulin (formerly FTS), act predominantly on suppressor T cells, at least at high dosage.

B10 THYMULIN : BIOCHEMISTRY AND BIOLOGICAL ACTIVITY. M. Dardenne, INSERM U 25, Hôpital Necker, 75015 PARIS, FRANCE.

The thymus produces several polypeptidic hormones able to induce immature lymphoid cells to express T-cell markers and execute T cell functions. One of these hormones, thymulin, formerly called Facteur Thymique Sérique (FTS), was initially isolated from pig serum. Its amino acid sequence was determined (<Glu-Ala-Lys-Ser-Gln-Gly-Gly-Ser-Asn) and the synthetic peptide was shown to be fully biologically active and capable of inducing several T-cell markers and promoting T-cell functions, including allogeneic cytotoxicity, suppressor function and interleukine-2 production. The synthesis of several analogous molecules demonstrated that minor changes in the C-terminal end result in the disappearance of biological and antigenic activities for certain analogues, while for others, they engender antagonistic activities. Recently, the presence of high affinity specific receptors on lymphoblastoid T-cell lines was demonstrated using (^{3}H) thymulin and direct evidence was found for the thymic origin of thymulin using polyclonal and monoclonal antibodies in an immunofluorescence technique. Finally, we demonstrated that thymulin binds the metal zinc which appears to be essential for the biological activity and antigenicity of the hormone. Zinc-deprived natural thymulin, obtained after incubation with the chelator Chelex 100, shows no activity in vitro and in vivo in the Thy-1 antigen induction assay. Biological activity is restored by the addition of zinc, which is inactive alone in the bioassay. Other metals can also restore to some extent thymulin activity (Al(III), Ga(III), Cu(II)), but inhibition studies using monoclonal antibodies produced against natural thymulin have revealed that zinc coupling provided a unique antigenic determinant, found on the natural peptide, but not on complexes of the synthetic peptide and other metal ions. Gel filtration studies using $^{65}ZnCl_2$ showed that at pH 7.5, thymulin binds one zinc ion with an apparent affinity constant Kd of $5 + 2 \times 10^{-7}$M. Study of in vitro and in vivo activities of the thymulin-zinc complex has indicated that the maximum activity is obtained at the molar metal ratio of 1:1. Moreover, by means of nuclear magnetic resonance (NMR) it was found that the conformational structure of the zinc-peptide complex is strikingly different from that observed when the metal-deprived peptide is studied : analysis of these spectra suggests that Zn(II) has a specific binding site involving the Asn^9 COO^- terminal group and both the hydroxyls of the two serine residues.

All these results indicate that natural thymulin binds zinc in vivo (probably within the thymus) and that this binding induces conformational changes necessary for the expression of biological activity.

B11 MOLECULAR BIOLOGY OF THE THYMOSINS, B.L. Horecker, Department of Biochemistry, Cornell University Medical College, New York, NY 10021

The name thymosin was first used to describe a factor with lymphopoietic activity in a preparation from calf thymus (1) and later to identify several chemically characterized peptides, isolated from a similar preparation called thymosin fraction 5 (2). Two of these peptides, thymosin α_1 and thymosin β_4, and structurally related peptides will be the subject of the present discussion.

Thymosin α_1, containing 28 amino acid residues, is one of several peptides in thymosin fraction 5 that appear to be proteolytic fragments derived from a larger, highly acidic native polypeptide named prothymosin α (pro α). The native polypeptide was isolated from fresh rat thymus using a radioimmunoassay for thymosin α_1. The procedure also yields a related polypeptide, named parathymosin α (para α), similar to prothymosin in size, amino acid composition and chromatographic behavior and containing homologous sequences. Pro α protects susceptible strains of mice against lethal doses of Candida albicans, associated with in vivo release of MIF and γ-interferon into the circulation and enhanced phagocytic activity of peritoneal macrophages. Para α is inactive in these tests but blocks the effects of pro α. Human pro α, structurally different from rat pro α, is less active in the mouse protection test. The cDNA for human pro α has been cloned.

Thymosin β_4 (thy β_4), containing 43 amino acid residues, is the most widely distributed member of a large family of closely related polypeptides. Its structure is fully conserved in vertebrate classes ranging from amphibia to mammals. A slightly different polypeptide, thymosin β_{11}, is present in bony fish. In mammals, thy β_4 is accompanied by a second related polypeptide, thy β_9 in bovine tissues and thy β_{10} in tissues of other mammals. The function of the β-thymosins remains unknown but accumulating evidence supports the view that they are not hormones or secretory peptides. 1. Thy β_4 is widely distributed in vertebrate tissues and is synthesized by cell lines, unrelated to the reticuloendothelial system. 2. The in vitro synthesis of thy β_4 from a 7-8 S mRNA does not involve formation of a precursor with a signal peptide sequence. 3. The sequence of a cloned cDNA for thy β_4 mRNA supports the conclusion that thy β_4 is synthesized as a 44-mer and not as a larger prepeptide. Screening of a rat genomal library for the thy β_4 gene yielded two clones having sequences similar, but not identical, to the thy β_4 cDNA sequence. Structural features in the 5'-region suggest that they may be pseudogenes. Several mRNA's related to thy β_4 are induced in human cells treated with interferon (3).

1. Goldstein & White (1966), Proc. Natl. Acad. Sci. USA 56: 1010-1017.
2. Hooper et al (1975), Ann. N.Y. Acad. Sci. 249: 125-144.
3. Friedman et al (1984) Cell 38:745-755; G.R. Stark, personal communication.

Interleukins and Gamma Interferon

B12 MULTIPLE BIOLOGICAL PROPERTIES OF RECOMBINANT HUMAN INTERLEUKIN-1,
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Host responses to infection and injury are characterized by a spectrum of changes in immunologic, metabolic, neurologic, hematologic and endocrinologic systems; grouped together, these changes are called the "acute phase" response. Interleukin-1 (IL-1), orginally described as an endogenous pyrogen and purified from monocyte supernates, is believed to be the mediator of many components of the acute phase response. Monocyte IL-1 exists as multiple charged species (pI 7, 6 and 5) and it is now clear that at least two of these are encoded by different genes. The pI 7 form was cloned from human monocytes (1) and the pI 5 form from mouse macrophages (2); although they share less than 25% amino acid sequences, they possess nearly the same spectrum of biological properties. IL-1 is unique among the lymphokines and monokines in that there is no signal peptide sequence which indicates the site for protease cleavage. Therefore, IL-1's purified from crude supernatants have been reported with various molecular weights which is likely due to various processing sites. Precursor IL-1 (31 kD) is cleaved into a 17.5kD peptide which is the predominant extracellular form. Although this cleavage product is the primary IL-1 form, this can be further cleaved into smaller peptide fragments (4,000 daltons) which retain biological activities. Of the two gene products, the pI 7 form is the major species and poly A RNA for this form can be as high as 5% 12 hours following stimulation. The pI 5 form seems to be a minor member of the IL-1 family, particularly in humans. Recombinant human pI 7 IL-1 is now being studied in a wide variety of assays which have confirmed the previous data employing purified monocyte-derived IL-1. Thus recombinant IL-1 induces fever, slow wave sleep, hypozincemia, hypoferremia, neutrophilia, hepatic acute phase protein synthesis, IL-2 synthesis, IL-2 receptors, bone resorption, fibroblast proliferation, collagenase production, and prostaglandin E in a variety of tissues. IL-1 is chemotactic for neutrophils, lymphocytes and monocytes and induces neutrophil degranulation and mast cell histamine release. It causes decreased appetite and decreased hepatic albumin synthesis. Endothelial cells increase procoagulant activity and expression of leukocyte adherence molecules which likely explains the increase in neutrophil adherence to IL-1-treated endothelial cells. IL-1 is also cytotoxic for a variety of cells and increases non-specific resistance to infection. These studies confirm the concept that IL-1 is a key mediator of host responses to infection, neoplastic change and injury.

1. Proc. Natl. Acad. Sci. USA. 81:7907, 1984.
2. Nature. 312:458, 1984.

B13 CLONING, EXPRESSION AND BIOLOGICAL ACTIVITIES OF HUMAN INTERLEUKIN-1α AND INTERLEUKIN-1β,
Steven Gillis, Carl March, Bruce Mosley, Michael Cantrell, Michael Deeley, Gary Braedt, Alf Larsen, Douglas Pat Cerretti, Kathryn Prickett, Shirley Kronheim, Kenneth Grabstein, Paul Conlon, Christopher S. Henney, Steven Dower, David Urdal, Anthony Allison[1], Tom Hopp, and David Cosman, Immunex Corporation, 51 University, Seattle, WA 98101, and [1]Syntex Corporation, 3401 Hillview Avenue, Palo Alto, CA 94303

Interleukin-1 (IL-1) is a potent mediator of the inflammatory response, and many diverse biological activities have been ascribed to it. We have used two strategies to clone human IL-1 cDNAs from lipopolysaccharide-stimulated human macrophages. The first used a hybrid selection assay to screen pools of cDNA clones for their ability to select RNA that could translate IL-1 biological activity in a rabbit reticulocyte lysate, and the second used oligonucleotide probes, derived from the amino acid sequence of purified IL-1, to screen the cDNA library. The two approaches led to the isolation of two distinct IL-1 cDNAs, IL-1α and IL-1β. Both cDNAs encoded proteins of 30,000 daltons which shared 26% amino acid homology. The biological activities of these proteins resided in the C-terminal 159 and 153 amino acids respectively (termed the mature parts of the molecules). We have constructed expression vectors designed to overproduce mature IL-1α and IL-1β in E. coli, and have purified the recombinant proteins to homogeneity.

Current research is directed in four areas: 1) the precise definition of the active regions of the IL-1 molecules using deletion and point mutants; 2) a comparison of the biological activities of the two proteins, namely pyrogenicity, bone demineralization, fibroblast proliferation, synovial cell prostaglandin release, endothelial cell proliferation, B-lymphocyte proliferation and induction of IL-2 synthesis by T-cells; 3) definition of receptors for IL-1α and IL-1β; and 4) generation of antisera and monoclonal antibodies reactive with IL-1α and IL-1β proteins. Results in these areas will be discussed.

B14 MOLECULAR CHARACTERIZATION OF INTERFERON-GAMMA AND ITS RECEPTOR, Patrick W. Gray, Genentech, Inc., 460 Point San Bruno Blvd., South San Francisco, CA 94080.

Interferon-gamma (IFN-γ) is a lymphokine secreted by mitogen-activated (or antigen-stimulated) T-lymphocytes. Cloning and expression of the IFN-γ cDNA demonstrated that the active protein has a monomer molecular weight of 17,000 daltons. The active form isolated from sera is a glycosylated dimer of approximately 50,000 daltons. IFN-γ is recognized by a cell surface receptor on monocytes. Binding to this receptor is specific and is not significantly inhibited by IFN-α or IFN-β. The IFN-γ receptor appears to be a protein of approximately 70,000 molecular weight. The availability of pure recombinant IFN-γ has aided the biological characterization of its many activities. In addition to antiviral and antitumor activities, IFN-γ plays an important role in the regulation of the immune system.

B15 HUMAN B AND T LYMPHOCYTE STIMULATING PROPERTIES OF INTERLEUKIN-2 MUTEINS, Peter Ralph, Ilona Nakoinz, Michael Doyle, Mei-Ting Lee, Department of Cell Biology; Kirston Koths, Robert Halenbeck, Department of Protein Chemistry; David F. Mark, Department of Molecular Biology; Cetus Corporation, Emeryville, CA 94608

IL2 acts directly on several cell types, being a T cell growth factor (TCGF), a growth and stimulating factor for NK cells, and inducing Ig production in subsets of B cells. Human T cells have high and low affinity IL2 receptors identified by anti-Tac antibodies. However, certain B cells typified by IgM line SKW6.4 and IgG line ARH-77 are stimulated by high concentrations (10^2-10^4 U/ml) of native and recombinant IL2 to secrete Ig, but do not bear Tac antigen nor have high affinity IL2 receptors (1). IL2 may trigger these B cells via weak binding to the receptor for B cell inducing factor (BIF, BCDF) that is different from IL2. To determine if altered IL2 configurations confer greater agonist or antagonist properties on the molecule, the effect of site-specific mutations and reduction of disulfide bonds on TCGF activity, stimulation of NK cells, and B cell differentiation was examined.

Recombinant des ala IL2 (rIL2), rIL2 with cys at position 125 replaced by ser (to ensure proper joining in the native cys58-cys105 configuration) (ser-IL2) (2), rIL2 with all three cys residues changed to ser (ser_3-IL2), and rIL2 with met_{104} changed to ala (ala_{104} IL2) were tested. Conventional JURKAT, rIL2, and ser-IL2 have similar specific activities in the TCGF and NK assays and are similar in inducing Ig secretion in the B cell line at high concentrations. Ala_{104}-IL2 also had similar activity in TCGF and B cell assays. Ser_3-IL2 and reduced ser-IL2 had much lower TCGF and B cell inducing activity. Ser_3-IL2 and reduced ser-IL2 did not block the TCGF or B cell inducing activity of rIL2, showing that the altered molecules do not have a high affinity for the bioactive acceptor sites for IL2 on either T or B cells.

We previously hypothesized separate sites on the IL2 molecule interacting with the Tac^+ IL2 receptor on T cells (and some B cells) and with a different, low-affinity receptor on B cells based on anti-IL2 neutralization studies (1). Separate sites on IL2 based on mutein studies will be discussed. Phase I and Phase II clinical trials involving over 400 cancer patients have been initiated using ser-IL2 to induce and maintain high levels of lymphocyte killer cells. The trials show that IL2 can be administered with relatively little toxicity and has resulted in regression of a variety of solid tumors.

ACKNOWLEDGMENTS We thank the staff of the Cetus Assay Lab for TCGF measurements, and Richard Robb for the JURKAT IL2.

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2. Wang, A., Lu, S.-D. and Mark, D.F. Science 224: 1431 (1984).

Interferons and Other Approaches

B16 ROLE OF MURAMYL PEPTIDE FOR PRODUCTION OF FUTURE SYNTHETIC VACCINES.
L. Chedid and F. Audibert - Institut Pasteur - Paris - France

A good conventional vaccine should give an early, high and durable response of the appropriate isotypes and also in certain cases induce cell-mediated immunity. Moreover, the immunogen shoud be effective in immunodeficient or immature hosts. Since such an immunogene rarely, if ever, exists an adjuvant is required to compensate the absence of one or several of the above-mentioned characteristics. An adjuvant may also be required in view of reducing the amounts of rare or costly antigens. These comments are particulary valid in the case of synthetic antigens which have by-passed the conventional route of vaccine development. Most synthetic vaccines have been constructed by coupling a protein carrier to a single synthetic peptide which copies a natural sequence of primary protein structure. These vaccines have required the addition of Freund's complete adjuvant (FCA) which is too toxic for use in humans.

Our purpose is to describe the role of synthetic adjuvants called muramyl peptides or MDPs in synthetic models represented by totally or partially synthetic constructs containing one or several specificities. It will also be shown how certain problems that can be raised by the use of carriers can be corrected or avoided.

All these examples should delineate various strategies allowing the use of clinically acceptable vaccines and underline the notion that, contrary to inert delivery systems, a synthetic immunoadjuvant intervenes actually in the reading of the epitopes and in the modulation of the antibody response.

Molecular Biology and Peptide Chemistry

B17 ANTIBODY DEFINED ACTIVE REGIONS OF HUMAN INTERFERONS.
Bruce W. Altrock, David Chang, Helen R. Hockman, Karen Duker and Ted Jones.
Amgen, Thousand Oaks, CA, 91320, and Amgen Development, Inc., Boulder, Co. 80301.

Monoclonal and polyclonal antibodies to specific domains of human interferons have been used as structural probes in the identification of biologically significant regions within these molecules. Antibodies were generated to specific synthetic peptide antigens representating selected amino acid sequences of human alpha and gamma interferons. Additionally, the epitope specificities of monoclones generated to various intact recombinant DNA-derived interferon species were investigated and mapped. Studies with these reagents have provided information on the posttranslational processing of IFN-γ. The abilities of these serologic reagents to neutralize biological properties of the interferons have been examined. In this fashion, regions of both alpha and gamma interferons were identified which were significant to the antiviral and antiproliferative activities of those molecules. The abilities of these reagents to block receptor binding were examined. The relation of predicted structural features of the interferons to serologically defined regions of biological significance are under further investigation.

B18 BIOLOGICALLY ACTIVE PROTEOLYTIC FRAGMENTS OF INTERLEUKIN-2

J. Browning and R. Mattaliano, Biogen Research Co. Cambridge, Ma.

Limited proteolysis was used to investigate the domain or region of interleukin-2 involved in biological activity. Recombinant IL-2 was digested with endopeptidase lys C and the resultant partial proteolytic fragments were purified using reverse phase HPLC. The first cleavages occurred at lys 32 and lys 76 with the cut at lys 76 resulting in a disulfide linked two chain molecule. Three fragments were characterized, 32-133 (I), 1-76-S-S-77-133 (II) and 32-76-S-S-77-133 (III). Cleavage points were determined by N-terminal sequence analysis of the purified fragments. Bioassays through the HPLC peaks yielded relative specific activities. The 'loop-nicked' molecule (II) had a specific activity identical or slightly improved over the parent molecule. The N-terminal deletion (I) retained 20% of the original activity, this activity was slightly improved by the addition of the loop-nick (III). These data suggest that the region around lys 76 in the disulfide bonded loop and N-terminal regions of IL-2 are not very critical for bioactivity. Genetic constructions of IL-2 lacking the N-terminal region (first exon) are typically inactive suggesting that the inactivity of these constructs stems from an inability to refold properly rather than a direct involvement of this region in receptor binding.

B19 IMMUNOCHEMICAL AND IMMUNOBIOLOGICAL CHARACTERIZATION OF GENETICALLY ENGINEERED HUMAN IL-2 MOLECULES.

Butler, L., DeRiso, P., Strnad, J., Belagagi, R., Van Frank, R., Smith, K., and R. Gadski. Lilly Research Laboratories, Indianapolis, IN and Dartmouth Medical School, Hanover, NH.

We have been investigating potential functional domains of the human interleukin-2 (IL-2) molecule utilizing genetic engineering techniques. These IL-2 constructions have been analyzed for changes in biochemical, immunochemical and biologic characteristics. Initial studies using constructions generated via exon deletion techniques have revealed probable binding sites of two neutralizing antibodies, DMS-1 and DMS-5. However, none of the unpurified exon deleted constructions appeared to competitively inhibit binding of radiolabelled IL-2 to the IL-2 receptor or possess biologic activity. Because of the changes in protein conformation that accompany exon deletion procedures, we attempted more subtle engineering of the molecule using site directed mutagenesis techniques. A number of constructions have been evaluated for changes in their capacity to bind DMS-1 and DMS-5 as well as biologic activity. Correlations have been established between the capacity to bind to the IL-2 receptor and biologic function.

B20 ANALYSIS OF THE FUNCTIONAL DOMAINS OF HUMAN AND MOUSE INTERLEUKIN-2 (IL-2) WITH MONOCLONAL ANTIBODIES,

R. Chizzonite, T. Truitt, W. Danho, P. Kilian, M. Gately, W.H. Tsien, J. Moschera, L. Collins, and G. Ju, Roche Research Center, Hoffmann-La Roche, Inc. Nutley, N.J.

Monoclonal antibodies (mAb) specific for natural and recombinant IL-2 have been developed and tested for inhibition of IL-2 activity by an IL-2 neutralization assay and an IL-2 receptor binding assay. The epitopes of 75 mAbs have been identified by ELISA reactivity on IL-2 synthetic peptides and by Western blot and Dot blot analysis on recombinant IL-2 analogues. The mAbs can be divided into 3 groups: Group #1 inhibit IL-2 activity in both functional assays, Group #2 do not inhibit IL-2 activity, and Group #3 inhibit mouse and human IL-2 activity but not rat IL-2 activity. Comparison of the epitope mapping data with inhibition of IL-2 activity highlights two epitopes which are important for human IL-2 activity: amino acids 1-19 and 30-60. Within the 30-60 area, antibodies 13D2 and 13A6 identify sequences 42-60 and 50-60 as neutralizing epitopes, respectively. Since mAb 13A6 also neutralizes mouse IL-2 activity, its epitope must be different from but close to the epitope of 13D2 which does not bind or neutralize mouse IL-2. Evidence that the 1-19 and 40-70 sequences are physically adjacent in the native molecule comes from epitope competition studies. Antibody 5B1, which binds residues 1-12, inhibits a mAb specific for residues 40-70 from binding IL-2. MAbs 17A1 and 3D5 which bind residues 21-123 and 71-87, respectively, are not blocked in the same assay. Of the 75 mAbs analyzed, more than 95% recognize epitopes which are concentrated between amino acids 1-19 and 40-80. MAbs specific to the C-terminal region were not isolated, and suggests that most of this region may be buried in the protein. The data are consistent with the model of IL-2 being folded so that the 1-19 and 40-60 sequences are closely aligned and may form contact points with the receptor.

B21 STRUCTURE-FUNCTION STUDIES OF A HEMOPOIETIC GROWTH FACTOR, INTERLEUKIN-3, USING AN AUTOMATED PEPTIDE SYNTHESIS APPROACH, Ian Clark-Lewis, Ruedi Aebersold, John W. Schrader+, Leroy E. Hood and Stephen B. H. Kent, California Institute of Technology, Pasadena, CA 91125, and +The Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia

Interleukin 3, a protein of 140 amino acids, was chemically synthesized using a fully automated peptide synthesizer and optimized solid phase chemistry methods. Extensive characterization of the synthetic product revealed that a high proportion of molecules had the correct primary structure and that the refolded crude material had the expected physiochemical properties. Techniques used were: Edman degradation sequence analysis, tryptic peptide mapping, amino acid analysis, SDS-polyacrylamide gel electrophoresis and reverse phase HPLC. Functional analysis of synthetic IL-3 and several analogues showed that 1) the crude synthetic material had high activity in the growth assay using IL-3-dependent cell lines and possessed the multiple activities attributed to IL-3; 2) an amino-terminal fragment had detectable activity; 3) the absence of the cysteine at position 17 of the molecule results in a greater than 1000 fold decrease in activity; 4) the stable tertiary structure of the complete molecule is required for maximal activity; and 5) the amino terminal 6 residues (the difference based on protein sequence data between the two forms of IL-3) was not required for activity. These results suggest that structure-function studies of proteins like IL-3 can be approached by automated peptide synthesis.

