DEFENSE MOLECULES

Organizers: John Marchalonis and Carol Reinisch

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Defense Mechanisms in Plants

CE 001 RECOGNITION MOLECULES IN PLANTS

R. Bruce Knox and Mohan B. Singh, School of Botany, University of Melbourne, Parkville, Victoria, 3052, Australia.

Pollen-pistil interactions provide an ideal system for the study of recognition molecules in plants and proteins and glycoproteins are the prime candidates. In the 1980's, research has focussed on the identification and cloning of genes from pollen and pistil regulating the interaction. On the female side, the S (Self-incompatibility) genes from a number of plants have been isolated, and the molecular basis of allelic specificity is being established. On the male side, several pollen-specific genes of undefined function in recognition have been identified in several plants. We have isolated cDNA clones for a gene of known function. Lol pI, that encodes the major allergenic protein of rye-grass pollen, responsible for hayfever and allergic asthma in humans. This gene is interesting as it involves the interaction of a plant protein with the human immune system. Molecular analysis of the ultimate barrier to fertilization - sperm-egg recognition - is being approached through identification of sperm surface determinants.

CE 002 GENES FOR PHYTOALEXIN DETOXIFICATION ON SMALL, DISPENSIBLE CHROMOSOMES IN THE PLANT PATHOGEN *NECTRIA HAEMATOCOCCA*, Hans VanEtten, Vivian Miao and Alan Maloney, Department of Plant Pathology, Cornell University, Ithaca, NY 14853.

Pathogenicity of the ascomycete *Nectria haematococca* on pea depends in part on the ability of the fungus to detoxify the pea phytoalexin pisatin. This reaction is catalyzed by pisatin demethylase, a substrate-inducible cytochrome P-450. The fungus possesses at least six genes for pisatin demethylase (*Pda* genes), any one of which is sufficient to confer pisatin demethylating ability; on pea seedlings however, high virulence is only associated with those genes conferring high levels of pisatin demethylating ability. Restriction and hybridization analyses using a cloned *Pda* gene suggests that there are two structural families of *Pda* genes, coincident with the different levels of virulence attributed to the activity levels of the Pda isozymes. These data suggest that fungal isolates carrying genes for efficient pisatin demethylating ability may have a selective advantage on peas over isolates which carry less effective *Pda* genes.

The *Pda* genes of *N. haematococca* at times show abnormal meiotic behavior, most often involving loss of the genes, as detected by pisatin demethylation, by hybridization with the cloned gene, or both. Although wild isolates of this pathogen have different electrophoretic karyotypes, most *Pda* genes map by hybridization to chromosomal DNA bands of less than 2 megabases resolved by pulsed-field gel electrophoresis. The meiotically unstable, anomalous inheritance of the *Pda6*-containing band suggests a mechanism by which *Pda* genes are deleted from the genome. Such small, dispensible chromosomes are similar in several respects to B chromosomes, but unlike conventional B chromosomes, the documented role of pisatin demethylation as a virulence factor in disease on pea suggests that the small *N. haematococca* chromosomes may contain genes conferring a phenotypic advantage to the fungus. Selective value of such genes as *Pda* in some contexts, e.g. on host plants, might maintain these chromosomes in the genome and increase diversity in the pathogen population.

Invertebrate Defense Mechanisms

CE 003 PEPTIDOGLYCAN-ELICITED ANTIBACTERIAL RESPONSES OF THE TOBACCO HORNWORM, *Manduca sexta*, Peter E. Dunn, Wei Dai, Virginia W. Russell, and Tony J. Bohnert, Department of Entomology, Purdue University, West Lafayette, IN 47907.