B22 HIGH LEVEL EXPRESSION OF RECOMBINANT INTERLEUKIN-2 RECEPTORS USING A BOVINE PAPILLOMA VIRUS DERIVED VECTOR LEADS ONLY TO LOW AFFINITY INTERLEUKIN-2 BINDING SITES, David Cosman, Janis Wignall, Andrew Lewis, Alan Alpert, Douglas Cerretti, Alf Larsen, Steven Dower, Steven Gillis and David Urdal, Immunex Corporation, Seattle, WA 98101

Interleukin 2 receptors on mitogen activated T cells or T cell derived cell lines have been shown to exist in two classes. The majority of receptors bind IL-2 with a relatively low affinity ($K_a \approx 5 \times 10^7$), while a small subclass of receptors bind IL-2 with high affinity ($K_a \approx 10^{12}$). No biochemical differences have yet been found between these two classes of receptors. We have cloned cDNAs encoding the human IL-2 receptor into a bovine papilloma virus derived vector, and transfected a mouse mammmary epithelial cell line (C127). Repetitive cycles of fluorescence-activated cell sorting, using a monoclonal antibody directed against the human IL-2 receptor, have led to the isolation of cell lines expressing between 2×10^5 and 5×10^6 molecules of IL-2 receptor per cell. However, all of these receptors bound IL-2 with low affinity. Similar results were obtained by expression of IL-2 receptor cDNAs isolated from HUT-102 T lymphoma cells, from normal, mitogen-activated T cells, or from a receptor cDNA in which serine-247, shown to be the site of protein kinase C-mediated phosphorylation, has been changed to an alanine residue.

B23 SERUM LEVEL HUMAN THYMOPOIETIN, Anthony Fuccello, Tapan Audhya and Gideon Goldstein, Ortho Pharmaceutical Corp., Raritan, N.J. 08869.

The measurement of human thymopoietin in human serum requires an assay with the ability to discriminate thymopoietin from splenin, a structurally similar peptide synthesized in the spleen. This degree of specificity has been successfully reported previously using monoclonal antibodies and bovine thymopoietin. We now report the preparation of a panel of anti-human thymopoietin monoclonal antibodies which do not cross-react with human splenin.

One of these monoclonal antibodies (HT2) was utilized to develop a competitive enzyme-linked immunosorbent assay (ELISA) for the measurement of thymopoietin in human serum. The sensitivity of the assay in 50 µgl ml serum, and the test can be performed in 3 hours.

B24 PROTECTION OF CATTLE AGAINST FOOT AND MOUTH DISEASE WITH A SYNTHETIC PEPTIDE, Charles Gale[1], Gerald Brooke[2], Timothy Doel[3], and Richard DiMarchi[2]. [1]Lilly Research Laboratories, Greenfield, IN; [2]Lilly Research Laboratories, Indianapolis, IN; [3]Animal Virus Research Institute, Pirbright, Surrey, United Kingdom.

A chemically synthesized peptide consisting essentially of two separate regions (residues 141-158, 200-213) from the 01K FMDV coat protein VP1, and free of any carrier protein elicted high levels of neutralizing antibody and protected cattle against intradermolingual challenge by innoculation with infectious virus. Comparative evaluation of this peptide with a single site peptide (residues 141-158) in guinea pigs suggests the importance of the VP1 carboxy-terminal residues in enhancement of the protective response.

B25 Induction of lymphokine and non lymphokine gene expression in human T blasts: effect of cyclosporin A. Angela Granelli-Piperno and Ralph M. Steinman, Rockefeller University, N.Y. N.Y. 10021.

The expression of lymphokine and non lymphokine genes have been studied in mitogen induced, human T lymphoblasts. Northern blotting analysis shows that c-fos mRNA peaks at 0.5 h and quickly disappeared, IL-2, γ-IFN and c-myc peaked at 3 h; IL-2 receptor and the 70K-heat shock protein (HSP) peaked at 20h or later.

The expression of IL-2 and γ-IFN mRNA's requires the synergistic action of lectin or OKT3 monoclonal antibody as was shown previously for the Jurkatt leukemia cell line. In contrast several other non lymphokine genes, c-fos, c-myc, IL-2 receptor and HSP were induced by lectin or PMA alone. Exogeneous IL-2 had little (IFN) or no (IL-2) effect on lymphokine mRNA. However IL-2 increased expression of c-myc, IL-2 receptor and HSP mRNA.

Cyclosporin A (CSA), a potent immunosuppressive agent strongly inhibited expression of IL-2 and γ-IFN. Induction of c-myc by lectin or by PMA was also blocked by CSA while c-fos, IL-2 receptor and HSP were unaffected. However CSA did not block the increase of c-myc and IFN mRNA's that were stimulated by exogeneous IL-2.

Taken together the data suggest that CSA act at a point between cell surface signalling and transcription, perhaps at a control site that is shared by c-myc and lymphokine promotors.

B26 **cDNA PREPARATIONS ENCODING THE T-CELL GROWTH FACTOR (INTERLEUKIN-2,IL-2) AND IL-2 RECEPTOR MODULATE T-LYMPHOCYTE RESPONSE TO HEPATITIS B VIRUS SURFACE ANTIGEN(HBsAg).** **Anwar A. Hakim. Loyola University Medical Center. Maywood. Illinois 60153.**

The study of the in vitro growth characteristics of T-lymphocytes(T-cells) contributes to the understanding of the critical elements necessary for DNA replication. T-cell growth factor (TCGF or IL-2) is produced by T-lymphocytes after antigen or mitogen stimulation, is required for the proliferation of activated T-cells and Natural Killer cells. IL-2 is an essential mediator of the immune response and is a requirement for the clonal growth of human lymphoblastic leukemia. T-cells secrete a variety of immunoregulatory polypeptides modulating cell responsiveness. T-cells from HBsAb-sero negative secrete traces, and the amounts of IL-2 increase by lymphocytes from HBsAb-sero positive prior and post vaccination with hepatitis B virus surface antigen(HBsAg). The in vitro proliferative response to HBsAg varied with the amounts of IL-2 secreted. IL-2 exhibits growth, inducing activity only on cells that have IL-2 receptors following antigen or lectin stimulation. mRNA preparations were isolated from T-cells obtained from the indicated groups of donors before and after vaccination with HBsAg and from patients with chronic active hepatitis (CAH-B). mRNA preparations were isolated from the T-cells before and after in vitro incubation with HBsAg. cDNA was prepared for each of these mRNA. Only cDNA complementary to mRNA from T-cells of HBsAb-sero positive vaccine recipients coded for IL-2. The amount of mRNA coding for IL-2 increased in activity and contration after in vitro incubation of the T-lymphocytes with HBsAg. The results indicate that IL-2 T-cell interactions displays all the characteristics ascribed to classic hormone receptor interaction, i.e. high affinity, saturability and ligand and tissue specificity.

B27 THYMOSIN MODULATION OF PITUITARY PEPTIDES. N.R. Hall, B.L. Spangelo, A. L. Goldstein. George Washington University, Washington, D.C. 20037

Thymosin peptides and lymphokine containing preparations were evaluated for their ability to modulate neuroendocrine circuits. This was accomplished by measuring pituitary and adrenal hormone secretion following the injection of thymosin fraction 5 (TSN-5) and purified component peptides both ip and into discrete brain regions and after adding them to cultures of pituitary or adrenal cells. Significant elevations in plasma ACTH, beta-endorphin and cortisol were time and dose dependent following the injection of TSN-5 into chronically cannulated monkeys (p 0.05). Activation of the pituitary-adrenal axis was also observed in rats and mice following the ip injection of TSN-5. No significant changes in C-AMP or corticosterone release were measured when a number of thymosin preparations were incubated with isolated adrenal fasciculata cells. However, a significant increase in ACTH and beta-endorphin release was observed when TSN-5 was incubated with cultured pituitary cells. Other studies have been carried out to evaluate the immunomodulatory potential of prolactin. In contrast to glucocorticoids, prolactin, which is also released in the presence of thymosin, exerts stimulatory effects upon measures of immunity. It was hypothesized that a component of TSN-5 is capable of activating the pituitary-adrenal axis at the level of the pituitary gland and possibly the central nervous system as well. In this capacity, it is proposed that thymosin peptides function as immunotransmitters to indirectly modulate functioning of the immune system. Other hormonal circuits may act in concert with the pituitary-adrenal axis to provide additional modulatory effects.

B28 NEUTRALIZING RABBIT ANTIBODIES TO SYNTHETIC IL-2 PEPTIDES, James Jenson, Waleed Danho, W-H. Tsien, and Maurice Gately, Hoffmann-La Roche Inc., Nutley,N.J. 07110.

Seventeen peptides which cover the entire human IL-2 sequence in an overlapping fashion were synthesized by the solid phase peptide methodology. The peptides were conjugated to KLH by the MBS method and used to immunize rabbits All 17 peptide-KLH conjugates gave antisera which reacted with free peptide in ELISA. The anti-peptide reactivity was specific in that antisera reacted only with the peptide used for immunization and corresponding overlap peptides Sixteen of the antisera also reacted with intact rIL-2 in ELISA and Western blots. The anti-protein reactivity could be inhibited with the peptide immunogen but not with unrelated peptides. All anti-peptide antisera were tested for neutralization of human rIL-2 in the CTLL bioassay and a radio-receptor binding assay. Rabbit antisera against peptides IL-2(27-41) and IL-2(23-41) neutralized both bioactivity and binding in a dose-dependant manner The neutralizing activity could be demonstrated with affinity purified IgG and was overridden with peptide IL-2(27-41). No other anti-peptide antisera exhibited detectable neutralizing activity. These results suggest that the IL-2(23-41) epitope could be located within or near the receptor binding site of IL-2.

B29 NEUROPEPTIDES AS MODULATORS OF LYMPHOCYTE FUNCTION. Howard M. Johnson. Department of Comparative and Experimental Pathology, The University of Florida, Box J-145, JHMHC, Gainesville, FL 32610

The immune and neuroendocrine systems are functionally and structurally related. This is illustrated under several conditions: (1) Neuroendocrine hormones such as ACTH, endorphins, enkephalins, thyrotropin, and arginine vasopressin can regulate important immune functions, examples of which are enhancement and suppression of antibody production, replacement of lymphokine requirement for production of other lymphokines, and enhancement or suppression of cytotoxic activity of lymphocytes. (2) In addition to responding to neuroendocrine hormones, lymphocytes also produce hormone-like activities such as ACTH, endorphins, and thyrotropin, suggesting that hormones are natural regulators of lymphocyte activity. There is evidence that lymphocyte-derived immunoreactive neuroendocrine hormones can perform the same functions as those of hormones derived from a neuroendocrine source. The fact that lymphocytes can produce hormones that act on the immune system makes these substances lymphokines by definition. Some of the immunoregulator functions of these neuroendocrine hormones-lymphokines are mediated by second messengers such as diacylglycerol, arachidonic acid and its metabolites, as well as by cyclic nucleotides.

B30 PARTIAL CHARACTERIZATION OF THE GENES ENCODING BOVINE AND HUMAN IMMUNOREGULATORY PEPTIDE HORMONES, Linda K. Jolliffe, Susan F. Trinker, Virginia L. Pulito, and Gideon Goldstein, Ortho Pharmaceutical Corporation, Raritan, NJ 08869

Several peptide hormones have been shown to be involved in T and B lymphocyte maturation. The best characterized peptide hormone, thymopoietin has been sequenced in both human and bovine systems and shown to induce prothymocyte differentiation (T. Audhya, D.H. Schlesinger, and G. Goldstein (1981) Biochemistry 20, 6195; M.P. Scheid, G. Goldstein and E.A. Boyse (1978) J. Exp. Med. 147, 1727). Characterization of thymopoietin mRNA was accomplished through the use of synthetic 60-mer oligonucleotide probes complementary to amino acids 17-36 of the human polypeptide and amino acids 30-49 of the bovine polypeptide. The human probe (H60) binds specifically to two species of poly (A) RNA from cultured human skin cells (3000 and 6000 bases). The bovine probe hybridizes to a single species in fetal calf thymic mRNA corresponding to 3000 bases. Southern blot hybridization analysis of bovine DNA digests with the B60 probe suggests there may be two gene copies present.

B31 INTERFERON-GAMMA BINDS TO HIGH AND LOW AFFINITY RECEPTOR COMPONENTS ON MOUSE MACROPHAGES. Patricia P. Jones and Ramani A. Aiyer, Dept. of Biol. Sciences, Stanford University, Stanford, CA 94305.

Interferon-γ, a glycoprotein secreted by activated T lymphocytes, activates many macrophage functions, and modulates immune responses. In the mouse macrophage cell line WEHI-3 murine interferon-γ (MuIFN-γ) induces the increased expression of Ia and H-2 antigens of the major histocompatibility complex (MHC). Using [^{125}I]-MuIFN-γ, we have identified specific receptors on WEHI-3 and other mouse cells. Binding of [^{125}I]-MuIFN-γ was: 1) time- and temperature-dependent, 2) species-specific, and 3) competitively inhibited by "cold" MuIFN-γ, but not MuIFN-α or MuIFN-β. Analysis of steady-state binding, at 37°C, yielded a curvilinear Scatchard plot, consistent with the presence of two classes of binding sites with K_ds of 9.1×10^{-11} M (500 sites/cell) and 2.7×10^{-9} M (4400 sites/cell). This result differs significantly from that obtained by various other groups that have reported only a single class of IFN-γ binding sites with K_ds ranging from 10^{-8} M to 10^{-10} M. Comparison of MuIFN-γ binding with the dose-response for MHC antigen induction on WEHI-3 cells indicates that the higher affinity sites most likely represent the physiologically relevant MuIFN-γ receptors. Furthermore, the half-maximal biological response occured at a fractional occupancy by MuIFN-γ of only 5% of the high-affinity receptors.

(Supported by: NIH, AI-19964)

B32 SITE-DIRECTED MUTAGENESIS OF THE HUMAN INTERLEUKIN-2 GENE PRODUCT: IDENTIFICATION OF AMINO ACID RESIDUES REQUIRED FOR BIOLOGICAL ACTIVITY

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We have used site-directed mutagenesis to locate the functional domains of the Interleukin-2 (IL-2) protein. A biologically active cDNA clone of human IL-2 was mutagenized at specific sites using synthetic oligonucleotides to incorporate nucleotide changes which resulted in defined amino acid substitutions and deletions in the mature protein. The IL-2 analogues were then produced in E. coli, and assayed for 1) ability to induce proliferation of IL-2 dependent cells, 2) ability to compete for binding to the IL-2 receptor, and 3) immunoreactivity with neutralizing monoclonal antibodies. Our analysis of over 40 different mutations demonstrates that the integrity of four regions of the IL-2 molecule are required for full biological activity: 1) the NH_2-terminus (residues 1 to 20), 2) an internal region (residues 30 to 60), 3) the COOH-terminus (residues 124-133), and 4) two of the three cysteine residues (58 and 105). The NH_2-terminal portion and the internal region are recognized by neutralizing anti-IL-2 antibodies. Deletion of the NH_2-terminal 20 amino acids or the COOH terminal 10 amino acids results in the loss of greater than 99% bioactivity. Amino acid substitutions at specific positions in these regions also result in proteins which retain less than 1% bioactivity. These data are consistent with the model of the IL-2 protein being folded such that the NH_2-terminus, the COOH terminus, and the internal 30 to 60 region are juxtaposed to form the binding site recognized by the IL-2 receptor.

B33 ISOLATION OF cDNA CLONES FOR HUMAN COLONY STIMULATING FACTOR-1 (CSF-1). E.Kawasaki, M.Ladner, P.Ralph, K.Warren, J.Van Arsdell, A.Wang, K.Wilson, A.Boosman, E.R.Stanley and D.Mark. Cetus Corp., 1400 Fifty-Third St., Emeryville, CA. 94608

Complementary DNA clones encoding human macrophage-specific colony stimulating factor-1 (CSF-1) were isolated. One cDNA clone codes for a mature polypeptide of 224 amino acids and a putative leader of 32 amino acids. This cDNA, which was cloned in the Okayama-Berg expression vector, specifies the synthesis of biologically active CSF-1 in COS cells, as determined by a specific radioreceptor assay, macrophage bone marrow colony formation, and antibody neutralization. Most of the cDNA isolates contain part of an intron sequence that changes the reading frame, resulting in an abrupt termination of translation; these cDNA's were inactive in COS cells. The CSF-1 appears to be encoded by a single-copy gene, but its expression results in the synthesis of several messenger RNA species, ranging in size from about 1.5 to 4.5 kilobases.

B34 CONFORMATION OF CYCLIC PENTA- AND HEXAPEPTIDES DERIVED FROM THYMOPOIETIN, Horst Kessler, Bernhard Kutscher, Armin Klein, Rainer Kerssebaum, Rainer Obermeier, Hubert Müllner, Jörg Lautz, Winfried van Gunsteren; J. W. Goethe University, D-6000 Frankfurt 50, W.-Germany; Hoechst AG, Frankfurt, W.-Germany; University Groningen, Holland

Cyclic penta- and hexapeptides, which contain the modified sequence of thymopoietin -Arg-Lys-Asp-Val-Tyr- have been studied by modern NMR spectroscopy including several two-dimensional techniques. Cyclic pentapeptides in which one amino acid is D-configurated prefer a βII'γ-conformation with the D-residue in i+1 position of the βII' turn. A systematic change of the amino acids and their configuration allows to build up molecules in which the different side chains have a limited spatial distribution. The most active compound turned out to be cyclo(-Arg-Lys-Glu-D-Val-Tyr-). The close proximity of the Arg and Tyr residue, seems to be essential for high activity. This arrangement cannot be reached in stretched conformation of the linear TPS sequence. Also in cyclic hexapeptides in which the above mentioned sequence is bridged by a Val or a Gly residue, the distance between Arg and Tyr seems to be too large to observe high biological activity.

B35 BIOLOGICALLY STABLE ANALOGS OF THYMOPENTIN, Daniel J. Kroon, Douglas Riexinger and George A. Heavner, Ortho Pharmaceutical Corporation, Raritan, New Jersey 08869

Analogs of the immunoregulatory peptide thymopentin with structural modifications designed to stabilize the molecule against digestion by peptidase were synthesized. Analogs were found that maintained activity in T-cell assay systems. The stability of these analogs against isolated enzymes and human serum was determined. The rate of clearance from circulation of several of the analogs after i.v. administration to guinea pigs was studied.

B36 STRUCTURE-FUNCTION RELATIONSHIPS FOR IL-2 and the IL-2 RECEPTOR. Li-Mei Kuo* and Richard J. Robb*. E. I. du Pont de Nemours, Central Research and Development, Glenolden, PA 19036*.

Interleukin 2 promotes proliferation and differentation of various activated lymphocytes by virture of its high-affinity interaction with a specific cell-surface receptor (the Tac glycoprotein). We have used synthetic peptides of IL-2 and its receptor in an attempt to map regions of the two molecules important for their functional interaction. Our results indicate that two different antibodies which react with peptides encoded within the N-terminal half of the IL-2 molecule are capable of blocking receptor association and bioactivity. A third antibody, reactive with the N-terminus of the molecule, was capable of inhibiting receptor association in short-term binding assays but had only a marginal effect upon the proliferation of IL-2-dependent target cells. One explanation of these findings is that the N-terminus of IL-2 contains one or more receptor contact sites. The results of the third antibody, however, emphasize the need for caution in quantitatively equating high-affinity receptor binding with the magnitude of the functional response. With regard to the IL-2 receptor, we have generated a number of polyclonal antibodies to synthetic fragments which are capable of recognizing purified, intact Tac protein in an Elisa assay. Only a few of these antibodies also recognize the receptor on whole cells and of these, none blocks the binding of radiolabelled IL-2 or anti-Tac monoclonal antibody or interferes with the proliferative response. Considering the location of the epitopes defined by these reagents, the N-terminal portion of the Tac protein is the most likely candidate for encoding the ligand binding site.

B37 CONSTITUTIVE AND INDUCED INTERLEUKIN-1 PRODUCTION BY A HUMAN ASTROCYTOMA CELL LINE. John C. Lee, Philip L. Simon, Peter Young and Gilliam Beattie, Smith Kline and French Laboratories, Philadelphia, PA 19101 and University of California, San Diego, CA 92093.

In addition to monocytes/macrophages, astrocytes and glial cells of different species have been suggested to produce interleukin-1 (IL-1) in vitro. In all cases, however, the evidence presented relied solely on the detection of an interleukin-1 like biological activity. We now report that a human astrocytoma cell line (T24) produces IL-1 constitutively and upon induction with phorbol myristic acetate (PMA) in vitro. The IL-1 activity was measured both by the conventional thymocyte co-mitogenesis assay as well as a modified assay measuring IL-1 dependent induction of IL-2 production by EL-4 cells. The active molecule had a molecular weight of 17 Kd on gel filtration and isoelectric point of 5.2. The activity was not neutralized by a goat antibody reacting against pI 7 IL-1. In contrast, a rabbit antibody reacting against pI 5 and pI 7 IL-1 did not neutralize the activity, suggesting that the astrocytoma derived IL-1 was of the pI 5 type. However, northern blot analyses of the m-RNA isolated from PMA induced astrocytoma cells using synthetic oligonucleotide probes corresponding to the published nucleotide sequence for both IL-1 forms indicate that m-RNAs encoding both IL-1 species were present. The reason for the inability to detect pI 7 IL-1 activity in culture supernatants is not presently known. These results provide unequivocal evidence that human astrocytoma cells synthesize both forms of IL-1 m-RNA and that only the biological activity of pI 5 form is detectable in culture supernatants.

B38 REGULATION OF T CELL FUNCTION WITH MONOCLONAL ANTI-THY-1 ANTIBODIES, D.N. Männel, W. Dröge, and W. Falk. German Cancer Research Center, D-6900 Heidelberg, F.R.G.

The effect on T cell activities of three different monoclonal rat antibodies (mAb) directed against the murine Thy-1 structure was studied. Two mAb recognized the nonpolymorphic determinant. One mAb was specific for Thy-1.2. All three mAb precipitated Thy-1 from T cell membranes. Also, soluble Thy-1 was precipitated from supernatants of stimulated T cells. The mAb in ng amounts induced IL2 production in T cell lines and proliferation in spleen and lymph node cell cultures. Thymocyte proliferation was induced by the addition of IL2 together with the mAb. Development of cytotoxic T cell responses in mixed lymphocyte bulk cultures as well as in lymphokine-activated thymocyte cultures was inhibited by the mAb. The cytolytic phase was not affected. The data suggest the participation of Thy-1 in a signal transducing event of T cell activation.

B39 CELLULAR INTERACTIONS OF TUFTSIN AND ITS AUGUMENTATION OF NATURAL CELL-MEDIATED IMMUNITY, K. Nishioka, K. Huang, J. Wagle, R. Banks, T. El-Hagin, S. Yim, A. Amoscato and J. Phillips, University of Texas M. D. Anderson Hospital and Tumor Institute, Houston, TX 77030

We originally reported the presence of tuftsin (Thr-Lys-Pro-Arg), derived from leukophilic IgG, as a substance in sera capable of stimulating neutrophil phagocytosis. We have demonstrated antitumor activity of this peptide in a variety of murine tumor models. In vitro cytotoxicity studies showed that tuftsin was able to stimulate granulocytes, monocyte-macrophages and NK cells. We prepared highly purified fluoresceinated (F) tuftsin (only at N-terminal), which was fully active in stimulating neutrophil phagocytosis. F-tuftsin specifically bound these effector cells, formed patches, and was internalized. No binding was observed on lymphocytes. To study the distribution of tuftsin, [L-3,4-dehydroproline^{3}]tuftsin, tuftsin containing double bond in proline ring for tritiation purpose, was prepared. This tuftsin analog, which showed higher phagocytosis stimulation than tuftsin, was then tritiated to produce ^{3}H-tuftsin, and utilized to examine the distribution pattern in mice. In the brain, high specific activity was found in the hypothalamus and pituitary gland. Short-term cultures of rat pituitary glands were then examined with F-tuftsin. While most pituitary secretory cells became labeled with tuftsin, fibroblasts were not. These results suggest possible neuromodulating function of tuftsin through hypothalamus-pituitary gland axis in addition to direct augmentation of the effector cells.(USPHS CA27330).

B40 DETECTION AND CHARACTERIZATION OF THE CELL SURFACE RECEPTORS FOR MURINE AND HUMAN GRANULOCYTE-MACROPHAGE COLONY STIMULATING FACTOR, Linda S. Park, Steven Gillis and David L. Urdal, Immunex Corporation, Seattle, WA 98101

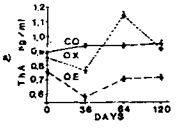
^{125}I-labeled recombinant murine and human GM-CSF were used to characterize receptors specific for this lymphokine on cells of both myelomonocytic and T cell origin. On all cell types examined, GM-CSF bound to a single class of high affinity receptor (1000-5000 receptors per cell for mouse and 100-300 receptors per cell for human) with a K_a of 10^8 - 10^9 M^{-1}. Both hormones exhibited complete species specificity. Binding was rapid and saturable at 37°C with a slow subsequent dissociation rate. Among a panel of lymphokines and growth hormones, only unlabeled GM-CSF was able to compete for binding of ^{125}I-GM-CSF to cells. Affinity crosslinking experiments with murine GM-CSF and the homobifunctional crosslinker disuccinimidyl suberate resulted in the identification of a murine receptor protein with a M_r of 130,000 on five out of seven cell types examined (P388D_1, PU5-1.8, Wehi-3B, LSTRA and LBRM-33). This protein was extremely sensitive to proteolysis and in the absence of protease inhibitors was degraded to a form with an approximate M_r of 70,000. A receptor protein of M_r 180,000, in addition to the M_r 70,000 protein, was found on bone marrow and P815 cells. The potential tissue-specific molecular heterogeneity associated with the GM-CSF receptor may help explain some of the diverse biological effects attributed to this lymphokine.

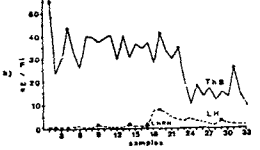
B41 STABLE MOUSE CELL LINE PRODUCING HUMAN INTERLEUKIN-1. R. C. Reuben, D. S. Neblock, G. A. Koch, R. H. Malavarca, P. J. Lisi, R. A. Zivin, P. E. Auron, C. A. Dinarello, and T. J. Livelli, Cistron Technology, Pine Brook, NJ 07058 and New England Medical Center, Inc., Boston, MA 02111. The cDNA of human interleukin-1 (pI 7), molecularly cloned from human monocyte mRNA (Auron et. al. PNAS 81:7907, 1984), was transfected into mouse L cell fibroblasts and a cell line isolated which constitutively synthesizes human interleukin-1. Cloned cells were screened by quantitation of Il-1 mRNA synthesis and by LAF (lymphocyte activating factor) assay. One clone selected for further study synthesizes 20-50 fold more Il-1 mRNA than human monocytes. This clone contains approximately 50 copies of the human Il-1 cDNA integrated into its genome. It synthesizes a 35 kilodalton protein which is not present in the parental cell line transfected with a related plasmid not containing the Il-1 cDNA. This protein converts to a smaller protein which stimulates mouse thymocytes to proliferate and human fibroblasts to synthesize PGE_2. The 35 kd protein and the proteolytic fragments derived from it react on a Western blot with rabbit polyclonal antibodies raised against human monocyte Il-1.

B42 LH, THYMOSIN, α_1 AND β_4, LEVELS IN COWS TREATED WITH LHRH OR ESTRADIOL, D. Vakharia, S. Echternkamp, M. Day, J. Kinder and T. Wise, Univ. Nebraska, Lincoln, and USDA ARS RLH US MARC, Clay Center NE 68933

In our attempt to understand the interrelation between thymosin α_1,ThA; or thymosin β_4,ThB, and luteinizing hormone (LH), we have undertaken studies wherein serum levels of ThA and ThB were monitored in animals whose LH levels were altered by either exogenous treatment with LHRH or estradiol(E). The data for a) LH and ThA levels in prepubertal normal (C, n=6), ovariectomized (OX, n=5) or OVX+E (OE, n=5) cows and b) LH and ThB levels in cows (n=3) injected with LHRH (25 µg) are presented. The hormone levels in a) sequential blood samples collected every 12 min for 8 hrs, various days until puberty and b) at every 15 min, 4 hrs before and after LHRH injection were quantitated by RIA. The temporal pattern for each animal was subjected to PULSAR algorithmic procedure to estimate mean (overall, OM, and baseline, BM) concentration, number of peaks (P) and mean amplitude (A) of P of each hormone and to study the pattern of hormone release. The difference in the pattern of hormonal release and the residual correlation between release of LH and ThA or ThB following treatment were calculated by subjecting the data to split-plot ANOVA and multivarient ANOVA. Unlike LH levels, ThA in OE cows were lower (P<0.1) than OX or C. The secretory pattern for ThA and LH was different between treatment (P<0.05). ThB did not rise following LHRH injection whereas LH did. There was a negative correlation between LH and ThB parameters (OM, BM, A; r=-.99, P<.04). The data suggests that with the increase in LH levels following estradiol or LHRH, there is corresponding drop in ThA or ThB, respectively.

a) LH data, see --
M. Day et al.,
Biol. Reprod. 31, 332-341, 1984.





B43 BINDING OF THYMOPOIETIN TO ACETYLCHOLINE RECEPTOR, Krishnamoorthy Venkatasubramanian, Tapan Audhya and Gideon Goldstein, Ortho Pharmaceutical Corporation, Raritan, N.J. 08869.

Thymopoietin, a 49 amino acid polypeptide hormone of the thymus, has a number of biological effects including effects on neuromuscular transmission, T cell differentiation and regulation of the immune response. The present study was aimed towards understanding the mechanism of action of thymopoietin (TP) in inducing the neuromuscular impairment. Acetylcholine receptor rich membranes were prepared from Torpedo californica and was used to study the binding characteristics of ^{125}I labelled thymopoietin. Thymopoietin not only binds to the acetylcholine receptor but actually competes for the α-bungarotoxin binding site. The binding of thymopoietin and α-bungarotoxin to the acetyl choline receptor was fully displaced by one another as well as by carbachol. On the other hand splenin, which differs from thymopoietin at position 34, and does not affect neuromuscular transmissions, did not compete for binding to acetylcholine receptor. These studies suggest that thymopoietin binds to the acetylcholine binding site of the acetylcholine receptor and this could be one of the mechanisms by which it induces neuromuscular impairment in certain thymic disorders, particularly myasthenia gravis.

Cellular Immunology

B44 NEUROENDOCRINE CELLS IN THE IMMUNE SYSTEM, Ruth Hogue Angeletti and William F. Hickey, University of Pennsylvania, Philadelphia, PA 19104

Immunochemical and immunohistochemical analyses of rat lymphoreticular tissues were carried out using antibodies to chromogranin, a secretory protein which is a marker for the diffuse neuroendocrine system. Strongly positive cells were present in spleen, lymph node, thymus, fetal liver and lamina propria. These chromogranin-positive cells were found to be negative when tested with antibodies for the Ia surface immunological marker. When these organs were gently dispersed and separated on a 1.077 g/ml Ficoll gradient, it was found that the chromogranin immunoreactive cells became enriched in the dense red cell pellets. The cells from the spleen have been further purified, and their immunological properties characterized. On the basis of the unexpected distribution of these neuroendocrine cells in all immunologically relevant structures, it is possible that they form an important link between nervous and immune systems.

B45 EFFECT OF THYMOPOIETIN (TP5) ON INTERLEUKIN-2 PRODUCTION BY NORMAL PERIPHERAL BLOOD MONONUCLEAR CELLS, Anthony C. Antonacci, MD, Joan Chiao, MD, Alyce Reid, RN, and Steve Calvano, PhD, The New York Hospital-Cornell Medical Center, New York, New York 10021

Thymic hormones are known to be critical to the development of adequate immune function particularly with respect to T-cell differentiation. Interleukin-2 is also an extremely important lymphokine responsible for complete proliferative and amplification responses. In this study we attempted to evaluate the effect of Thymopoietin (TP5; Ortho Pharm.) on IL-2 production by normal peripheral blood mononuclear cells.

Ficoll-hypaque separated mononuclear cells from forty-one normal controls were quantitated by light scatter analysis on an Ortho Spectrum III flow cytometer. 1×10^6 lymphocytes/ml were cultured for 48 hours at 37°C / 5% CO_2 in 5% FCS-CM under four conditions: 1) -PHA/-TP5; 2) -PHA/+TP5; 3) +PHA/-TP5; 4) +PHA/+TP5. Final concentrations of purified PHA and TP5 were 1 ug/ml and 100 ug/ml, respectively. Cultures were centrifuged and serial dilutions of each supernatent were assayed for IL-2 production using an IL-2 dependent murine cell line, CTLL-2. Units of IL-2 were calculated by log/logit method relative to commercially obtained rat TCGF. Quality control supernatents were run for each assay with a % coefficient of variation over 8 assays equal to 14%.

IL-2 production is summarized below as mean units ± S.E.:

	-PHA / -TP5	-PHA / +TP5	+PHA / -TP5	+PHA / +TP5
IL-2 Prod.	.13 ± .05	.15 ± .06	15.54 ± 2.3	14.36 ± 2.2
P. Value	N. S.		N. S.	

No statistically significant differences were observed among treatment groups, suggesting that TP5 does not exert a direct cellular effect on IL-2 production in normal peripheral blood lymphocytes.

B46 CELL FREE T LYMPHOCYTE ACTIVATION SIGNAL, Arthur A. Vandenbark and Halina Offner, Veterans Administration Medical Center, Portland, OR 97207

Activation of a myelin basic protein specific T lymphocyte line (BP-1) requires presentation of an epitope surrounding residue 79 of guinea pig basic protein (GP-BP) by accessory cells which express major histocompatibility complex (MHC) gene products. To determine if the T cell activation signal was APC associated or was shed into the medium, supernatants from an APC population pulsed with GP-BP were collected and used to stimulate resting BP-1 cells. Supernatants containing GP-BP but not Bovine-BP or PPD induced highly significant proliferation of the BP-1 line cells, and transfer of these supernatant activated cells produced clinical signs of experimental autoimmune encephalomyelitis and delayed type hypersensitivity reactions to GP-BP. The production of stimulatory supernatant activity occurred optimally over a 6 hour period, and could be enhanced by the addition of factor(s) from activated T lymphocytes. The GP-BP component was not inhibited by either of two monoclonal antibodies directed at determinants which flank the epitope recognized by BP-1 cells. However, activity was inhibited by an anti-I-A but not an anti-I-E monoclonal antibody, suggesting the involvement of a Class II MHC gene product in T cell activation. These results suggest that in the presence of BP, activated APC produce and shed the encephalitogenic T cell activation signal into the surrounding medium without need for direct T cell-APC contact.

B47 INFLUENCING OF ANTIBODY PRODUCTION WITH THYMOPENTIN; AN IN VIVO MODEL FOR IMMUNOMODULATION, Bolla, K., *Duchateau, J., **Cappel, R., Cilag Ltd. Technical Center, CH-Schaffhausen, *University Hospital St. Pierre, Brussels and **Inst. Pasteur, Dept. Virology, Brussels.

Drugs or biological substances which - depending on dose, route of administration, timing of dose and immune status of the treated subject - can either stimulate or inhibit various immune processes are generally accepted as immunomodulators. The influence of these substances on antibody production provides a well measurable model for demonstration and study of various factors on which the effects of immunomodulators depend.

Based on the result of many years' investigations into thymopentin, evidence shows that the four above-mentioned characteristic features, which influence - or even determine - the effect of an immunomodulator, can be restricted to only two of them. The term "dose-dependence" probably includes the influence of the route of administration, while the dependence on the immune status of the subject treated expresses the importance of timing. General aspects of thymopentin-influenced antibody production, elaborated in human and animal models, are presented. The validity of these aspects is demonstrated by clinical investigations into vaccination against hepatitis.

B48

Surface Ia antigens have a functional role in induction of murine B lymphocyte proliferation. Bondada Subbarao and Arthur R. Baluyut; Sanders Brown Research Center on Aging and Dept. of Medical Microbiology and Immunology, University of Kentucky, Lexington, KY.

Several stimuli that induce B lymphocyte activation have been shown to increase the amount of cell surface Ia on the B cells. Some of these stimuli are divalent antibodies to surface immunoglobulin, lipopolysaccharide, and a T cell derived lymphokine, called BSF-p1. However, thus far this hyper Ia expression has not been shown to have any direct functional role in B lymphocyte activation. Here we show that B cell Ia antigens may provide an activation signal for growth. We find that under serum free conditions, anti-IgM and anti-Ia antibodies induce very little B cell growth by themselves but synergize with each other in inducing B cell proliferation. This synergistic action of the anti-Ia with anti-IgM antibody does not require the presence of T cells, is allele specific, and can be observed with IA as well as IE region specific monoclonal antibodies. Furthermore, the effect of anti-Ia is not due to increased secretion of interleukin 1 since no significant increase of IL-1 is found in these cultures as determined by the thymocyte costimulation assay. Our results support a recent proposal by Corley et al. that Ia molecules act as transducers for B cell activation, based on their studies with a B cell lymphoma.

B49 DEXTRAN SULFATE ENHANCEMENT OF LIPOPOLYSACCHARIDE INDUCED PROLIFERATION OF RAT LYMPHOCYTES. MECHANISM OF ACTION. H.G. Archie Bouwer, Tom Thieme and Steven Hefeneider. Providence Medical Center and Vet. Ad. Hosp. Portland, OR.

We have studied lipopolysaccharide (LPS) induced proliferation of rat lymphocytes and found that serum free tissue culture medium will support significant cell division in the presence of this mitogen. When the compound dextran sulfate (DxS), which itself is not mitogenic for rat lymphocytes, is added to LPS stimulated cultures, significant augmentation of proliferation results. An intact T-cell compartment is not required for this augmentation in that lymphocytes derived from athymic rats proliferate and respond to the influence of DxS to the same degree as euthymic derived lymphocytes. A DxS free supernatant from DxS stimulated spleen cell cultures is able to substitute for DxS in all stimulatory activity. This supernatant possesses interleukin 1 (IL-1) activity. However, purifed recombinant IL-1 does not cause enhanced proliferation when added to LPS stimulated cultures as is seen with DxS or DxS free supernatants. A supernatant from DxS stimulated adherent spleen cells will also cause enhanced proliferation to similar levels compared with culture supernatants from DxS stimulated "intact" spleen cell cultures. The DxS stimulated adherent cell supernatant was however virtually devoid of detectable IL-1 activity. The active component present in the adherent cell derived culture supernatant appears to be distinct from IL-1.
Supported by Grant # NS 16731

B50 INTERLEUKIN-2 INDUCES IgM SECRETION IN LY1+ NEOPLASTIC MURINE B CELLS (BCL_1). Kathryn H. Brooks, Jonathan W. Uhr and Ellen S. Vitetta, Department of Microbiology, University of Texas Health Science Center, Dallas, Texas 75235

Two neoplastic B cell clones have been used to examine the effect of interleukin-2 (IL-2) on B cell differentiation. Both clones express high levels of sIgM but relatively little sIgD, however, they differ in their expression of the Ly1 marker. The Ly1+ BCL_1-derived clone secretes IgM in response to purified and recombinant IL-2. This differentiative response can be blocked with anti-IL-2 monoclonal antibody. In contrast, the Ly1- AKR-225-derived clone does not differentiate in response to IL-2 or IL-2 plus recombinant IFNγ. Both the BCL_1 clone and the AKR-225 clone can differentiate in response to B cell stimulatory factor(s) present in supernatant (SN) from the alloreactive T cell line, PK 7.1. This PK 7.1 SN lacks significant IL-2 activity but does contain the B cell differentiation factor, BCDFμ. The differential activation requirements of these Ly1+ and Ly1- B cells is intriguing in light of both the association of Ly1+ B cells with autoimmune disease and the production of a B cell growth factor by the Ly1+ BCL_1 clone but not the AKR-225 clone.

B51 T CELL/ENDOTHELIAL CELL INTERACTIONS IN VIVO, Denis R. Burger, Blayne A. Standage, and R. Mark Vetto, Veterans Administration Medical Center, Portland, OR 97201

We have proposed a hypothesis in which vascular endothelial cells (VE) rather than or in addition to bone marrow (BM) derived cells play an integral part of antigen presentation in cell-mediated immune phenomenon including delayed-type hypersensitivity (DTH). The hypothesis suggests that VE present antigen in context of Ia on the lumenal surface of capillary vessels. Antigen-specific T cells trigger the antigen-armed VE resulting in the release of factors which lead to the subsequent sequelae known collectively as cell-mediated immunity. In order to test the validity of this hypothesis in vivo, an adoptive transfer system was utilized where DTH was passively transferred to chimeric rats which were constructed so that bone marrow-derived cells and non-bone marrow-derived cells were of different RT-1 haplotypes (RT-1 is the MHC of the rat). The results indicate that when the antigenically naive recipients received immune donor lymphocytes, DTH responses were only observed when donor cells and recipient endothelium shared a RT-1 haplotype. Transfer of DTH was not observed even when donor cells and recipient BM-derived cells were compatible if the endothelial cells were histoincompatible. Thus the role of non-bone marrow derived endothelial cells in the DTH reaction is critical.

B52
THE ROLE OF IL-2 IN PROLIFERATION OF HUMAN ACTIVATED B CELLS, Yong Sung Choi, Mary Carroll and Pamela Trail, Alton Ochsner Medical Foundation, New Orleans, LA 70121

Although rapid progress has been made in characterizing IL-2 molecules biochemically and cloning the IL-2 gene, the progress of BCGF research has been hampered due to the lack of a reliable assay method and of sufficiently large quantities of purified BCGF. It is crucial to have a BCGF-specific assay system for biochemically purifying BCGF because conventional methods for protein purification cannot separate IL-2 from BCGF. The target cells currently used for the assay (i.e. SAC or anti-μ antibody stimulated B cells) cannot distinguish IL-2- from BCGF-dependent proliferation. Hence we have developed a simple reproducible method for specifically measuring BCGF activity in the presence of (without the interference of) IL-2. Using this assay method and recombinant IL-2, we show the experimental evidence that IL-2 has a definite synergistic effect on BCGF-induced proliferation of activated B cells and that BCGF is a distinct lymphokine from IL-2.

B53 THE EVOLUTION OF IMMUNOREGULATORY POLYPEPTIDES: STRUCTURE AND FUNCTION OF IL2-LIKE MOLECULES IN THE AMPHIBIAN, XENOPUS.
Nicholas Cohen and David Watkins, University of Rochester, Rochester, NY 14642

Supernatants (SNs) from PHA-stimulated Xenopus splenocytes (chicken erythrocyte absorbed) costimulate thymocytes, induce blast cell proliferation, and facilitate the establishment of MLR alloreactive and cytotoxic T cell lines. These SNs do not cause proliferation of mammalian T cells and mouse and human IL2-rich SNs and recombinant human IL-2 do not stimulate Xenopus T cells. Ammonium sulfate precipitation of and Xenopus SNs passage over a Sephacryl-200 sizing column showed that the stimulatory activity was in the 20-40 kD fraction. Further characterization using DEAE-Sephadex followed by SDS-PAGE of the resulting active fraction, revealed a band in the 15-25 ID range using ^{125}I labelling. Although PHA stimulates larval and adult splenocytes as well as adult thymocytes, it does not stimulate larval thymocytes. IL-2 rich SNs plus PHA, however, do co-stimulate larval thymocytes. Preliminary studies suggest deficient IL2 production by PHA-stimulated adult as well as larval thymocytes relative to larval and adult splenocytes. Splenocytes from adult frogs that were thymectomized (txd) at day 10 of larval life do not respond well to PHA and Con A. Culturing of these splenocytes with PHA and an IL2-rich SN for 6 days renders them responsive to PHA and Con A. Additionally, splenocytes from such txd frogs are capable of producing an IL2-rich SN.

B54 FURTHER CHARACTERIZATION OF IL-B4 -- FORMERLY TERMED B CELL-DERIVED ENHANCING FACTOR (BEF), Paolo del Guercio, John F. Marcelletti, Riaz I. Zuberi, Marie-France del Guercio and David H. Katz, Medical Biology Institute, La Jolla, CA 92037.

IL-B4, formerly termed B cell-derived enhancing factor (BEF), is a soluble mediator constitutively and exclusively produced by cells of the B cell lineage, and acting on T cells by reducing the activation of suppressor cells. As a result, antigen-driven antibody responses both in vitro and in vivo are markedly increased when IL-B4 is added to spleen cell cultures or mice are injected with IL-B4 before immunization. IL-B4 is a regulatory molecule distinct from Ig and lacks Ig determinants. Monoclonal antibody (MF.414-13.49) (μ,κ) was generated by fusing a murine myeloma line with spleen cells from rats immunized with mouse IL-B4. Purified MF.414-13.49 was highly active in inhibiting IL-B4 regulatory activities in vitro. In solid phase assay, the antibody recognizes IL-B4, but not IL-1, IL-2, γ-interferon, or any of the mouse Ig isotypes. In further studies, anti-IL-B4 mAb was used for purification of IL-B4 from crude supernatant fluids. IL-B4 purified by affinity chromatography, which as determined by SDS-PAGE contains 3 distinct molecular weight species of proteins, exhibits IL-B4 immunoregulatory properties. It does not mimic the activity of IL-1, IL-2, BCGF or γ-interferon, nor does it show TRF effect-like activity. These studies confirm earlier but less direct observations, and clearly indicate that IL-B4 is a novel lymphokine with unique regulatory properties.

B55 IA-POSITIVE CELLS, IL-1, γ-IFN AND T-CELL DEVELOPMENT IN FETAL THYMUS ORGAN CULTURES. D. DeLuca Medical University of S.C., Charleston, S.C. 29455

A fetal organ co-culture system has been developed to study the cellular interactions involved in the development of T-cells in a closed system where cell yields during differentiation can be easily monitored. The organ culture system has been used to determine if monoclonal anti-IA antibodies, interleukin 1 (IL-1) and gamma interferon (IFN) (or polyclonal antibodies to these agents) affect the growth and development of functional T-cells in fetal thymus organ cultures. Anti-IA antibodies specifically inhibit the development of total cells and MLC reactive T-cells in fetal thymus organ culture. Ia-positive cells normally found in these tissues were no longer detectable after anti-IA treatment, inspite of the fact that the reagents were used without complement. Fetal thymus organ cultures treated with anti-IA antibodies were cultured with IL-1. This treatment reversed the inhibition of functional T-cell development by anti-IA antibodies, and restored Ia expression. IFN added to anti-IA treated cultures also restored Ia expression. Goat polyclonal anti-IL-1 antibodies and rabbit anti-IFN were added to fetal thymus organ cultures to determine if IL-1 or IFN were physiologically involved in the development of T-cells in our system. The development of total cells and MLC reactive T-cells was inhibited by the addition of anti-IL-1 and anti-IFN antibodies. Normal IgG did not affect T-cell growth. These results support the involvement of Ia-positive non-lymphoid cells in the development of T-cells in the fetal thymus, possibly through the production of IL-1. The continued expression of Ia on the surface of non-lymphoid cells may be stimulated by IFN.

B56 INFLUENCE OF THYMOPENTIN ON CANDIDIN-INDUCED PROLIFERATION AND PWM-INDUCED IgG PRODUCTION OF HUMAN LYMPHOCYTES. Duchateau, J., Collet, H., *Bolla, K., University Hospital St. Pierre, Brussels, Belgium and *CILAG Ltd. Technical Center, CH Schaffhausen.

The influence of various doses of thymopentin (0.01 ng/ml to 10 μg/ml) on human blood lymphocytes was tested in two in vitro models. The maximal effects, i.e. stimulation of candidin-induced proliferation and inhibition of PWM-induced IgG production, were observed at two different concentration ranges of thymopentin. Low concentrations of thymopentin (1 and 10 ng/ml) enhanced the candidin-induced proliferation, while high concentrations of thymopentin (1 and 10 μg/ml) significantly reduced the PWM-induced IgG production by about 70%. The observations are discussed in connection with results of in vivo experiments and clinical investigators.

B57 PROBABLE INVOLVEMENT OF Ca^{2+}/PHOSPHOLIPID (C-KINASE)-DEPENDENT PHOSPHORYLATION IN T CELL ACTIVATION, Robert M. Galbraith, Andre E. Nel, Walter Dirienzo, Gregory R. Lattanze, Marie W. Wooten, and Henry C. Stevenson. Medical University of South Carolina, Charleston, South Carolina 29425; Cold Spring Harbor Laboratory, New York, New York 11724; National Cancer Institute, Frederick, Maryland 21701.

The activities of immunoregulatory moieties frequently involve binding to specialized membrane recognition or receptor sites, but the mechanisms operating in their subsequent biological effects are unclear. This study focussed on the role of Ca^{2+}/phospholipid dependent kinase (C-kinase) related to T cell activation induced by monoclonal antibodies to T3 antigen. Human peripheral blood T lymphocytes obtained by leukopheresis, elutriation and E-rosette formation (volume $<$ 170 cu. mm) were unstimulated, or stimulated with either anti-T3 or the phorbol ester TPA (a known C-kinase activator). SDS-PAGE and autoradiography of soluble abstracts after addition of [γ-^{32}P]-ATP showed several major C-kinase substrates MW 20-56K. This profile was altered after cell stimulation with either TPA or anti-T3. Separation of cells into cytosol and Triton-soluble membrane fractions (TSM) indicated translocation of C-kinase activity from cytosol to TSM upon stimulation. This was confirmed by quantitative assays employing exogenous substrate (pmol ^{32}P/ug protein/min). Similar results were found in intact T ceils labeled with ^{32}P orthophosphate. Parallel studies with polymyxin B (PMB), a C-kinase inhibitor, showed coordinate inhibition of phosphorylation and of IL-2-mediated response as assessed by ^{3}H-thymidine uptake. These results indicate a role for C-kinase in T lymphocyte activation. Signal-dependent phosphorylation may be useful in the evaluation of other stimulatory and suppressive factors.

B59 INTERACTION OF DISTINCT SOLUBLE FACTORS TO INDUCE SPECIFIC ANTIBODY SECRETION
Michael G. Goodman, Scripps Clinic and Research Foundation, La Jolla, CA 92037

B lymphocytes require a source of T cell-like help to respond to most antigens. T cell-derived lymphokines and nucleosides such as 8-mercaptoguanosine are effective sources of such T cell-like help. Addition of T cell-derived lymphokines to antigen-activated B cells together with 8MGuo results in synergistic differentiation, amplifying the sum of the individual responses 2-4 fold. Lymphokine activity must be added at onset of culture for optimal synergy with 8MGuo, whereas the nucleoside can be added up to 48 hours later with full synergy. The nucleoside also provides a T cell-like differentiation signal to B cells from immunodeficient xid mice, indicating that Lyb 5^- B cells are receptive to such signals. This observation distinguishes a subset of nucleoside-responsive B cells from those activated by soluble anti-μ followed by BSF-1, IL-1, and B cell differentiation factors, since these latter B cells are Lyb 5^+. Moreover, at least a subset of the B cells recruited by the synergistic interaction of lymphokines and nucleoside is distinct from that responsive to 8MGuo + antigen insofar as G-10 nonadherent xid B cells fail to respond to either 8MGuo or lymphokines alone, but do respond in the presence of both. This subset can be demonstrated among normal B cells by limiting dilution analysis. Thus, the precursor frequency of antigen-reactive B cells in the presence of lymphokines or nucleoside alone increases substantially when both are present together. Together with the kinetic data, this suggests that lymphokines actively induce element(s) limiting the capacity of a distinct B cell subpopulation to respond to 8MGuo.

B60 HYBRIDOMA-DERIVED CYTOTOXIC T LYMPHOCYTE DIFFERENTIATION FACTOR,
Roberta L. Hayes, and Janet M.D. Plate, Rush Medical College, Chicago, IL 60612.

We have previously described the requirement for a cytolytic T lymphocyte (CTL) differentiation factor in addition to interleukin-2 (IL-2) during the generation of primary 2,4,6-trinitrophenyl-(TNP)-specific murine CTL in vitro which we have designated CTL-DF. We now report the isolation of a murine T cell hybridoma, D6T-5C12.1E8, that produces CTL-DF upon antigenic stimulation. The factor activity produced by this hybridoma is distinct from interleukins 1,2 and 3, colony-stimulating factors 1 and 2, and the interferons (IFN). None of these latter factors are present in detectable quantities in the 1E8 hybridoma supernate. In addition we have analyzed the RNA isolated from restimulated hybrid cells for transcription of the murine IL-2, IL-3 and gamma IFN genes. CTL-DF is neither antigen-specific, nor MHC-restricted in its activity. CTL-DF appears to function in precursor CTL differentiation and maturation resulting in effector killers in the presence of low concentrations of IL-2. Large amounts of IL-2 have been shown to activate nonspecific induction of memory CTL, possibly due to the triggering of the endogenous production of differentiation factors by memory helper cells. CTL-DF quite substantially decreases the amount of IL-2 required to activate CTL, and thus may be a corequirement for the effective immunotherapy of cancer and immunodeficiency diseases.

B61 BIFUNCTIONAL LYMPHOCYTE REGULATION BY HUMAN Fc FRAGMENTS AND SUBFRAGMENTS DERIVED FROM HUMAN IgG_1,
Monte V. Hobbs, William O. Weigle and Edward L. Morgan, Scripps Clinic and Research Foundation, La Jolla, CA

Fc fragments of human IgG_1 and the synthetic peptide, p23, representing residues 335-357 in the CH_3 domain of IgG_1 were able to increase levels of secreted immunoglobulin (Ig) in murine spleen cell cultures. Analysis of peptides based on the p23 sequence revealed that the carboxyterminal region of the molecule is required for B cell activation. B cell activation by Fc fragments was macrophage- and T cell-dependent whereas activation by p23 was only T cell-dependent. Induction of Ig secretion by both stimulators was influenced by endogenous oxidative products of arachidonic acid metabolism, as evidenced by the augmentation of Ig secretion in cultures treated with prostaglandin synthetase inhibitors. Both Fc fragments and p23 induce the release of PGE from splenic macrophages and the $P388D_1$ macrophage-like cell line. Addition of exogenous PGE_2 to culture reduced the ability of Fc fragments or p23 to stimulate the secretion of Ig by murine B cells. In addition to activating the arachidonate metabolic pathway, Fc fragments stimulate the secretion of interleukin 1, a potent immunomodulator. These data suggest that B cell activation by Fc fragments and subfragments is influenced by the concomitant induction of interleukin-1 and suppressive prostaglandins.

B62 TUMOR PROMOTERS IN CONJUNCTION WITH CALCIUM IONOPHORES MIMIC ANTIGENIC STIMULATION BY REACTIVATION OF ALLOANTIGEN-PRIMED MURINE T LYMPHOCYTES, Noah Isakov and Amnon Altman, Scripps Clinic and Research Foundation, La Jolla, CA, 92037.

Activation of allospecific primed T cells can be triggered in vitro by the specific antigen or interleukin 2 (IL2). Antigen binding to its receptor induces transmembrane Ca^{2+}-dependent signals which involve phosphatidylinositol turnover. Exposure of murine primary mixed lymphocyte culture (1°MLC) cells to phorbol ester or teleocidin tumor promoters in conjunction with Ca^{2+} ionophore could mimic antigenic stimulation and lead to T cell proliferation, IL2 production and receptor expression, and enhanced cytotoxic activity. The ability of phorbol ester derivatives to synergize with Ca^{2+} ionophore in induction of 1°MLC cell activation correlated with their ability to bind to and activate protein kinase C. In addition, the synergistic effect of Ca^{2+} ionophore and TPA was blocked by either a Ca^{2+} chelator (EGTA) or cAMP, which is thought to inhibit phosphatidylinositol metabolism. To further analyze T cell activation by TPA and Ca^{2+}, ionophore, we tested their effect on functionally distinct T cell subpopulations, i.e., cloned helper (Th) and cytotoxic (Tc) cells specific for class I alloantigens. TPA plus Ca^{2+} ionophore induced cell proliferation and IL2 production by Th cells, but not by Tc cells. Activation of mixed clones of Th and Tc cells, but not of Tc cells alone, resulted in cytotoxic activity which could be blocked by anti-IL2 receptor antibodies. Thus, an increased concentration of intracellular Ca^{2+} in conjunction with PKC activation can bypass the signal provided by antigen-receptor interaction on Th cells, but does not substitute for IL2 in activating cytotoxicity by isolated Tc cells.

B63 A NOVEL IMMUNOREGULATORY ACTIVITY BY T CELLS DERIVED FROM MURINE PEYER'S PATCHES H. Kawanishi, K. Koyama, S. Senda and J. Kiely. Gut Mucosal Immunity Laboratory SUNY at Stony Brook and Northport VA Medical Center, New York 11768. USA.

Much evidence suggests that Peyer's patch (PP) lymphocytes are capable of mounting both humoral and cell-mediated immune responses to luminal antigenic stimuli. To shed further light on T-B cell interactions in gut-mucosal immune-associated processes, we studied in vitro the effect of a variety of Con A-activated immunoregulatory T cell subsets on immunoglobulin (Ig) production by LPS-activated B cells. Three types of immunoregulatory effector T cells from PP T cells, helper (Th), suppressor (Ts) and contrasuppressor (Tcs), were developed and isolated. PP-derived B cells were then co-cultured with these immunoregulatory T cells and LPS at various ratios for 7 days, secreted class-specific Igs were quantitated by the ELISA double-antibody sandwich method. The data showed that the B cell Ig production was under regulation of these T cells. In addition, effector cell-inducer T cell subsets, a Ts inducer (Tsi) and a Tcs inducer (Tcsi), also appeared to be operable. Thus, similar to the Ag-specific system polyclonally activated PP T cells are able to execute their novel immunoregulatory functions in response to intraluminal non-specific stimuli.

B64 **8-BROMOGUANOSINE ACTIVATES NATURAL KILLER CELLS AND MACROPHAGES VIA PRODUCTION OF INTERFERON. Gloria C. Koo, Marvin Jewell, Christine L. Manyak, Nolan H. Sigal and Linda S. Wicker. Department of Immunology Research, Merck, Sharp & Dohme Research Laboratories, P.O. Box 2000, Rahway, New Jersey 07065.**

It has been shown that 8-Bromoguanosine (8-BrGuo) activates B cells. We found that it activates Natural Killer (NK) cells and macrophages as well by induction of interferon (IFN) production. Culturing spleen cells with 8-BrGuo for 16-18 hours induced cytotoxic activity to YAC cells. The cytotoxic cells expressed Qa-5, AsGm-1 and NK-1.1 antigens. Similarly preincubation of peptone elicited peritoneal macrophages with 8-BrGuo induced macrophage cytolytic activity to P815 cells in an 18 hour assay. Supernatants from these cultured cells contained IFN. Anti-IFN antibodies to α, β and γ-IFN abolished these inductive events. We proposed that 8-BrGuo constitutes a class of immunoregulants whose actions are primarily mediated by IFN.

B65 Augmentation of Growth Factor Production by Thymic Hormones. N.M.Kouttab,F.A.Natar & A.L.Maizel, UT M.D. Anderson Hospital and Tumor Institute, Houston, Texas 77030

Partially purified thymic extracts, and highly purified or synthetic thymic peptides were examined for their ability to augment the production of B and T cell growth factors (BCGF and TCGF, resp.) by normal human peripheral mononuclear cells (PBMC). The hormones tested included, Thymosin fraction 5 (MW 1000-15000; a gift from Dr. A. Goldstein); Thymostimulin (also called TP-1; several peptides with MW <10,000; homogenously purified peptide thymosin α1 (MW 3108; a component of thymosin fraction 5; also a gift from Dr. A. Goldstein); and a synthetic thymic peptide Thymopentin (MW 600;TP-5; a gift from Dr. G. Goldstein). PBMC were incubated with various concentrations of thymic hormones in the presence or absence of phytohemagglutinin (PHA). The presence of growth factors in PBMC supernatants was measured by their ability to induce DNA synthesis (measured by ^{3}H-Tdr incorporation) in B and T lymphocytes. The results showed that thymosin, TP-1 and TP-5 were capable of augmenting the production of BCGF and TCGF. TP-5, however, was effective at 10-100 fold lower concentrations as that required by thymosin or TP-1. In contrast thymosin α1 did not show any augmenting ability suggesting that a different component in thymosin fraction 5 is responsible for this function. Augmentation of growth factors was evident by 24 hr and continued to be evident at 48 hr but became negligible by 72hr after incubation. Only stimulated PBMC exhibited this augmenting effect. No effect of thymic hormones on the test cells was observed. These observations suggest that thymic hormones can regulate the function of apparently mature activated T lymphocytes. Such regulatory mechanisms may impart a therapeutic potential to thymic hormones in diseases presenting with T cell dysfunctions.

B66 MONOCLONAL ANTIBODIES AGAINST SURFACE STRUCTURES INVOLVED IN ACTIVATION AND DIFFERENTIATION OF MURINE THYMOCYTES, Peter H.Krammer and Andreas Immelmann, Institute for Immunology and Genetics, German Cancer Research Center, Heidelberg, FRG

To identify cell surface molecules involved in lymphokine activation of CTL, monoclonal antibodies were raised against murine thymocytes. Rat x mouse hybridoma supernatants were screened for their ability to block the differentiation of CTLp without affecting the effector phase of CTL-mediated killing. Affinity-purified antibodies from positive hybridomas were further characterized: the antibodies do neither interfere with the IL-2-dependent growth of a long-term T cell line nor with the proliferation of lipopolysaccharide stimulated spleen cells. Proliferation of concanavalin A stimulated thymocytes is substantially inhibited whereas activation of thymocytes by phytothemagglutinin or phorbol-myristat-acetat is unaffected. The monoclonal antibodies also strongly inhibit the IL-2-dependent induction of thymocyte proliferation. Therefore, we are further testing the hypothesis that they detect the IL-1 receptor on T helper cells which help CTLp to mature into CTL. This hypothesis is supported by studies which showed that the antigen detected by the monoclonal antibodies is present on a fraction of activated T cells, shortly after mitogen activation, on long-term T cell clones, on T cell tumors (murine and human), and on a fraction of T cells from a fibroblast tumor. Since preliminary data show that the cell surface molecule seems to be essential for growth of normal and malignant T cells, its characterization by means of molecular biology will determine our efforts in the future.

B67 MITOGENIC LECTINS CAN INDUCE INTERLEUKIN-2 RECEPTORS ON ANTIGEN-SPECIFIC HUMAN T_H-LYMPHOCYTES IN THE ABSENCE OF ACCESSORY CELLS, David H. Margolin and Elaine DeFreitas, The Wistar Institute of Anatomy and Biology, Philadelphia, Pa. 19104.

T_H-lymphocytes activated by antigen or lectin are dependent on the binding of interleukin-2 (IL-2) to its cell surface receptor (IL-2R) for progression through the cell cycle. IL-2R is not found on resting T cells, but appears after activation. We have studied the requirements of antigen-specific, IL-2 dependent, T_H-lymphocytes for activation by appropriate antigens or by the mitogens, phytohemagglutinin (PHA) or concanavalin A (Con A), and determined the kinetics of IL-2R induction. Using a monoclonal antibody to the IL-2R and indirect immunofluorescence detected by a cytofluorograph, we find: 1) IL-2R induction by antigen requires the presence of syngeneic accessory cells (AC) (eg., gamma-irradiated peripheral blood adherent mononuclear cells) whereas IL-2R induction by PHA occurs maximally in the absence of AC. Although the presence of AC has little effect on PHA-induced IL-2R expression, their presence enhances DNA synthesis, especially at low mitogen concentrations. By contrast, Con A-induced IL-2R expression is augmented by AC when submitogenic dosed of Con A are used. This effect of AC is not attributable to endogenous IL-2R production since all experiments were performed in the presence of a saturating concentration of IL2. This system may be valuable in the dissection of the signals required by T cells for induction of the IL-2R and for subsequent clonal expansion. Supported by NIH grant AI-19987-01. D.M. supported by Medical Scientist Training Program, NIH grant 5T32GM07170.

B68 T-CELL REGULATION OF HUMAN B-CELL DIFFERENTIATION, FOCUS ON THE ROLE OF IL-2

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We previously reported that the production of IL-2 is an early and essential event in pokeweed mitogen (PWM) driven immunoglobulin (Ig) synthesis by human B cells. An active role for IL-2 in in-vitro Ig-synthesis was even more clearly shown by the $T4^+$-dependent innate Ig-inducing capacity of IL-2. We now provide evidence that two functional activities, viz. IL-2 production and the capacity to deliver the actual helper signal for B-cell differentiation, both required for T-helper activity on Ig-synthesis, are independent and distinct steps. Monoclonal antibodies directed against lymphocyte function associated antigen 1 (LFA-1) inhibited PWM-induced IL-2 and Ig-production, but only marginally inhibited IL-2 induced T-cell dependent Ig-synthesis. This dissociation was further demonstrated with mature "clonal" neoplastic $T4^+$ cells of a series of 7 patients with T-cell malignancies. The neoplastic $T4^+$ cells of 3 patients produced large amounts of IL-2 and provided helper activity on both PWM- and IL-2 driven Ig-synthesis. The neoplastic $T4^+$ cells of 4 patients did not produce IL-2 and did not support PWM-driven Ig-synthesis. The $T4^+$ cells of these 4 patients, however, provided excellent helper activity on IL-2 driven Ig-synthesis. These findings emphasize the role of IL-2 in T-cell dependent Ig-synthesis and clearly show that IL-2 production is required for helper activity in the PWM-driven system and that IL-2 induces T-helper cells to provide a signal for B-cell differentiation.

B69 IMMUNOREGULATORY PROPERTIES OF HUMAN COMPLEMENT FRAGMENTS C3a AND C5a,

Edward L. Morgan, Scripps Clinic and Research Foundation, La Jolla, Ca

The complement (C) system has traditionally been viewed in the context of a nonspecific resistance mechanism. There is accumulating evidence, however, that C components may be involved in the regulation of specific humoral and cell-mediated immunity. Bioactive C fragments, C3a and C5a, have recently been shown to differentially influence *in vitro* immune function. C3a was found to be a potent suppressor of antigen-specific and polyclonal antibody responses. In contrast, C3a was unable to suppress antigen- or mitogen-induced B and T cell proliferation. Analyses of synthetic peptides based on the sequences of C3a revealed that the carboxyterminal region of the molecule is responsible for immunosuppression. C3a-mediated suppression occurs through the activation of a nonspecific suppressor T cell pathway. Activation of suppressor T cells may involve prostaglandins (PG) since indomethacin abrogates C3a-mediated suppression. In contrast to the results obtained with C3a, C5a was found to augment both *in vitro* humoral and cell-mediated immune responses. Enhancement of antibody responses by C5a requires the presence of macrophages. Coculture of human monocytes or murine splenic macrophages with C5a results in the secretion of interleukin-1 (IL-1), a potent immunomodulator. In addition, C5a stimulation of human monocytes results in the activation of the arachidonate metabolic pathway with preferential release of leukotrienes (LT). LT have also recently been shown to modulate various immune responses. Regulation of immune function by C components may form part of an *in vitro* nonspecific immunoregulatory network.

B70 DEFINITION OF TWO TYPES OF MURINE HELPER CLONE BY LYMPHOKINE ACTIVITY PROFILES, PROTEIN BIOSYNTHESIS AND B CELL HELPER FUNCTION.

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Two types of murine T cell (T_H1 and T_H2) have been found in a large panel of antigen-specific T cell clones. Both types of T_H were L3T4+, Lyt2-, and were restricted to or specifc for the I region of the MHC. T_H1 clones produced Interleukin 2 (IL2), Interleukin 3 (IL3), interferon gamma (IFN-γ), and granulocyte-macrophage colony stimulating factor (GM-CSF) in response to antigen or lectin stimulation, whereas T_H2 cells produced IL3, B cell stimulating factor 1 (BSF-1) and two novel activities: a T cell growth factor distinct from IL2, and an activity that synergized with IL3 to give full stimulation of certain mast cell lines. The secreted proteins induced by lectin were analyzed by biosynthetic labeling and SDS gel electrophoresis; characteristic differences were seen between T_H1 and T_H2 clones. A 15 kd band was seen only in supernatants from T_H2 clones, and the set of bands at 16-17 kd due to IFN-γ were produced only by T_H1 clones. Some T_H1 cells could give either antigen linked or unlinked help to B cells, depending on the assay conditions. The major isotypes were IgM and IgG. T_H2 cells also gave help to B cells, stimulating secretion of IgM, IgG_1 and IgE antibody. Most T_H1 clones gave a DTH reaction when injected with antigen into footpads, whereas no T_H2 clones have yet given DTH reactions.

B71 GANGLIOSIDES INHIBIT ENCEPHALITOGENIC T LYMPHOCYTES, Halina Offner and Arthur A. Vandenbark, Veterans Administration Medical Center, Portland, OR 97207

Gangliosides were evaluated for their ability to inhibit the phenotype and function of an encephalitogenic T helper lymphocyte line from Lewis rats (BP-1), which responds specifically to guinea pig myelin basic protein (GP-BP). After activation for 3 days with GP-BP, the BP-1 line induced a lethal form of experimental autoimmune encephalomyelitis (EAE) in recipient rats 3-6 days after intraperitoneal injection. Incubation of activated BP-1 line cells with 250 uM gangliosides for 1 hr prior to injection prevented EAE completely in 5/14 recipients and markedly reduced the severity of clinical signs and histologic lesions in the rest. Similar treatment of BP-1 cells with galactocerebroside had no inhibitory effect. Both individual and mixed gangliosides inhibited accessory cell dependent activation of BP-1 cells with GP-BP. Gangliosides also inhibited BP-1 activation with a cell free supernatant containing accessory cell-processed GP-BP and rat Ia molecules, suggesting that the inhibition was not restricted to accessory cell function. In addition to inhibiting antigen dependent proliferation, gangliosides inhibited IL-2 dependent cell growth. Futhermore, individual and mixed gangliosides blocked binding of anti-T helper cell antibody, but not anti-T total, or anti-Ia antibodies to the BP-1 line. Similarly, gangliosides inhibited selectively the binding of anti-T helper antibody to human blood lymphocytes. Taken together, the immunomodulatory properties of gangliosides on T effector cell function lend biologic importance to the increased levels of gangliosides which have been reported in human diseases with immunoregulatory abnormalities.

B72 Genetic events and signals involved in T-cell differentiation.

D. Pardoll, Fowlkes B., Sant A., Lechler R., Germain R. and Schwartz R.

Although it has been well established that T cell differentiation occurs predominantly in the thymus, the molecular mechanisms involved in development and generation of the repetoire remain unknown. The isolation of the genes encoding the clonotypic T cell receptor provides an opportunity to study the early genetic events of intrathymic differentiation. We have utilized a highly sensitive in situ hybridization technique to analyze the timing and regulation of expression of the three T cell receptor genes (Tcrα,Tcrβ and Tcrδ) as well as IL2, IL2 receptor and Thy 1. The major advantage of in situ hybridization is that gene expression is assessed at the single cell level. Thus, small numbers of cells can be easily analyzed and infrequent events among a cell population can be detected. Thymocyte development in the fetal mouse was studied back to the 11th day of gestation when stem cells from the fetal liver first begin to enter the thymic rudiment. The three T cell receptor genes appear to be turned on sequentially and in tightly synchronized waves. Tcr appears to be expressed very early in T cell differentiation. Its message can be found in early thymocytes that are not yet expressing Tcrα or Tcrβ. Tcrβ is next turned on between day 12 and day 13 of gestation. The majority of day 14 thymocytes express Tcrγ and Tcrβ but do not express Tcrα. This early stage of differentiation is very similar to the immature dull lyl subpopulation isolated from the adult thymus. Immature thymocytes, which express IL2 receptor, grow well in short-term culture using a combination of PMA, ionomycin and recombinant IL2. The affects of these compounds on thymocyte development in vitro and in organ culture will be presented.

B73 RESTORATION OF IMMUNOLOGICAL RESPONSES BY THF IN MICE INFECTED WITH MCMV.

B. Rager-Zisman, E. Katorza, P. Cesar, R.N. Apte, M. Pecht*, Y. Burnstein and N. Trainin*, Ben Gurion University of the Negev, Dept. of Microbiology and *Weizmann Inst. of Science, Dept. of Cell Biology.

Human cytomegalovirus (CMV) is a ubiquitous herpes virus which may cause severe infections in the immunocompromised patient and is one of the most frequently isolated viruses from patients with AIDS. Because of its similarities to human CMV, murine cytomegalovirus (MCMV) infection in mice has been used extensively as a model to study the pathogenesis and the immune response to CMV. It has been shown by several investiagors that T cell functions are critical for the recovery from this virus infection. In addition, it has also been found that MCMV itself induced a depression of T cell functions. In the present study, we examined the effect of treatment with the thymic humoral factor (THF), a thymic hormone, on the immune responses of MCMV infected mice. THF was shown to have immunomodulatory properties, particularly on T cell functions and antiviral activity. When Balb/C mice were infected with the Smith strain of MCMV a marked depression to Con A was detected. The diminished responses to LPS, PHA and Con A were first observed 3 days after virus infection and lasted for 10 days thereafter. Daily treatment of the infected mice with THF resulted in reconstitution of the Con A responses to normal levels. Similar results were obtained when IL-2 production was measured. Contrary to other thymic hormones, THF treatment did not augment NK activity or had any effect on interferon responses of the infected mice. Results of these experiments suggest that the antiviral effect of THF is mediated by restoration of T cell competence.

B74 Monoclonal Anticodies to Intracellular High MW BCGF (BCGF II) Recognize the low MW Secreted Form of Human BCGF (BCGF I). Chintaman G. Sahasrabuddhe, Barbara A. Martin and Frances M. Davis, University of Texas System Cancer Center, M.D. Anderson Hospital, Houston, Texas 77030

Human B cell growth factor is a protein of MW 12000-14000, secreted by activated T-lymphocytes. We have previously shown that a high MW intracellular protein present in T-lymphocytes exhibits BCGF activity and that the size of messenger RNA coding for human BCGF is sufficiently large to direct the synthesis of this high MW protein. We have now purified this intracellular protein to homogeneity. This protein has a MW of 60,000 as analyzed by SDS-PAGE and an isoelectric point of 6.3. The purified protein was used to immunize two mice. The spleen cells from immunized mice were used to form hybrids by fusion with SP2/o -Ag14 cells. From a total of 3600 hybrid colonies initially plated, only 280 (7%) were found to secret mouse IgG. Of these 280 IgG secretors, 5 (2%) recognized purified high MW BCGF in an ELISA. Antibodies from four colonies also recognized the secreted low MW BCGF in an ELISA, demonstrating the presence of shared epitopes on these molecules. The two antibodies tested could remove both high as well as low MW BCGF from solution as monitored by BCGF biological activity in a microculture assay. These results strongly support the hypothesis that intracellular high MW BCGF is a precussor to extracellular low MW BCGF.

B76 **B CELL GROWTH AND DIFFERENTIATION INDUCED BY ETAF.** David W. Scott and P.S. Pillai, Immunology Unit, University of Rochester Cancer Center, Rochester, NY

We recently showed that B cell growth and differentiation could be induced by a new cytokine, called ETAF (epidermal cell-derived T cell activating factor). ETAF is a 15-20 Kd IL-1-like factor produced by murine and human keratinocytes and epidermal carcinoma cell lines. In this study, ETAF was purified by gel chromatography followed by HPLC of uninduced supernatants from the human squamous cell carcinoma line, COLO-16 and provided by Dr. Dan Sauder, McMaster Univerisity (Hamilton, Ontario). In contrast to IL-1, which can augment the anti-IgM-stimulated B cell growth, ETAF stimulates thymidine incorporation in the absence of anti-mu. Moreover, ETAF was able to drive hapten-purified B cells to antibody secretion in the absence of either T or accessory cells. While ETAF contains IL-1 activity as measured in a thymocyte co-stimulator assay, it lacks classical IL-2 and BSF functional activity, the latter measured by augmentation of class 2 antigen expression in unprimed B cells. Finally, the target population for ETAF appears to be J11D+ resting B cells.
(Supported by USPHS grant AI-20757 and ACS Institutional Grant IN-18.)

B77 T-LEUKEMIA DERIVED SUPPRESSOR LYMPHOKINE (TLSL): BIOLOGY AND BIOCHEMISTRY, David J. Tweardy, Daniela Santoli and Giovanni Rovera, The Wistar Institute, Philadelphia, PA 19104

The human T-leukemia cell line, Jurkat, spontaneously released a non-interferon suppressor lymphokine, $TLSL_{Ju}$, able to inhibit proliferation of a variety of normal and malignant hemopoietic cells including T lymphocyte responses to mitogens and antigens. Titration curves indicated that the inhibitory activity in the crude supernatant preparations against susceptible target cells ranged between 10^{-3} and 10^{-7}. Unlike lymphotoxin and tumor necrosis factor, $TLSL_{Ju}$ was cytostatic with maximal inhibition of proliferation occurring on day 4-5 without a significant loss in cell viability. Treated tumor target cells accumulated either in the G_1 or in the S phase of the cell cycle. The effect of $TLSL_{Ju}$ on the target cells was irreversible; brief (1 h) incubation of susceptible cells with $TLSL_{Ju}$ resulted in inhibition of proliferation. $TLSL_{Ju}$ was inactivated by heat and extremes of pH and did not bind to lectins. $TLSL_{Ju}$ was purified to homogeneity from serum-free culture supernatants using a purification scheme consisting of ammonium sulfate precipitation, reactive blue 2-agarose, octyl-sepharose, hydroxylapatite and Mono-Q anion-exchange chromatography. Purified $TLSL_{Ju}$ was homogeneous by the criteria of sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), autoradiography of iodinated protein and high pressure gel filtration chromatography. The protein had a molecular weight of 90 kDa by high pressure gel filtration chromatography and 45 kDa by SDS-PAGE under reducing and non-reducing conditions. These features along with the amino terminal sequence demonstrate that $TLSL_{Ju}$ is distinct from the suppressor/cytotoxic lymphokines thus far described.

B78 Increased T helper cell generation after low dose Cyclosporin A treatment
P. Wassmer, M. Kennedy, P. Erb, Inst. Microb., Univ. Basel, Switzerland

Cyclosporin A (Cs A) at lower concentrations (25 to 50 ng/ml) enhanced the in vitro generation of a secondary anti-SRBC plaque forming cell (PFC) response, while 100 ng/ml was found to be suppressive. In contrast, cell proliferation, as well as IL-2 secretion were suppressed with increasing doses of Cs A, suggesting a differential effect of Cs A on cell proliferation and IL-2 secretion versus the induction of thymus dependent PFC. We next analysed the effect of Cs A on T helper cell (Thc) generation (macrophage - T cell cooperation) and in T - B cell cooperation for antibody response. Thc were generated by incubating nylon wool purified T cells, together with macrophages and KLH for 5 days. PFC were produced by incubating Thc in the presence of primed, T cell depleted spleen cells and TNP-KLH for another 5 days. Cs A at concentrations up to 200 ng/ml did not suppress, but enhance the generation of Thc, while T - B cooperation was blocked at 100 ng/ml Cs A. These results taken together with our previous observations that 1) small, but not large T cells express helper function, 2) anti-IL-2 receptor antibodies are unable to block Thc generation and 3) that only certain Ia^+ cells induce Thc, strongly support our hypothesis that the activation requirement for Thc is different than for other T cell functions.

B79 IN VITRO GENETIC RECONSTITUTION OF THE MOLECULES INVOLVED IN IMMUNE RESPONSE, Motoo Watanabe, Nobukata Shinohara*, Atsuo Ochi, and Nobumichi Hozumi, Research Institute, Mount Sinai Hospital, 600 University Avenue, Toronto, Canada M5G 1X5. *Immunology Branch, National Institutes of Health, Bethesda, MD 20205.

T cell - B cell (T-B) interaction is regulated in an antigen specific and MHC restricted manner. We are studying several mechanisms involved in T-B interaction at the molecular level, by utilizing monoclonal T cell and B cell lines. Recent progress in DNA-mediated gene transfer has enabled us to construct B cell lines carrying in vitro manipulated Ia and immunoglobulin (Ig) genes with defined hapten specificity. B cells were transfected with antibody genes specific for a defined hapten and were analyzed for antibody receptor mediated antigen presentation. We have found that transfected antigen specific B cells are able to present antigen to T cells 10^3 - 10^4-fold more efficiently than that of non-specific presentation. We have constructed a vector carrying both $I\text{-}A_\alpha^k$ and $I\text{-}A_\beta^k$ and have succeeded in functional expression of $I\text{-}A^k$ molecules on thesurface of B lymphoma cells.

The results on T cell activation, B cell activation and B cell differentiation based on in vitro genetic reconstitution experiments will be discussed.

B80 ACTIVATION OF IL-1-DEPENDENT AND IL-1-INDEPENDENT T CELL LINES BY CALCIUM IONOPHORE AND PHORBOL ESTER, Albert Zlotnik and Barbara Diane, DNAX Research Institute, Palo Alto, CA 94304.

The IL-1-dependent T cell lymphoma LBRM33-1A5.47, which requires phytohemagglutinin (PHA) and interleukin-1 (IL-1) in order to produce interleukin-2 (IL-2), was compared to the T cell hybridomas DO-11.10/54.4 and 3DO-54.8. The latter hybridomas do not require exogenous IL-1 to produce IL-2 in response to Concanavalin A (Con A) or Ovalbumin (OVA)/$I\text{-}A^d$. We studied the activation of those T cell lines by the calcium ionophore A23187 and phorbol myristate acetate (PMA). LBRM-33-1A5.47 produces IL-2 when given A23187 and PMA simultaneously. Furthermore, A23187 can replace PHA, and PMA can replace IL-1, in the activation of LBRM-33-1A5.47 to IL-2 production. Parallel experiments with 3DO-54.8 and DO-11.10/54.4 showed a strong response to A23187 and PMA used simultaneously. IL-1 replaces the enhancing effect of PMA in the activation of these hybridomes. These observations suggest a common pathway of activation which involves the signals provided by A23187 and PMA, for the T cell lines studied.

B120 A Recombinant Human Granulocyte-Macrophage Colony-Stimulating Factor (rH GM-CSF) Stimulates in vitro Mature Human Neutrophil and Eosinophil Function, Surface Receptor Expression and Survival. ANGEL F. LOPEZ‡, D. JAMES WILLIAMSON*, C. GLENN BEGLEY*, NICOS A. NICOLA*, GORDON WONG†, STEVEN C. CLARK† and MATHEW A. VADAS‡
‡Division of Human Immunology, Institute of Medical & Veterinary Science, Adelaide 5000, South Australia. *The Walter & Eliza Hall Institute of Medical Research, Melbourne 3050, Victoria, Australia and †Genetics Institute, Boston, Massachusetts 02115, USA.

An rH GM-CSF purified from COS cells transfected with a human GM-CSF cDNA is shown to be a powerful stimulator of the function of mature human eosinophils as well as neutrophils. The purified rH GM-CSF enhanced the cytotoxic activity of neutrophils and eosinophils against antibody-coated targets and was more effective on eosinophils than on neutrophils. rH GM-CSF also enhanced the phagocytosis of serum-opsonized yeast by both cell types in a dose-dependent manner, FMLP-stimulated degranulation of cytochalasin B-pre-treated neutrophils and FMLP-stimulated superoxide production. rH GM-CSF was found to selectively enhance the surface expression of granulocyte functional antigens (GFA) 1 and 2, the complement receptor type 3 (CR3) but not β_2 microglobulin. Furthermore, rH GM-CSF was found to enhance the survival of both neutrophils and eosinophils in vitro. These experiments show that a purified colony-stimulating factor obtained as a single gene product in addition to stimulating the proliferation and differentiation of neutrophil and eosinophil progenitor cells, can act on mature cells by 1) stimulating function; 2) enhancing the expression of functional surface antigens, and 3) prolonging survival.

B121 IMMUNOREGULATION IN RHEUMATOID ARTHRITIS, Eric M. Veys and Stefaan Suykens, Department of Rheumatology, University of Ghent, B 9000 Ghent, Belgium.
Actually, the heterogeneity of the rheumatoid arthritis (RA) population is widely accepted. The major problem is that we are still unable to distinguish subgroups based on clinical or biological variables. Considered as an entire group, active RA patients present immune dysregulation characterized in peripheral blood (PB) by an increase of OKT4+ cells and a decrease of OKT8+ cells. However, some patients (+ 10%) present reverse changes and have more OKT8+ than OKT4+ cells in PB. No clinical or other biological abnormalities allowed us to consider them as a separate subgroup, but they all appeared to be resistent to classical, slow-acting, antirheumatic drugs (goldsalts, D-Penicillamine, levamisole). As a group, patients in an inactive disease stage do not differ from a normal control population regarding the profile of their T cell subsets. Considering individual values however, we did not find a correlation between T cell subsets and respective variables of disease activity (sedimentation rate, CRP, joint indices, morning stiffness). Low doses of prednisolone were found to produce an immune regulation depending on the start conditions. OKT4+ cells are significantly more redistributed in patients starting with high OKT4+ values, whereas in patients with high OKT8+ cells before drug intake, these cells are more influenced than the OKT4+ cells. Levamisole, when taken since more than 3 months, normalized the OKT4+/OKT8+ cell ratio for at least 48 hours. Similar results were obtained with diethyldithiocarbamate in RA patients treated since 6 months. The clinical significance of T cell subset changes by pharmacological compounds in RA remains questionable.

B122 CSA STIMULATES THYMIC EPITHELIAL FUNCTION AND PROLIFERATION.
M. Dardenne, W. Savino, G. Feutren and J.F. Bach. Hôpital Necker - INSERM U25 - 75743 PARIS Cedex 15

When injected daily in normal young or ageing mice Cyclosporin A induces a significant stimulation of thymic hormone (thymulin) secretion, as measured by the peripheral level of the hormone and the number of thymulin-producing cells in the thymus. This stimulation is dose dependent and reversible after the end of treatment. Similar findings have been observed in primary cultures of human thymic epithelial cells in which Cyclosporin A increases the percentage of thymulin-producing cells, evaluated by immunofluorescence, as well as the amount of hormone released into the supernatants. Conversely no effect is observed on the expression of class II MHC antigens. Importantly, Cyclosporin A also increases the proliferation of cultured human thymic epithelial cells, as assessed by bromodeoxyurine and ^{3}H-thymidine incorporation. This effect seems to be specific for CsA since an inactive analog, namely CsH does not induce such proliferation.

The present study strongly suggests that Cyclosporin A can stimulate proliferation and endocrine function of thymic epithelial cells. Its action on the immune system is thus not restricted to the lymphoid cells and might partially be mediated by its effect on the thymic epithelium.

B123 INTRACELLULAR PATHWAYS FOR THYMULIN SECRETION:AN IMMUNOHISTOCHEMICAL EVIDENCE. Wilson Savino and Mireille DARDENNE, Hôpital Necker, INSERM U25,75743 PARIS Cx 15.

Thymulin is a zinc containing thymic nonapeptide that induces T cell markers and functions on immature T lymphocytes. It is produced by thymic epithelial cells (TEC),and immunoelectron microscopic data suggest that it is stored within cytoplasmic vesicles.However, little is known concerning the intra-cellular pathways for the secretion of this hormone.We approached this problem by blocking experimentally the movement of secretory vesicles in primary cultures of human TEC. These cultures were subjected to:a) colchicine, a microtubule inhibitor able to prevent directioned vesicle movement ; and/or b) cytochalin B, a microfilament inhibitor,also acting as a blocking agent but at the final step of secretory vesicle transport towards the cell membrane, preventing its fusion with the vesicle ; or c) monensin, a ionophore that specifically perturbates the traffic of Golgi-derived vesicles.

Both cytoskeleton inhibitors partially blocked thymulin secretion in the culture supernatants, and this effect was dose-dependent. Moreover, the percentage of thymulin-containing cells (evaluated by indirect immunofluorescence with a zinc specific anti-thymulin monoclonal antibody)as well as the fluorescence intensity within the cells was significantly higher than what was observed in control cultures.Similar results were obtained with monensin.

These results, together with the recent identification of high molecular weight proteins reacting with anti-thymulin antibodies, suggest the following intracellular pathway for thymulin secretion:A precursor is synthetized at the level of the granular endoplasmic reticulum, migrates to the Golgi complex, from which hormone containing vesicles are released. The secretory vesicles then uptake zinc, move towards the cell membrane eventually fusioning with it and characterizing the classical phenomenon of exocytosis.

B124 INDUCTION OF MATURE IMMUNOCOMPETENT CELLS IN THE THYMUS WITH THYMIC HORMONES, THYMOSIN ALPHA-1 AND ALPHA-11. R. NETA, S.D. DOUCHES, Armed Forces Radiobiology Research Institute, Bethesda, MD 20814-5145

Thymic cells from C_3H/HeJ mice 10-14 weeks old are more responsive to Con A, PHA, and IL-1 than age identical counterparts of C57B1/6 mice. These differences in responsiveness are detected in normal thymuses and in thymuses of 4.5 Gy ^{60}Co irradiated animals. Such differences in responses do not depend on arachidonic acid metabolites since the inhibitor of cyclooxygenase pathway (indomethacin) and of lipoxygenase pathway (nordihydrogueretic acid) do not significantly change these responses. Administration of thymic hormones thymosin α_1, and thymosin α_{11} (two synthetic polypeptides kindly provided by Drs. Horacker and Felix of Hoffman La Roche) into C57B1/6 mice increases responses of thymic cells to Con A and IL-1 at 6, 9, and 13 days after irradiation. Similar treatment of C_3H/HeJ mice (high responders to Con A and IL-1) does not affect the level of responses of irradiated; control and treated mice. We conclude that the recovery of the thymus of the low responder strain may be accelerated by exogenous thymic hormones. It is therefore possible that the difference in endogenous levels of thymic hormones in the two strains determine the levels of more mature T-cells in the thymus.

Animal Models and Clinical Studies

B81 EFFECTIVENESS OF LIPOSOMES AS POTENTIAL CARRIERS OF ANTIGENS AND ADJUVANTS, Carl R. Alving, Roberta L. Richards, Joel Moss, Loren I. Alving, John D. Clements, Tetsuo Shiba, Shozo Kotani, Robert A. Wirtz and Wayne T. Hockmeyer. Walter Reed Army Institute of Research, Washington, DC 20307-5100, National Institutes of Health, Bethesda, MD 20205, and Osaka University, Osaka, Japan.

Liposomes were investigated as carriers for inducing rabbit antibody responses against two separate antigens: cholera toxin (CT), and a synthetic albumin-conjugated 16-mer peptide derived from Plasmodium falciparum sporozoites. The influence of liposomal lipoidal adjuvants, including lipid A and lipophilic MDP, was also examined. The Plasmodium peptide antigen was completely nonimmunogenic alone, but was highly immunogenic in liposomes. Antibodies against the Plasmodium antigen reacted with the Plasmodium peptide but not with the albumin carrier to which the peptide was attached. The antibodies also reacted with intact Plasmodium sporozoites. CT was immunogenic alone, but was much more immunogenic in liposomes. When lipid A was included in the liposomes the immune response against each of the liposome-associated antigens was greatly increased. The anti-CT immune response induced by liposomes containing lipid A was 160-fold to 650-fold greater than the response induced by CT alone. We conclude that liposomes can serve as effective carriers of antigens and adjuvants, and the immune response generated by liposomes and liposomal adjuvants may be greatly enhanced. There may be special benefits in using liposomes with antigens, or antigens combined with protein carriers, that have a low degree of immunogenicity.

B82 CELL-MEDIATED IMMUNE RESPONSES IN MELANOMA PATIENTS UNDERGOING ACTIVE IMMUNOTHERAPY WITH VACCINIA MELANOMA ONCOLYSATES (VMO), Jerry A. Bash, Marc K. Wallack and Eleuthere Leftheriotis, Mount Sinai Medical Center, Miami Beach, Florida 33140

Lysates prepared from vaccinia-infected melanoma cell lines (VMO) have previously been shown to induce production of antibodies against uninfected melanoma target cells in melanoma patients undergoing active VMO immunotherapy. In the present trial, the development of cell-mediated immunity was also studied. Stage I and Stage II melanoma patients at high risk for recurrence after surgery were treated with weekly injections of 2mg VMO in a Phase Ia/Ib multiinstitutional trial. Serum obtained before and at 3 month intervals during treatment was tested in a Staphylococcus protein A(SpA) binding assay for antibody reactive with melanoma cell lines. Peripheral blood mononuclear cells (PBM) obtained from patients at the same time points were cryopreserved in liquid nitrogen until assayed simultaneously along with normal control PBM. Assays for T lymphocyte proliferative responses (3H-thymidine incorporation) included measurement of nonspecific blastogenesis induced by phytohemagglutinin (PHA) as well as specific blastogenic responses to VMO and mitomycin-C treated melanoma cells in 3-5 day microcultures. Effector cells cytolytic for K-562 or melanoma lines were measured in 4hr 51-chromium release assays directly or after in vitro stimulation of PBM with melanoma antigens in the presence of purified human interleukin-2 (IL-2). Nonspecific responses to PHA and NK activity were generally increased during VMO treatment. Melanoma-restricted proliferative responses and CTL activity were also demonstrable and sometimes preceded the appearance of melanoma-restricted antibody production. Further studies are being performed to characterize the development of humoral and cellular responses in these patients. These studies will then be extended into a large randomized Phase III trial of VMO to correlate immune responses with clinical course.

B83 IMMUNE IMPAIRMENTS IN ASYMPTOMATIC HOMOSEXUALS PRECEDE THEIR EXPOSURE TO HTLV. Zvi Bentwich,* Rimona Burstein,* Zvi Ben Ishai,** Marit Pecht,*** Nathan Trainin,*** Stanley Levin,* and Zeev Handzel*, *Kaplan Medical Center, Rehovot, **Rambam Medical Center, Haifa, and ***Weizmann Institute, Rehovot, Israel.

Israel is still a low incidence country for AIDS, with only 17 AIDS patients diagnosed to date. It thus offers an optimal background to determine the temporal and causal relationships between immune impairments, HTLV and development of AIDS. A cohort of 400 healthy asymptomatic male homosexuals (MHS) have been studied and followed up during the last two years. In 40% of these significant decreases in the peripheral blood lymphoid populations, impaired lymphoid cellular function and activation of the interferon system were found. Seropositivity for HTLV1, HTLV3 or both was found in 23%, 13.5% and 8.0%, respectively. No difference in the prevalence of either immune derangements or interferon system activation was observed between HTLV seropositive and seronegative individuals. However, of the five subjects from the group who developed AIDS or ARC, all were seropositive for HTLV1 and HTLV3. These results indicate that immune impairments in MHS precede the exposure to HTLV viruses and probably account for their increased susceptibility to these viruses and for developing AIDS.

B84 CONTINOUS T CELL LINES GROWN FROM FINE NEEDLE ASPIRATES OF HUMAN RENAL ALLOGRAFTS DURING REJECTION. S. Caillat, L. Chatenoud, H. Campos, H. Kreis, J.F. Bach. INSERM U 25, Hôpital Necker, 161, rue de Sèvres, 75015 Paris, France.

The functional analysis of cells infiltrating renal allografts (obtained by fine needle aspiration FNA) may promote new insights into the intricate mechanisms mediating cellular rejection. FNA cells were recovered from 10 patients undergoing acute rejection (before the onset of anti-rejection treatment). The cells were grown in a conditioned medium (CM) supplemented with 1 Unit/ml of recombinant Interleukin 2. Feeder cells, either autologous peripheral blood lymphocytes or donor splenocytes were added every two weeks. Thus, continuous T cell lines were obtained that could be analysed for their phenotypes and functions.

At one month of culture, in most cases, an homogeneous phenotypic pattern was observed, namely : 80% to 96% of cells reacting with the monoclonal antibodies OKT3 (anti-total human T cells) and OKT8 (anti-cytotoxic/suppressor T cells). Furthermore, these lymphocytes were found to express Ia antigens and Il-2 receptor. In parallel, the specific and non-specific functional capacities of these expanded cells were tested. In all the cases, the T cell lines obtained exhibited a specific cytotoxic activity against donor splenocytes, but not against non specific target cells including HLA mismatched human cells and the B cell line LHN13 (activated killer sensitive). The specificity restriction of this cytotoxicity was studied. Moreover, these FNA expanded cells showed a specific proliferative response in secondary MLC against donor cells, as well as a spontaneous and specific induced Il-2 production.

B85 THYMOPENTIN AND HERPES SIMPLEX VIRUS-2 MODULATE THE SERUM LEVEL OF INTERFERON γ IN MICE. Cappel, R., Huygen, K., *Bolla, K., Inst. Pasteur, Dept. Virology, Brussels, *Cilag Ltd. Technical Center, CH-Schaffhausen.

The influence of three doses of HSV-2 (LD-50, LD-10 and LD-10/100), seven doses of thymopentin (from 10ng/kg up to 10mg/kg), and the appropriate controls on the basal level of interferon γ (IF-γ) in mouse serum was investigated. Six mice a day per group were sacrificed for ten days, after a single injection of viruses or thymopentin. The IF-γ levels in the animals' sera were individually tested.

The two lower doses of HSV-2 increased the IF-γ level (maximally 7-fold) for 8 days. The highest dose (LD-50) induced, if at all, only a minimal increase (30%) on days 3 and 4 after infection. Thymopentin increased the basal level at all doses investigated. Maximal increase (about 4 times the basal level) was observed with 100 ng/kg and 1 mg/kg of thymopentin. The increase achieved with thymopentin seems to be of longer duration than the increase induced by viruses. The possible interrelationship between virus- and thymopentin-induced increase in the IF-γ level are discussed.

B87 THE EFFECT OF BSF-1 AND IFN-γ UPON MOUSE IMMUNOGLOBULIN ISOTYPE EXPRESSION, Robert L. Coffman[1], Joanne Carty[1], Junichi Ohara[2], Martha W. Bond[1], and William E. Paul[2]. [1]DNAX Research Institute of Molecular and Cellular Biology, 901 California Avenue, Palo Alto, CA 94304-1104. [2]Laboratory of Immunology, NIAID, NIH, Bethesda, MD 20205.

A subset of helper T (T_H) clones produce an activity which enhances the IgE response of LPS-stimulated B cells by more than 100-fold. This IgE-enhancing activity copurifies through several fractionation steps with the lymphokine BSF-1. Three preparations of highly purified BSF-1 from EL-4 lymphoma cells enhance IgE production to the same extent as the T_H supernatants. In addition, a monoclonal antibody to BSF-1, 11B11, totally inhibits the enhancing activity in T_H supernatants. BSF-1 also causes a 10-fold increase in IgG_1 and a 10-fold decrease in IgG_3 production (as previously reported, E.S. Vitetta, et al., J. Exp Med. in press), but has little effect upon IgM, IgG_{2A}, IgG_{2B}, or IgA levels in LPS-stimulated cultures. IFN-γ totally reverses the BSF-1 mediated enhancement of IgE and IgG_1 at concentrations which have relatively little effect on other isotypes. Both BSF-1 and IFN-γ appear to act directly upon B cells. Thus, BSF-1 and IFN-γ can dramatically and selectively affect the distribution of isotypes in these cultures, although they cause little change in total immunoglobulin levels.

B88 SUPPRESSOR T CELLS IN INDUCED AUTOIMMUNE HAEMOLYTIC DISEASE

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CBA mice injected with rat erythrocytes (rbc) develop anti-erythrocyte autoantibodies in addition to anti-rat rbc antibodies. After 8-12 weeks generation of suppressor cells which are able to suppress the autoantibody response can be demonstrated by adoptive transfer of suppression into naive mice. Using the adoptive transfer assay system, suppressor cells have been phenotyped previously as $Ly\text{-}1^+2^-T$ cells (1) or $Ly\text{-}1^+$ B cells In contrast, using an in vitro culture system in which dissociated spleen cells from autoimmune mice form autoantibodies in response to rat rbc antigens, we have found that $L3T4^-Ly2^+$ T cells are responsible for autoantibody-specific suppression, while addition of $L3T4^+$ T cells, and B cells results in enhanced autoantibody responses. By fusing suppressor T cells with BW 5147 cells, we have produced T cell hybridomas which elaborate autoantibody-specific suppressor factors able to suppress in vitro T cell proliferation as well as in vitro autoantibody responses.
Thus suppressor effector cells in this autoimmune model are $L3T4^-$ $Ly2^+$ T cells, which may function through secretion of antigen-specific suppressor factors.
(1) A Cooke et al (1978) Nature 273:154.
(2) G J Watt et al, Eur. J. Immunol. in press.

B89 EFFECT OF THE TP5 ANALOGUE OF THYMOPOIETIN ON THE REJECTION OF MALE SKIN BY FEMALE MICE, Elizabeth DePirro, Ellen H. Goldberg, and Gideon Goldstein, University of New Mexico School of Medicine, Albuquerque, NM 87131, and Ortho Pharmaceutical Corp., Raritan, NJ 08869.

The TP5 pentapeptide (thymopentin) analogue of thymopoietin was shown to affect the capacity of C3H/HeJ female mice to reject C3H/HeJ male skin (the H-Y rejection response). Whereas C57BL/6 (B6) female mice almost always reject B6 male skin, young C3H/HeJ (C3H) female mice seldom reject C3H male skin. That this difference is due to an immunoregulatory balance was apparent from studies which showed that the incidence of rejection is increased in aged or thymectomized C3H female mice. Furthermore, both of these conditions are associated with changes in the relative proportions of functionally distinct T cell sets.

Therapy with TP5 has been shown to substantially reduce the capacity of aged C3H females and young, thymectomized C3H females to reject male skin (down-regulated), whereas the H-Y rejection response of young, thymus-intact female mice was heightened by treatment with TP5 (up-regulated). In all cases, mice were inoculated weekly with 1 µg of TP5. In attempts to determine the immunoregulatory effects of various doses of TP5 on male skin rejection by thymectomized and thymus-intact C3H females, doses ranging from 10^{2} µg down to 10^{-5} µg were inoculated at weekly intervals. Results suggest that there is a wide dose range for up-regulating immune reactivity, with a more narrow and higher dose range for down-regulatory effects.

B90 PROLONGED EXPRESSION OF INTERLEUKIN-2 RECEPTORS ON T CELL LINES FROM MULTIPLE SCLEROSIS PATIENTS. Elaine DeFreitas* and Magnhild Sandberg-Wollheim,† The Wistar Institute, Philadelphia, PA 19104, USA* and Department of Neurology, University of Lund, Sweden.†

When activated with antigen or mitogen, T lymphocytes express membrane receptors which bind Il2, resulting in cellular proliferation. After a limited time period, normal T cells become refractory to receptor-saturating concentrations of Il2 due to down regulation of Il2 receptors (Cantrell and Smith, 1983, J. Exp. Med. 158:1895). We have evaluated the expression of the Il2 receptor on antigen- and mitogen-activated T cells from blood and cerebrospinal fluid of normal and multiple sclerosis (MS) patients using the monoclonal antibody anti-Tac (Uchiyama et al., 1981, J. Immunol. 126:1393). Il2-propagated T cell lines from normals showed maximal binding of anti-Tac 3 days after antigenic stimulation. Continuous supplementation with recombinant Il2 resulted in proliferation and detection of Tac-positive cells until day 9-11. Thereafter, all cell lines were Tac-negative and did not synthesize DNA. In contrast, T cells from MS patients showed maximal expression of Il2 receptors as early as day 2 and a significant proportion proliferated and remained Tac-positive for more than 22 days after restimulation. After Il2 removal, Tac-positive and DNA synthesizing normal T cells were undetectable within 48 hrs. In contrast, Tac-positive MS T cells were still present 4-6 days after Il2 removal. MS T cells stimulated with mitogen on normal allogeneic monocytes showed the same pattern of prolonged Il2 receptor expression as antigen specific lines stimulated with autologous monocytes.

Supported by NIH grant AI-19987-01* and the Swedish Medical Research Council.†

B91 IN VIVO ACTIVATION OF CYTOLYTIC ACTIVITIES DURING IL2 CLINICAL TRIALS. M.V. Doyle, S. DeGroat, R. Lowitz*, D. Ben-Zev* and E.C. Bradley. Cetus Corporation, Emeryville, CA 94608, and *Cancer and Aging Research Institute, Walnut Creek, CA 94598.

Thirty patients with progressive, metastatic, solid tumors which were refractory to conventional therapies were entered into a Phase II clinical trial of recombinant des ala ser_{125} IL2. Patients received daily subcutaneous injections of IL2 with an escalating dose schedule of 10^{5} to 10^{6} units/$meter^{2}$ over a period of twelve weeks. Natural killer (NK) and lymphokine activated killer (LAK) cell activities were measured prior to and every two weeks throughout the course of the trial using NK sensitive K562 cells and LAK sensitive Daudi cells in a 4 hour ^{51}chromium release assay. Initial results demonstrate that, while many of the patients presented with abnormally low levels of spontaneous NK cell cytotoxicity, during the first month of IL2 administration at 10^{5} units/$meter^{2}$ there was a significant elevation and maintenance of that activity. However, LAK cell cytotoxicity was not detected in these patients either prior to or during the first month of IL2 therapy, although such activity could be generated by treatment of the patients' lymphocytes with IL2 in vitro. The completed set of NK and LAK cell assay data through the entire IL2 dosing schedule and the relationships between the duration of treatment, doses of IL2 and cytolytic activities will be discussed.

B92 ANTI-IDIOTYPE APPROACH TO REGULATION OF AUTOIMMUNE DISEASE, Donard S. Dwyer, University of Alabama at Birmingham, Birmingham, AL 35294

A panel of monoclonal antibodies (Mabs) has been accumulated that react with the acetylcholine receptor (AChR) from the mammalian neuromuscular junction and P_2 protein from peripheral nerve myelin. The AChR is the target of an autoimmune attack in myasthenia gravis, whereas an autoimmune response against the P_2 protein may occur in certain inflammatory neuropathies.

Pretreatment of mice with a combination of syngeneic monoclonal anti-idiotypes (ids) from the AChR system produces a reduction in the subsequent autoimmune response to AChR. The suppression appears to be mediated in part by the induction of endogenous anti-ids. Representatives of these anti-ids as well as rheumatoid factors and epibodies have been isolated from anti-id treated mice by hybridoma technology. Although a dominant id has not been identified, anti-id treatment can nevertheless reduce the autoimmune response to AChR.

In the myelin antigen system, 16 monoclonal anti-ids have been produced which react with a panel of 5 Mabs against P_2 protein. Most of these anti-ids recognize the immunizing antibody alone, however one anti-id reacts with 3 of the 5 Mabs whereas a second anti-id recognizes a common determinant found on 4 of the 5 antibodies. These results offer hope that a dominant id will be identified in this antigen system. Studies to examine the regulatory role of these anti-ids are underway.

B93 THYMIC HORMONE TREATMENT OF VIRUS ASSOCIATED IMMUNODEFICIENCIES, M. Fiorilli, L. Palmisano, and F. Aiuti, Department of Clinical Immunology, Universtiy of Rome 'La Sapienza', Rome, Italy

Viral infections may cause as well as result from immunodeficiency. In both cases, treatment with thymic hormones might prove to be a clinically valuable tool by acting at several levels, e.g. by enhancing cytotoxic T-cell activity, interferon production and natural killer cell activity. We report the results of a number of clinical trials of thymic hormone therapy in patients with viral infections. An open label, randomized trial, involving patients with recurrent Herpes Simplex virus type-I (HSV) infections and underlying immunodeficiencies (primary or secondary), showed a significant reduction of the relapse rate in treated vs control patients. A double-blind study was conducted in a total of 40 patients with recurrent HSV keratitis. A significant reduction of the number of relapses ($p<0.05$, Fisher exact test), an increase of the relapse-free period ($p<0.05$, log rank test), and an earlier normalization of the number of circulating T-cells, were observed in treated vs placebo patients. A randomized study was done in 32 patients with HBsAg+/HBeAg+ chronic active hepatitis. The rates of disappearance of HBsAg and HBeAg, and of appearance of anti-HBsAg antibodies, were significantly higher in treated patients ($p<0.02$, $p<0.02$, and $p<0.01$, respectively; Kaplan-Meyer test) than in controls. The SGOT values at six months were also significantly improved by treatment (intra-group analysis: $p<0.01$ reduction in the treatment group, $p<0.05$ increase in the control group; inter-group analysis:<p 0.01). Preliminary data are available from an ongoing randomized study on thymic hormone treatment for HTLV3-associated lymphadenopathy syndrome in drug abusers and male homosexuals.

B94 RESISTANCE TO EAE: REGULATION BY NON-MHC GENES, Mary Pat Happ, Peter Wettstein, and Ellen Heber-Katz, The Wistar Institute, Philadelphia, Pa. 19104

Experimental Allergic Encephalomyelitis (EAE) is a primary laboratory model for studying CNS autoimmune diseases. In the rat, some strains are resistant to the induction of EAE; this phenomenon has been attributed to the MHC. The congenic rat strain, BN.B1L, bears the MHC of the susceptible Lewis strain on a BN-derived background, a resistant strain. We found that the BN.B1L does not develop clinical symptoms of EAE although it does appear to possess the appropriate T cell specificities. After a period of in-vitro culture with myelin basic protein, a BN.B1L T lymphocyte population can be induced to transfer a weak form of the disease, with poor efficiency. When the in-vitro cultures are supplemented with a Con A-induced supernatant containing IL-2 and interferon, the transfer of disease is unaffected. However, supplementation with LPS dramatically increases the efficiency of transfer. Preliminary data indicates that the induction of Ia may be involved. We are currently examining the possibility of a BN background-derived gene which may control Ia expression, and the regulation of such a gene by LPS or other immunomodulators. We are also examining the contribution of the T cell repertoire encoded by the resistant BN background.

B95 DEVELOPMENTAL ABNORMALITIES OF T-CELL-RECEPTOR GENES IN AUTOIMMUNE MICE.

Yasuhiro Hashimoto and Mark I. Greene, University of Pennsylvania, Philadelphia, PA 19104

The developmental expression and rearrangement of the T cell receptor genes have been studied. Beta and gamma subunits are expressed relatively abundantly in immature thymocytes; Lyt2(-), L3T4(-). Conversely, alpha and beta subunits are expressed abundantly in mature thymocytes; Lyt2(+), L3T4(+) while the gamma subunit is not expressed at significant levels.

Some autoimmune mice have developmental deficiencies in their T cells. In autoimmune mice with lpr/lpr or gld/gld genotypes, the major population of peripheral T cells is immature; Lyt2(-), L3T4(-).

We investigated the rearrangement of the T-cell receptor gene in the immature T cell of C3H/HeJ-gld/gld which is a known autoimmune model. Rearrangement of T-cell-receptor gene within a heterogeneous population of T cells is detected on Southern blots as the disappearance of the germline configuration bands. Southern analysis suggests that, in C3H/HeJ-gld/gld peripheral lymph nodes, the T-cell-receptor germline configuration is less conserved than in T cells from C3H/HeJ-+/+ peripheral lymph nodes.

We also studied the expression of T cell receptor genes in C3H/HeJ-gld/gld during development. Northern blots show that T cells from gld/gld mice express the alpha gene at a much higher level than control C3H/HeJ. Differences in the expression of the beta and gamma genes were not observed between gld/gld mice and control C3H/HeJ mice.

B96 THE ROLE OF MAST CELLS IN THE DEVELOPMENT OF ALLERGIC ENCEPHALOMYELITIS.

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In the rat model of Experimental Allergic Encephalomyelitis (EAE) we have been able to adoptively transfer clinical disease into bone marrow chimeras constructed by infusing bone marrow cells derived from F_1 (Lewis X Brown-Norway) animals into lethally irradiated P_2 (Brown-Norway) recipients. These chimeras developed paralytic disease 5 days following transfer of basic protein sensitized spleen cells. The spleen cells were obtained from P_1 (Lewis) animals previously injected with Myelin-derived basic protein emulsified in Complete Freund's Adjuvant. The development of disease in these chimeras questions the mechanisms of lymphocyte trafficking since the transferred cells are interacting in vivo with allogeneic endothelium. A possible explanation of disease transfer in this donor-recipient combination evokes the involvement of the mast cell as a source of mediators that alter the endothelial barrier and allow semi-allogenic cell interaction at the developing CNS lesion. We have completed a series of experiments that indicate a key role for the mast cell in the initial cell interactions leading to clinical disease. Moreover, all of our observations are consistent with the existence of mast cells with receptors for T cell derived antigen specific factor(s). When mast cells come into contact with antigen a triggering event ensues which leads to capillary permeability and subsequent lymphocyte interaction with antigen. These observations are consistent with those of P.Askenase in the mouse model of dermatitis.

Supported by Grant # NS 16731

B97 NOVEL IMMUNOREGULATORY T CELL CLONES INVOLVED IN B CELL RESPONSES TO EBV AND PWM

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Human T cell responses to Epstein-Barr virus infection are clinically important and involve interactions of T cells with resting B cells, activated B cells and virus-infected B cells. We have studied the immunoregulatory properties of T4+ inducer cell clones which recognize EBV transformed autologous B cells. The T cells proliferated in an MHC class II restricted fashion to autologous BCL. Two novel types of clones were identified. A relatively frequent clone was T3+, T4+, $T8^-$, Leu 8+, able to elaborate IL-2 and γ IFN in large amounts, and able to directly suppress polyclonal B cell IgG production in response to either PWM or EBV. Although activation of the clone was MHC-restricted, suppression of IgG secretion was unrestricted. The clone was noncytolytic. A second, less frequent clone was T3+, T4+, $T8^-$, Leu 8+, able to make IL-2 but not γ IFN and able to directly help B cells to make IgG. Helper function was dependent on the state of B cell activation: the clone proliferated in response to and helped PWM-activated B cells, but not resting B cells. When the two types of T4+ clones were mixed, the suppressive function of the first clone predominated. These studies appear to indicate that the human T4+ inducer cell population contains previously unrecognized subsets with important B cell regulatory properties.

B98 EVIDENCE OF A NEW ENDOTHELIAL CELL RECOGNITION SYSTEM CONTROLLING LYMPHOCYTE TRAFFIC INTO THE INFLAMED SYNOVIUM

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Lymphocyte traffic is controlled in part by selective lymphocyte recognition of high endothelial venule (HEV) cells at sites of lymphocyte exit from blood. In rheumatoid and other inflammatory arthritides lymphocyte infiltrates are found in the synovium that frequently contain vessels morphologically resembling the HEV in lymph nodes. The functional capacity and specificity of these HEV to interact with lymphocytes was studied in rheumatoid synovium using an in vitro frozen section assay of lymphocyte-HEV binding. We found that lymphocytes bind specifically to synovial HEV and that the lymphocyte-endothelial recognition system in the synovium is distinct from those previously described for peripheral lymph nodes or mucosal lymphoid tissues. Such a unique endothelial specificity associated with synovium infiltrates, or chronic inflammation in general, could selectively direct the extravasation of pathologically important effector cells to inflamed tissue.

B99 POSSIBLE RELATIONSHIP OF AN ANTIGEN-SPECIFIC SUPPRESSOR FACTOR TO PHOSPHOLIPASE INHIBITORY PROTEIN.

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Glycosylation inhibiting factor, GIF, a phosphorylated fragment of lipomodulin, has recently been shown in vivo to have immunosuppressive properties. Biochemical characterization of GIF released from an ovalbumin specific T-cell hybridoma, 231F1, revealed that this factor shared common properties with T-cell suppressor factors, (TsF). The majority of the GIF released by 231F1 following antigen stimulation had a molecular weight of 30 - 40 kd, and, like other TsFs, bound to antigen and had I-J determinants. Following reduction of this factor with dithiothreitol, GIF activity was retained by a 15 kd molecule which no longer bound antigen but could be absorbed by an anti-I-J^b monoclonal antibody. In addition, the smaller molecular weight GIF (15K) was constitutively secreted by 231F1. Thus, it appears that the antigen-specific GIF will dissociate into 2 chains, a 15 kd chain bearing I-J determinants and a separate antigen-binding chain which appears to require antigen stimulation for expression. Because of the similarities between antigen-specific GIF and antigen-specific TsF, we suggest that lipomodulin, or a fragment of lipomodulin, may be a component of antigen-specific TsFs. Preliminary evidence indicates that at least one other TsF, a TsF specific for anti-G-T, has GIF-like activity. Furthermore, both the 15 kd and 40 kd GIF molecules from 231F1 bind monoclonal anti-lipomodulin antibodies and exert phospholipase A2 inhibitory activity upon dephosphorylation. These findings suggest intriguing possibilities for investigating the biochemical basis of immunosuppression.

B100 DOWN REGULATION OF Ia ANTIGEN EXPRESSION ON INFLAMMATORY MACROPHAGES BY FACTORS PRODUCED DURING ACUTE INFECTIONS WITH Rickettsia tsutsugamushi.

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Acute, lethal infections of mice with R. tsutsugamushi are associated with a marked inflammatory exudate. An early (5 days after infection) increase in the number of cells expressing Ia antigen was seen; however, as the infection progressed the number and proportion of Ia antigen-bearing macrophages (MØ) declined to baseline levels. Ascites fluid produced during the infection was found to contain high levels of IFN gamma that was capable of inducing Ia antigen expression on the MØ-like tumor cell, J774. The presence of IFN gamma in the exudate suggested the possibility that expression of Ia by the inflammatory MØ was somehow blocked. High levels of corticosterone and prostaglandins were found in the ascites fluid. The inflammatory MØ population was found to produce significant levels of PGE_2, which inhibited the expression of Ia by J774 cells. MØ isolated from infected mice could not be induced to express Ia antigen when incubated with various sources of IFN gamma. If these cells were treated with indomethacin prior to the addition of IFN gamma a significant increase in Ia antigen expression occurred. These data suggest that the lack of Ia antigen expression by MØ responding to a fulminant infection even in an environment rich in IFN gamma, may be due to the production of PGE_2 by cells in the population. Increased levels of corticosteroids in the exudate may also contribute to the suppression of Ia antigen expression in this model.

B101 EVIDENCE FOR INCREASED PRODUCTION OF IFN-GAMMA AND OTHER FACTORS SUPPRESSING HEMATOPOIESIS IN APLASTIC ANEMIA PATIENTS, Rolf Kiessling, Tamas Laskay, Mona Hansson, Anna Porwit and Magnus Björkholm. Karolinska institute, Karolinska hospital and Danderyd hospital, Stockholm, Sweden.

Previous studies have pointed at the ability of T-cells as well as NK-cells to suppress stem cells in the bone-marrow of aplastic anemia patients and healthy donors. We have studied the involvement of soluble factors produced by lymphocytes in the stem cell inhibition. Lymphocytes from aplastic anemia (AA) patients or healthy controls were cultured with or without PHA, and the culture supernatants tested for both its ability to inhibit GM-CFC , as tested in conventional agar assays, and for its contents of various types of interferons (IFN), as measured in anti-viral assays and in RIA assays. We found that a) in line with what has been shown by others, AA-patients produce 10-100 times more IFN-gamma than do healthy donors. Low levels of IFN gamma was found in sera from AA-patients.
b) A stem cell inhibitory factor(s) distinct from IFN was produced by AA-patients as well as to a lower degree also by healthy lymphocyte donors. This non-IFN factor from AA patients is active both against the more immature (day 14) as well as the more mature (day 8) GM-CFC progenitor cell, while the factor from healthy donors only inhibits day 8 colonies.
The role of IFN-gamma as well as of other lymphokines in the pathogenesis of AA will be discussed.

B102 Suppression of IgG_1 antibody responses during a malaria infection.

Jean Langhorne and Klaus Eichmann, Max-Planck-Institut für Immunbiologie, Freiburg, FRG.

Infection of mice with Plasmodium chabaudi adami results in a non-specific B cell response and a parasite-specific antibody response, both of which apparently lack significant IgG_1 components. In addition, during the first 15-20 days of infection, when this effect is most pronounced, there is an accompanying decrease in the number of Fc receptor-bearing spleen cells able to bind IgG_1 immunoglobulin. Studies of an in-vivo antibody response to sheep erythrocytes (SE) during infection indicate that the IgG_1 component of the SE-specific PFC response is also preferentially depressed, whereas that of the remaining IgG isotypes is relatively unaltered. This effect can also be reproduced in in vitro experiments by the addition of spleen T cells from infected mice to SE-primed lymphocytes in Mishell-Dutton cultures. Thus, these data indicate that there is selective suppression of IgG_1 immunoglobulin, regardless of antigenic specificity, during infection with Plasmodium chabaudi adami.

B103 EFFECT OF SAF IN ANIMAL MODELS, Catherine Lau, Ortho Pharmaceutical (Canada) Ltd., Don Mills, Ontario M3C 1L9

The in vivo suppressive effects of the Suppressor Activating Factor (Lau et al., J. Imm. 134:3155, 1985) were tested in several animal models. Bone marrow graft, treated for four hours in vitro with SAF, reconstituted successfully the immune response in histoincompatible recipients with little or no graft vs. host disease. In vivo administration of SAF also significantly prolonged histoincompatible skin graft survival in Balb/c mice. SAF, when given intraperitoneally, effectively arrested the production of autoantibody in C_3^H mice with induced autohemolytic anemia. The suppression on heterologous antibody levels was, however, only minimal. Furthermore, mice that were injected intraperitoneally with SAF for up to three months, manifested no unfavorable side effects.

Currently, SAF is under intensive biochemical characterization. The biological activity of the more purified molecule will be discussed.

B104 PRELIMINARY PHYSICOCHEMICAL CHARACTERIZATION OF THE T CELL-SELECTIVE, IgE-INDUCED REGULANT EIR_T, John F. Marcelletti and David H. Katz, Medical Biology Institute, La Jolla, CA 92037.

Appropriate levels of IgE activate a cascade of cellular and molecular interactions which function to maintain normal homeostatic balance in the IgE antibody system. During this process soluble mediators are produced which we have termed IgE-induced regulants (EIR). One such regulant is derived from Lyt-2^+ T cells and is referred to as EIR_T. Paradoxically, this regulant functions to further enhance IgE synthesis by inducing production of a potentiating IgE-binding factor denoted enhancing effector molecule (EEM). We have begun initial physicochemical characterization of the EIR_T conventionally derived from Lyt-2^+ T cells ($cEIR_T$) and from a monoclonal T cell hybridoma ($mcEIR_T$). Both species of EIR_T were shown a) to be a protease-sensitive molecule with a molecular mass of 45-60 kd, b) to induce the enhanced expression of Fc receptors for IgE ($Fc\varepsilon R$) selectively on Lyt-2^+ T cells, and c) to cause Lyt-2^+ T cells to release EEM. Finally, culture supernatant fluids containing $cEIR_T$ were found to exert potent inhibition of in vivo IgE antibody responses, whereas similar fluids containing $mcEIR_T$ potentiated IgE synthesis. This inconsistency was shown to be the consequence of suppressive factor of allergy (SFA) in the former preparation, and the lack of such contaminants in the latter. We conclude that EIR_T is a molecule with the physiological function of selectively potentiating IgE antibody synthesis.

B105 T-CELL REPERTOIRE TUNING BY THE MHC IN PROTECTION AGAINST LETHAL VIRUS INFECTION AND IN REJECTION OF H-Y DISPARATE SKIN GRAFTS. THE ROLE OF CYTOKINES.
C.J.M. Melief, W.M. Kast, C.J. Boog and L.P. de Waal. Central Lab. Netherl. Red Cross Blood Transf. Service, Amsterdam, The Netherlands

The in vivo importance of MHC control of T-cell responses will be illustrated by two examples. First, an all-or-none-CTL response against Sendai virus correlated with a three amino acid difference in the crucial class I restriction element (K^b) for this response, is associated with a tenfold difference in the susceptibility to lethal pneumonia induction by virulent Sendai virus, the CTL nonresponder (bm1 mutant) being more susceptible. This is the first demonstration of an association between MHC and susceptibility to a natural pathogen of high virulence linked to CTL nonresponsiveness on the basis of class I MHC mediated regulation. The role of interferon-γ in T-cell protection against virulent virus will be discussed. The second example concerns the inability of bm12 mutant mice with a three amino acid substitution in their class II $I\text{-}A^b$ molecule to reject H-Y incompatible skin grafts. We now report that both the CTL nonresponsiveness to H-Y and the failure to reject H-Y incompatible skin grafts can be abolished by immunization with antigen-bearing dendritic cells (DC). The abolition of the CTL response defect was not due to activation of helper-independent CTL, but to the arousal of a dormant T_H repertoire. Immunization with antigen-bearing DC holds promise for situations in which specific abolition of low responsiveness is desired, such as states of immunodeficiency or instances of weak immunogenicity. The participation of cytokines (IL-1, IL-2) in the abolition of non-responsiveness will be discussed.

B106 Induction of proliferation and thymus-dependent responses in athymic nude mice by Pseudomonas exotoxin A. Michael L. Misfeldt and Peter S. Holt, University of Missouri-Columbia, Columbia, Missouri 65212.

Pseudomonas aeruginosa produces a number of extracellular enzymes and/or toxins which appear to play a significant role in the ability of the organism to cause disease. We have examined one of these extracellular products, exotoxin A, for its ability to act as a biological response modifier. Depending on the immunocompetence of the host, exotoxin A may exert its effect through different immune cell populations. In immunocompetent euthymic mice, exotoxin A was observed to suppress the immune response to both thymus-dependent (TD) and thymus-independent (TI) antigens by activating a suppressor T cell. In immunoincompetent athymic nude mice, exotoxin A was unable to induce suppression of the immune response to the thymus-independent antigen, TNP-Ficoll. Instead, exotoxin A was observed to enhance the immune response to both TI and TD antigens in nude mice. This enhanced response was found to be antigen specific and not due to polyclonal B cell activation. We also observed that exotoxin A was capable of inducing specific cells to proliferate in athymic nude splenocytes as well as in euthymic thymocytes. Within the athymic nude splenocyte population, it was observed that an Ig^-, Ia^- cell was induced to proliferate by exotoxin A. In euthymic thymocytes, exotoxin A activated a cortisone-resistant, peanut agglutinin binding negative T cell. Toxoiding the molecule had little effect on the induction of proliferation. However, cyclosporin A addition eliminated the activation of both athymic splenocytes and euthymic thymocytes. Therefore, exotoxin A is an immunoregulator which has the potential to be a valuable probe in exploring T cell differentiation and/or activation.

B107 The effect of cyclosporine (CsA) on the effector phase of autoreactive DTH. D. Naor and A. Langer. The Lautenberg Center for Immunol., Hebrew Univ.-Hadassah Med. School, Jerusalem, Israel. X-irradiated (250rad) A mice injected with syngeneic concanavalin A (Con A)-induced lymphoblasts and footpad challenged seven days later with syngeneic lipopolysaccharide-induced lymphoblasts presented 24h to 72h after challenge significant footpad swelling response, accumulation of ^{125}IUdR or massive cellular infiltration into the injection site. Since the immunological activity was transferred by Lyt-1$^+$ T cells, we designated it as syngeneic delayed type hypersensitivity (syn-DTH). This DTH was induced and elicited by antigens of the syngeneic lymphoblasts and not by contaminants attached to them. Multiple doses of 60 mg/kg of CsA given daily in the time interval between immunization and challenge, inhibited the syn-DTH. Multiple injections of CsA before or close to the induction phase of the syn-DTH was ineffective, whereas single or multiple injections of CsA close to the effector phase (the challenge time) markedly reduced the syn-DTH. Even a single injection of CsA 24h after the challenge, efficiently reduced the 48h syn-DTH. These facts may suggest re-evaluation of the CsA treatment strategy, which has been essentially based on in vitro experiments. T cells (Lyt-1$^+$) from x-irradiated mice immunized with Con A-induced lymphoblasts and injected with CsA, failed to efficiently transfer the syn-DTH response to native recipients. Since the nonspecific footpad swelling response of x-irradiated mice challenged with lymphoblasts alone is resistant to the standard protocol of the CsA treatment, we suggest that CsA inhibits the ability of T cells to produce or release lymphokines at the effector phase of the DTH. The autoreactive DTH response was also blocked, in more specific manner, by suppressive factor obtained from normal T cells or T cell hybridoma.

B108 RADIOPROTECTION BY INTERLEUKIN-1. *R. Neta, *S. Douchess, and $^{+}$J.J. Oppenheim, AFRRI*, Bethesda, MD 20814-5145 and BRMP, NCI^{+}, Frederick, MD 21701

Protection from lethal effects of ionizing radiation has been achieved by administration prior to irradiation of a number of immunomodulatory substances such as LPS, BCG, MDP, or glucan. However, it is not known what events induced by these substances lead to the radioprotective effect. Since these immunomodulatory substances induce the release of endogenous cytokines, we investigated whether any cytokines mediated radioprotection. We presently report that administration of a single dose of recombinant interleukin-1 (IL-1) (generously supplied by Drs. Benjamin and Lomedico of Hoffman LaRoche) protects mice in a dose and time dependent manner from lethal effects of ionizing radiation. In contrast, administration of a protein obtained from E. coli extract containing the plasmid without IL-1 cDNA did not confer radioprotection. Administration of 2000 units of IL-1 20 hrs prior to 950 R irradiation with a ^{60}Co source protected 82 +/-11% C57Bl mice (n=42) from one LD100/16 dose (dose of radiation that kills 100% mice in 16 days). The protective effect was greatly reduced when IL-1 was administered either at 45 hrs (12%), 4 hrs (30%) before or 2 hrs (0%) after irradiation. Similar protection was demonstrated for DBA/1 strain in which 1050 R was the LD100/15 dose yet which require lower doses of IL-1 for similar radioprotection (1000 units of IL-1 for 88% survival). Thus the identification of IL-1 as a radioprotection cytokine permits elimination of the use of exogenous microbial compounds which have deleterious side effects and furthers understanding of the pathways mediating radioprotective events.

B109 USE OF OKT3 FOR REVERSAL OF ACUTE AND STEROID AND ATGAM RESISTANT REJECTION, Douglas J. Norman, John M. Barry, Gideon Goldstein, Beth Funnell, Karen Henell; Laboratory of Immunogenetics and Transplantation, Oregon Health Sciences University, Portland, Oregon

Seventy-four patients were treated with OKT3 to reverse acute renal allograft rejection. Fifty-four were given OKT3 as the primary treatment for the first rejection and 20 as rescue treatment after prednisone and AtGam failed to reverse rejection. Ages were 2 - 66 years.

Rejection reversed in 100% of related donor recipients (n=12) and non-diabetic cadaver recipients under 50 years (n=32) both in the primary treatment and rescue treatment groups. Reversal was 80% with primary treatment and 50% with rescue treatment in diabetic and old cadaver recipients (n=30). Rejection reversed by the third day of treatment in 90% of patients. Re-rejection was successfully treated with conventional therapy in most cases and correlated with the severity of first rejection and anti-OKT3 antibody production. One year graft survival was 100% among related donor recipients, 83% among non-diabetic cadaver kidney recipients under 50 years and 74% among cadaver and older recipients who did not develop a complication of OKT3. First dose symptoms were common and are presumed to be due to release of T cell contents upon opsonization. Complications included pulmonary edema (1), allograft thrombosis (2), a TIA (1) and herpes esophagitis (1). No patient died during therapy and one year patient survival was 98.5%.

In conclusion, OKT3 proved to be the most effective agent which we have used to reverse acute rejection. Long term survival has been excellent without the use of cyclosporine.

B110 BIOLOGICALLY BASED IMMUNOMODULATORS IN THE THERAPY OF RHEUMATIC DISEASE, Seth H. Pincus, and John R. Ward, Division of Rheumatology, University of Utah School of Medicine, Salt Lake City, Utah 84132.

We have organized a meeting with the above title to be held December 9-10. This workshop will be attended by 100 scientists and will consider a variety of new agents including cyclosporin, monoclonal antibodies, interferons and interleukins. We will primarily focus on the treatment of rheumatoid arthritis and systemic lupus erythematosus. Attendees will include rheumatologists, molecular biologists, immunologists and pharmacologists. They are drawn from universities, pharmaceutical houses, and genetic engineering companies. At the conclusion of the meeting, a questionnaire will be completed by every attendee. We will ask the attendee to identify those agents that look most promising, agents that may have detrimental effects, how agents should be screened for therapeutic efficacy. and how long it will be before clinical trials begin with these materials. We would like to present the results of this poll.

B111 IN VIVO ACTIVITY OF A CHEMICALLY SYNTHETIZED HEMOPOIETIC GROWTH FACTOR (IL-3). John W. Schrader, Ian Clark-Lewis, Leroy Hood, Steven B.H. Kent, Hermann J. Ziltener, The Walter and Eliza Hall Institute of Medical Research, Melbourne 3050, Australia, and The California Institute of Technology, California CA. 91125.

The complete 140-amino acid sequence of panspecific hemopoietin (PSH) or IL-3 was synthesized using an automated synthesizer (see Clark-Lewis et al. ibid). Mice were injected with subcutaneous injections of 10^4 ED_{50} units (assayed in vitro on a PSH-dependent cell-line) for four days, three times daily. There was a greater than 100-fold increase in the numbers of mast cell precursors and increases in hemopoietic colony-forming cells in the spleen, which also increased in weight. Histological examination indicated increases in the numbers of mast cell and megakaryocytes in the spleen, and mast cells in the gut wall. At the injection-site, there were accumulations of mast cells, eosinophils, neutrophils and monocytes. These data directly demonstrate that the in vivo administration of PSH resulted in effects on the broad range of hemopoietic cells that were predicted from in vitro experiments. The clearance from the blood of intravenously-injected, biologically active, synthetic PSH was biphasic and resembled that of natural PSH. Polyclonal and monoclonal antibodies raised against synthetic PSH are being used to probe both the structure of the native molecule and its role in allergy, inflammation and neoplasia of the hemopoietic system. Analysis of the in vivo activity of chemically synthesized peptide analogues should rapidly yield information on the structural features critical for activity and lead to the design of more effective therapeutic agents.

B112 EARLY EVENTS OF B CELL ACTIVATION INDUCED BY NON-TRANSFORMING EBV, R.Szigeti, M.G.Masucci, I.Ernberg, G.Klein and E.Klein,Dept.of Tumor Biology,Karolinska Inst.S-104 01 Stockholm 60, Sweden

The early events of viral transformation were analyzed by infection of purified B lymphocytes with the transforming B95-8 derived and the non-transforming P3HR1 derived and UV-inactivated B95-8 viruses. Increased expression of Class I. and Class II. MHC antigens and activation-specific surface markers,as well as transferrin receptor were detected already 24 hrs after infection, i.e. prior to virally induced DNA synthesis. The activated B lymphocytes were able to produce the lymphokine, leukocyte migration inhibitory factor (LIF). The activation was independent of the expression of viral functions and was not affected by UV-irradiation of the B95-8 virus. Similar signs of activation were observed after exposure of the B cells to P3HR-1 virus. The results suggest that only binding of the virus to the specific receptor already activates the B cells to entry the early cell cycle phase. LIF production and low level of 3H-thymidin incorporation could be observed after exposureof the B cells to rabbit-anti-human gp140 antibody reacting with the C3d receptor, whereas no thymidin uptake, but high LIF production and Ig-secretion were found when certain oligopeptides, representing parts of the CH2 or CH3 domains of IgG-Fc, were used for activation.

B113 ANTIBODIES FROM OKT3 TREATED PATIENTS DO NOT BLOCK ANTIGEN BINDING OF OTHER CD3 MONOCLONAL ANTIBODIES, Mary Anne Talle, Mary Makowski, Catherine Blynn and Gideon Goldstein, Ortho Pharmaceutical Corp., Raritan, N.J. 08869.

The use of murine monoclonal antibody OKT3 for reversal of acute renal rejection results in the rapid appearance of neutralizing anti-OKT3 antibodies in most patients[1]. In one unusual patient, however, an anti-OKT3-isotype serum was generated in the absence of an anti-OKT3 idiotype response. The serum from this patient did not inhibit the binding of OKT3 to its target antigen and allowed persistant high levels of circulating OKT3[2]. We tested several patient sera containing anti-OKT3 idiotype activity for their ability to inhibit binding of OKT3 and other monoclonal antibodies directed to the T3 (CD3) antigen. Each monoclonal antibody in the panel was mitogenic for T cells and competed for binding with OKT3 yet anti-OKT3 serum blocked binding and function of only OKT3, not the other antibodies in the panel. The lack of anti-OKT3 idiotype cross reactivity with other anti-T3 antibodies was demonstrated both by immunofluorescence competition and failure of patient sera to reverse an anti-T3 mediated block of CTL. These findings have clinical implications.

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2. Baudrihaye, M., Chatenoud, L., Kreis, H., Goldstein, G., Bach, J-F. (1984). Eur. J. Immunol. 14:686-691.

B114 Reversal of steroid resistant rejection with OKT3 in Azathioprine and Cyclosporine treated renal allograft recipients. J.R. Thistlethwaite, A.O. Gaber, B.W. Haag, J.K. Stuart and F.P. Stuart, Dept. of Surgery, University of Chicago

The monoclonal antibody OKT3 has been used to treat steroid resistant renal allograft rejection in 11 patients with a follow-up of 2-7 months from time of monoclonal antibody therapy. Baseline immunosuppression was azathioprine and prednisone in 4 LRD recipients, cyclosporine and prednisone in 6 CD recipients and a combination of cyclosporine, azathioprine and prednisone in 2 CD recipients. All patients also received ATG at the time of transplantation as part of routine immunosuppression. Five first, 5 second and 1 third rejection episodes were treated after methylprednisilone pulses failed to lower the serum creatinine. Rejection was reversed in all 11 patients. This is more comparable to the 94-100% rate of reversal of rejection which has been reported when OKT3 is used as the initial treatment of first rejection episodes, than the 60% rate of reversal when OKT3 is used for salvage of rejection episodes resistant to all other therapy. Mean time from initiation of therapy to reversal of rejection was 4.6 days, slightly longer than that observed when OKT3 is used as the initial antirejection therapy. Surprisingly, there has been only 1 (9%) recurrent rejection after OKT3 therapy. Only 1 patient death has occurred; this was unrelated to OKT3 therapy in a recipient whose last measured serum Cr was 1.2 mg/dl. Two cytomegalovirus and 1 bacterial infections have occurred in the 11 patients during the follow-up period. We conclude that OKT3 is safe and effective when used to treat steroid resistant rejection even when the baseline immunosuppression regimen includes cyclosporine.

B115 CELLULAR REGULATION OF THE IMMUNE RESPONSE IN PLASMODIUM FALCIPARUM MALARIA IN VITRO. Marita Troye-Blomberg, Lalitha Kabilan, Gudrun Andersson, Peter Perlmann University of Stockholm, Department of Immunology, S-106 91 Stockholm, Sweden.

T-cell responses in vitro in patients acutely infected with P.falciparum malaria after exposure to crude P.falciparum antigen are generally suppressed. This suppression was at least in part a deficient production of IL-2. To elucidate the effect on immune regulation of different malaria antigens T-cellsfrom acutely infected patients were exposed either to the crude malaria antigen or to a glycophorin purified preparation of a major merozoite derived polypeptide antigen, Pf 155. Nine of twelve donors investigated thus far responded by increased DNA synthesis and proliferation. However, while those stimulated with crude antigen showed the typical suppression, those stimulated with Pf 155 were not suppressed. T-cell stimulation with Pf 155 was well correlated with the presence of elevated anti-Pf 155 antibodies in the serum of the lymphocyte donor. A mixed T-B lymphocyte system was established to directly investigate T-cell regulation of antibody formation. In responding donors, stimulation with crude antigen induced secretion of measurable amounts of anti-malarial antibodies, including antibodies to Pf 155 when the lymphocytes were derived from donors having elevated levels of serum antibodies to this antigen. We are now establishing malaria specific T-cell clones and study the effects of Pf 155 and fragments thereof on the induction of T-cell proliferation, αFNγ secretion and/or antibody formation in vitro.

B116 ASSAY FOR HOMOPHILE IgG RF IN THE MOUSE, Alison J. Venn and David W. Dresser, National Institute for Medical Research, London, NW7 1AA, U.K.

An assay capable of detecting cells secreting homophile IgG rheumatoid factor (RF) has been developed in a murine system. The chief difficulty in detecting homophile IgG RF plaque forming cells (PFC) is in distinguishing between target and effector IgG. This distinction has been achieved using an anti-allotype developing serum of xenogeneic origin in a PFC assay.

An antiserum produced in rabbits immunized against a mouse immunoglobulin of the Igh-1^b allotype, has been shown to be specific for Igh-1^b, both by the ELISA assay and in plaque assays, using as target cells sheep red blood cells coated with mouse IgG (a allotype) or a goat anti-mouse IgG for reversed plaques.

So far, the production of cells producing homophile IgG RF has been investigated in Igh^b mice.

B117 IMMUNOLOGIC EFFECTS OF A 64 KD GLYCOPROTEIN (gp64) PURIFIED FROM HUMAN CYTOMEGALOVIRUS (HCMV). C.L. Wright, S.J. Forman, J.A. Zaia, B.R. Clark, K.G. Blume. City of Hope National Medical Center, Duarte, California 91010.

HCMV infects 60-90% of the world's population and is associated with significant morbidity and mortality in certain high risk groups, including bone marrow transplant patients and AIDS patients. We have purified one of the 30 to 35 structural polypeptides which is abundant in the dense bodies of this virus and are studying its role in the immunologic effects induced by HCMV. We measured responses to gp64 and found it capable of inducing proliferation in PBMC, induction of IL-2 receptor expression, IL-2 secretion and IFN production only in individuals seropositive for past infection with HCMV. These responses were found to be similar quantitatively and kinetically to those elicited by HCMV. In addition, we studied the ability of gp64 to induce specific suppression of the mononuclear cell proliferation induced against whole HCMV antigen: PBMC were cultured 6 days with either gp64, HCMV, HSV, or VZV, harvested and co-cultured with fresh autologous PBMC in ratios of 1:1, 1:5, and 1:10. HCMV, gp64, HSV, VZV, and PWM antigens were then added for stimulation. The percent suppression induced by gp64 was similar to HCMV. This gp64 mediated suppression was found to be specific in decreasing the proliferative response to HCMV in that it had very little or no effect on stimulation induced by other antigens or mitogens. These experiments suggest that a single protein of HCMV is capable of inducing similar immune responses as the virus itself and may have an important role in mediating the immune response to HCMV infection. The protein may also have a role in the design of CMV specific vaccines.

B118 **RECEPTOR IMPLANTATION FOR REPAIR OF IMMUNODEFICIENT LYMPHOCYTES,** I. Zan-Bar and T. Chitlaru, Weizmann Institute of Science, Rehovat, Israel

We examined the effect of inserting purified membranal receptors into the membranes of nonresponding cells using Sendai virus as vehicles. Our purpose was to elucidate the function of particular cell membrane receptors and to attempt to overcome immunodeficiencies caused by nonfunctional receptors needed for maturation and differentiation processes. B cells, derived from C3H/HeJ LPS nonresponder mice, when modified by B membrane derived from C3H/eb responder strain acquired LPS responsiveness. Moreover the modified C3H/HeJ cells acquired anti-TNP-LPS antibody response when incubated in culture with the antigen for 3 days. The acquired capability to respond to TNP-LPS was long-lasting and could be demonstrated in CeH/HeJ mice injected with the cells even 60-90 days after cell transfer. B cells, derived from CBA/N anti-Ig nonresponder mice, when modified by purified membranal IgM (mIgM) derived from normal B lymphocytes acquired proliferative response to soluble anti-IgM antibodies as well as to Sepharose beads expressing low epitope density of anti-IgM antibodies. This finding indicates that inserting particular membranal receptors into lymphocytes can endow them with a new function. Moreover, this temporary change in membranal composition enables maturation-arrested lymphocytes to mature and to differentiate into long-lasting, functioning memory B cells.

B119 **IgD SECRETION AS A MARKER OF B CELL ACTIVATION IN AIDS, Susan Zolla-Pazner, Hiroshi Mizuma, Wafaa El-Sadr, Sandra Sharpe and Stephen Litwin, New York VA Medical Center, NYU Medical Center, New York, NY 10016 and Guthrie Research Institute, Sayre, PA 18840.** **IgD levels are elevated in the serum of many patients with AIDS. This is consistent with the elevated levels of other Ig isotypes which are a frequent finding in patients with AIDS and AIDS-related disease states. In a study of 15 patients with AIDS, 9 patients with AIDS-related complex (ARC) and 12 high risk patients (homosexual men and drug users with mild clinical symptoms or laboratory abnormalities consistent with AIDS virus infection), 36%, 56% and 42%, respectively, had serum IgD levels above 78.6 μg/ml, the mean + 2SD derived from the serum of 25 normal controls. Since, however, serum IgD has a rather short half-life and its level can be profoundly affected by altered catabolism and by genetic factors, we studied the spontaneous synthesis and secretion of IgD by peripheral blood mononuclear cells from patients and controls. Levels in excess of 10 ng IgD/10^6 cells/24 hr were achieved by the cells of only 1 of 25 control subjects. Cells from 5 of 12 AIDS patients, 3 of 6 ARC patients and 2 of 9 high risk patients produced >10 ng IgD/10^6 cells/24 hr with levels of 55 ng being reached by cells of two AIDS patients. The elevated IgD production could represent a direct effect of the AIDS virus on B cells, an unusual response of B cells to the AIDS virus or a manifestation of T cell regulatory dysfunction. In further studies, when secretion of IgM and IgD was measured simultaneously, it was found that only cells from patients which made elevated levels of IgM made elevated levels of IgD. The results suggest that IgD secretion is coordinated with elevated IgM production and that, in this disease, both IgM and IgD may be secreted by individual B cells.**

Late Additions

B125 SYNTHESES, BIOLOGICAL AND IMMUNOLOGICAL PROPERTIES OF HUMAN AND RAT GASTRIN /G-17/ AND MINIGASTRIN /G-14/ I. LEU-ANALOGUES, Lajos Balaspiri, Kalman Kovacs, Geza Remak, Janos Lonovics and Vince Varro, Medical University of Szeged, Szeged, Hungary.

Using the segment condenzation from 3 fragments and the Solid Phase Peptide Synthesis methods we have synthesized the 4 peptides in title with application of the base-labile 9-fluorenyl-methyloxycarbonyl /Fmoc/ group for protection of α-amino functions, of the t-butyl group for slide-chain protection in trifunctional amino acids, of the pentafluorophylesters of these amino acids for most of the couplings.

we also have examined different biological and immunological properties of the smaller fragments and of the 4 non-sulfated gastrins using the ^{125}I-labeled derivatives, too.

We shall report the details of both synthetic approaches, of different purifications /LC/, of purity controls /HPLC/, of bioassays and other biological studies, and of immunological properties of the mentioned fragments and peptides.

B126 MODULATION OF CLASS II ANTIGENS EXPRESSION ON HUMAN THYROID TISSUE. IN VIVO AND EXPERIMENTAL STUDIES AFTER TRANSPLANTATION TO NUDE MOUSE, Marie-Christine BENE, Jacques LECLERE, Gilbert FAURE. Université Nancy I, Faculté de Médecine. NANCY. FRANCE.

Current hypotheses consider class II molecules epithelial expression a critical feature in auto-immune diseases such as Graves' thyroiditis. We investigated the frequency of this characteristic on a series of human thyroid samples from 60 Graves' disease patients, and an equivalent series of tissues from healthy individual or subjects with other immune and non-immune diseases of this gland. Class II molecules of more than one locus appeared expressed with a wide topography in 20% of Grave's disease patients, was more focal in 55%, and was absent from epithelial cells in 25%. In normal and non-autoimmune tissues, this feature was constantly absent. A model of thyroid tissue survival by transplantation into nude mice was designed to analyze the role of the extra-thyroid environment, and investigate the effect of immune effectors. This model allowed us to demonstrate that both pathognomonic lymphoid infiltrates and class II expression disappear within 10 days after grafting, while the human tissue remains functional. No significant modification further appears for at least 70 days. Daily injection of Graves' disease patient serum into the grafted mouse maintains class II epithelial expression. Such serum is also able to re-induce this feature on a "normalized" graft, as well as on normal thyroid. Thyroid stimulating hormone (TSH) has no effect in similar conditions, while gamma interferon also induces a re-expression of class II molecules by normal or normalized thyroid tissue.

B127 INTERRELATIONSHIPS BETWEEN HYPOTHALAMUS PITUITARY ADRENAL AXIS AND THE IMMUNE SYSTEM, H. Besedovsky, A. del Rey and E. Sorkin, Schweiz. Forschungsinst., 7270 Davos

Glucocorticoid hormones exert well-known multifacetted effects on immunity. We have shown that fluctuations in endogenous blood levels of glucocorticoids are relevant for the continuous endocrine surveillance of the immune cell network, e.g. by controlling the number of immunoglobulin secreting cells.On the other hand, the immune response against non-infective antigens can bring about an increase in corticosteroid blood levels proportional to the magnitude of the response. Also experimental infections, e.g. by inoculation into mice of Newcastle Disease virus, cause an increase in ACTH and corticosterone in blood. The rise in ACTH and glucocorticoid observed in the mentioned situations is mediated by at least two different immune cell derived messengers: a) a glucocorticoid increasing factor (GIF) of lymphoid cell origin, b) a monokine (IL -1). We have shown that natural or recombinant IL -1 causes a remarkable activation of the pituitary - adrenal axis when administrated in subpyrogenic doses.
These data led us to postulate the first immunoregulatory circuit linking neuroendocrine structures and the immune system. Activated immune cells release factors (GIF, IL -1) which increase glucocorticoid blood levels via the hypothalamus-pituitary axis. Such increased glucocorticoid levels contribute to regulate the immune response by affecting the function of different types of immunological cells and the production and/or function of several lympho-monokines. The relevance of this ciruit in physiological and pathological conditions will be discussed.

B128 IMMUNOREGULATION BY TWIN-α_1, THE HEAD-TO-HEAD DIMER OF THYMOSIN-α_1.

Christian Birr[1,2], Thomas L. Ciardelli[3], Thomas Nebe[2], Wolfgang Heinzel[2], Michael Young[4], Anthony D.Ho[5] and Bernd Stehle[5].

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Based on our hypothesis on the feed-back control of immune balance by enzymatic processing of thymic hormones, interferons and interleukins at their receptor sites we will present data on a synthetic succinoyl-bridged dimer of thymosin-α_1 synthesized in our laboratory. Special interest is focused on interactions of the molecule with T lymphocytes. Data obtained by flow cytometry and biochemical markers support our view of a more potent biological activity of the dimer as compared to thymosin-α_1. Whether this is brought about by an increased binding energy by multiple attachment to receptor sites, by receptor crosslinking, or by different enzymatic degradation will be the object of future experiments, including other dimeric variants of thymosin-α_1.

B129 AUTOIMMUNITY IN NON OBESE DIABETIC (NOD) MICE, Edward H. Leiter, David V. Serreze, Phuoc H. Le, D. L. Coleman, and Leonard D. Shultz, The Jackson Laboratory, Bar Harbor, ME 04609

The Non Obese Diabetic (NOD) mouse is a model for autoimmune-related type 1 insulin-dependent diabetes. An extended prodromal state is characterized in NOD mice of both sexes by insulitis and the presence of serum autoantibodies against pancreatic beta cell antigens (insulin and p73, a glucose-inducible beta cell retroviral antigen). NOD females show a 100% incidence of diabetes by 23 weeks of age, with peak onset between 14-20 weeks; males show a more protracted onset, with 82% diabetic by 32 weeks. A related, but histoincompatible sister strain, Non Obese Normal (NON), develops neither insulitis nor diabetes. We have found by cytofluorometric techniques two to three times more T-lymphocytes in blood and spleen of NOD mice compared to NON, and decreased numbers of surface immunoglobulin positive lymphocytes. The increase in T-lymphocyte number appears to include both Lyt-1 and Lyt-2 phenotypes. NOD T- and B-lymphocytes exhibit normal responsiveness to mitogens. However, both NOD and NON suppressor cell function, as assessed by a syngeneic mixed lymphocyte reaction (SMLR), were depressed in comparison to other inbred strains in our laboratory. Depressed SMLR in NOD mice suggest that autoimmunity may, in part, entail a defect in suppressor cell formation or function.

B130 THE USE OF RECOMBINANT INTERLEUKIN-2 (RIL-2) AND LYMPHOKINE-ACTIVATED KILLER (LAK) CELLS IN THE THERAPY OF CANCER J.J. MULE AND S.A. ROSENBERG, Surgery Branch, National Cancer Institute, Bethesda, Maryland 20892

The in vitro incubation of murine or human lymphocytes in RIL-2 for 3-4 days gives rise to LAK cells that are specifically cytotoxic in vitro to fresh noncultured, autologous, syngeneic and allogeneic primary and metastatic tumor cells (regardless of natural killer cell susceptibility) but not to normal cells. In the mouse, LAK cells are Thy-1$^+$, Lyt-2$^+$, L3T4$^-$ and Ia$^-$. Human LAK cells express the OKT3$^+$4$^-$8$^+$ phenotype. Mice with established 3-day pulmonary or hepatic metastases from MCA-105 sarcoma were treated with varying doses of RIL-2 in combination with intravenously infused LAK cells. The ability of the combination therapy to reduce the number of established metastatic nodules was highly reproducible. The extent of reduction of metastases was directly dependent upon the dose of RIL-2 administered; RIL-2 (or saline) was given intraperitoneally approximately every 8 hours for 5-7 days and comparisons were made between mice receiving LAK cells (with or without RIL-2) and those treated with saline alone (no LAK cells). In the lungs, results from 74 determinations of % reduction of metastases from 44 consecutive experiments showed that at doses of 0-1,200 U of RIL-2 plus LAK cells, the % reduction in pulmonary nodules was 20.2 ± 5.1% (mean ± SEM). Mice receiving LAK cells plus 6,000-15,000 U or 20,000-50,000 U of RIL-2 had reductions of 81.1 ± 3.3% and 88.3 ± 1.9%, respectively. LAK cells plus 100,000-125,000 U of RIL-2 reduced the numbers of established pulmonary metastases by 97.2 ± 0.5%. In the liver, results from 63 determinations of reduction of metastases from 31 experiments showed that at doses of 0-5,000 U of RIL-2 plus LAK cells, the % reduction in hepatic metastases was 29.9 ± 7.0%. Mice receiving LAK cells plus 7,500-10,000 U or 25,000-50,000 U of RIL-2 had reductions of 75.8 ± 8.5% and 92.9 ± 1.8%, respectively. LAK cells plus 100,000 U of RIL-2 reduced the number of established hepatic metastases by 96.8 ± 1.0%.

Immunotherapy with the combination of LAK cells plus RIL-2 was highly effective in reducing established pulmonary or hepatic metastases from four MCA sarcomas, one colon adenocarcinoma, and two melanomas, regardless of the immunogenicity of the tumors in vivo. The use of allogeneic LAK cells was found to be nearly as effective as syngeneic LAK cells in reducing tumor metastases. The systemic administration of RIL-2 served to maintain the in vivo cytolytic activity and proliferation/expansion of the transferred LAK cells. At the sites of regressing tumor in the lungs and liver, activated lymphocytes (LAK cells) were isolated and shown to mediate strong lysis of fresh tumor cells in vitro. These findings provide the rationale for our ongoing clinical trials of LAK cells plus RIL-2 in the therapy of pulmonary and hepatic metastases in humans.

B131 THE EFFECTS OF CD4 AND CD8 ANTIBODIES ON THE IMMUNE RESPONSE IN RHESUS MONKEYS; M. Jonker, R. van Lambalgen, F.J.M. Nooij and B. den Butter, Primate Center TNO, Rijswijk, The Netherlands

Monoclonal antibodies specific for subpopulations of T lymphocytes might be ideal agents to manipulate the immune response in transplantation, autoimmunity or immunodeficiency. Since CD4 and CD8 monoclonal antibodies react with functionally different T cell subsets, these antibodies are good candidates for this purpose. When injected i.v. CD4 antibodies usually coated CD4 positive lymphocytes or modulated the CD4 antigen, but did not specifically remove these cells from the circulation. On the other hand, CD8 antibodies either eliminated the CD8 positive cells from the circulation, or these cells were coated by the antibody. Both types of antibodies were immunosuppressive in skin and kidney allografted rhesus monkeys. When kidney allograft recipients had received pretransplant blood transfusions, no significant additional effect of CD4 or CD8 treatment was seen on top of the so-called blood transfusion effect. However, a mixture of CD4 and CD8 antibodies abrogated the blood transfusion effect. This showed that the favourable effect of blood transfusions is mediated by both CD4 and CD8 cells. CD4 antibodies also very effectively reversed an ongoing autoimmune respons in monkeys that suffered from experimental allergic encephalomyelitis (EAE). These results show that EAE is (in part) mediated by CD4 positive T cells and that CD4 antibodies can treat this type of autoimmune disease.