The immediate response of tobacco hornworm larvae to the presence of bacteria in the body cavity is directed at entrapping and sequestering free bacteria and is mediated by circulating hemocytes. As a result of this response, hemocytes are depleted from circulation leaving the insect vulnerable to subsequent infection. As a secondary defense, the initial hemocytic responses are supplemented by the induced synthesis of several bacteria-regulated hemolymph proteins, including lysozyme, cecropin-like peptides (CLPs), and an attacin-like protein (ALP). Previous studies have demonstrated that regulation of the synthesis of these proteins by the presence of bacteria is mediated by the peptidoglycan component of the bacterial cell wall (1). Studies of the tissue sources of these peptidoglycan-induced hemolymph proteins have identified fat body as a major supplier (2). Recent studies utilizing cDNA probes to examine expression from the peptidoglycan-inducible genes in various tissues have identified Malpighian tubules as an additional peptidoglycan-sensitive tissue. Subsequently, *in vitro* culture techniques have demonstrated the peptidoglycan-regulated synthesis and secretion of the lysozyme and ALP proteins from Malpighian tubules. We are currently determining if this secretion is directed into the hindgut or into the hemolymph. The effects of peptidoglycan are not limited to regulation of specific protein synthesis. Doses of peptidoglycan, which induce lysozyme, CLP, and ALP synthesis, also affect feeding behavior of treated larvae. Specifically, larvae cease feeding for 12-24 h after treatment. Preliminary data suggest that cessation of feeding may modify the gut environment so that defensive proteins secreted into the hindgut would not be degraded by proteases.

(1) Kanost, M.R., Dai, W., and Dunn, P.E. (1988) Arch. Insect Biochem. Physiol. 8:147-164.
(2) Dunn, P.E., Dai, W., Kanost, M.R., and Geng, C. (1985) Dev. Comp. Immunol. 9:559-568.

CE 004 THE INDUCIBLE HUMORAL IMMUNE RESPONSE TO SOLUBLE PROTEINS IN THE AMERICAN COCKROACH, Richard D. Karp, Department of Biological Sciences, University of Cincinnati, Cincinnati, OH. 45221.

Relatively recent advances indicate that the immune capabilities of insects are far more sophisticated than previously believed. Results from our lab have described the existence of a protective factor in American cockroaches which is induced by the injection of inactivated protein toxins. Studies revealed that the response displayed both specificity and memory, and that the mediating factor was a large protein that could precipitate its corresponding antigen. Since the injection of saline does not induce this activity, our first approach for identifying the mediating factor was to compare the protein composition of immune hemolymph to that of control hemolymph to determine if any unique or enhanced proteins were associated with immunization. SDS-PAGE analysis of immune hemolymph from female cockroaches injected with honeybee venom toxoid indicated that: 1) unique protein bands were induced with M_r=220kD and 162kD; and, 2) 4 constitutive proteins (M_r=115kD, 102kD, 95kD and 45kD) were present in enhanced amounts. It was subsequently found that the 115kD band was present only in females. Other experiments, using more defined antigens such as phospholipase A_2 and creatine phosphokinase, indicated that the appearance of the unique 220kD and 162kD bands and the enhanced 95kD band, was only associated with the use of whole bee venom. Only the 102kD protein consistently showed enhanced levels irregardless of the antigen used for immunization. We have switched to studying native hemolymph proteins, since the 102kD band is difficult to separate from the 115kD and 95kD bands, and the denatured protein may no longer be active. Non-reducing PAGE analysis revealed 5 well resolved bands in immune and control hemolymphs. Bands 2 and 4 were found to be present at enhanced levels in immune hemolymph as compared to saline-injected control hemolymph. Passive transfer studies using proteins eluted from bands 1, 2, 3, and 4 showed that only bands 2 and 4 conferred protection against a lethal challenge of antigen. Experiments combining immunoblotting with autoradiography, as well as affinity chromatography studies, have revealed that the binding activity resided in bands 2 and 4. SDS-PAGE analysis of bands 2 and 4 indicated that they are made up of the same constituents, which included the 115kD and 102kD protein bands. Band 2 may prove to be a version of band 4 that either is more heavily glycosylated, or is multimeric in nature. A consistent picture has now emerged from the *in vitro* and *in vivo* data, giving us a clear indication of where to concentrate our attention in our efforts to isolate and characterize the inducible humoral factor.

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CE 005 INVERTEBRATE DEFENSE MECHANISMS AND THE ROLE OF NON-SELF RECOGNITION MOLECULES,
Norman A. Ratcliffe, Biomedical and Physiological Research Group, School of Biological Sciences, Singleton Park, Swansea, SA2 8PP, Wales, U.K.
This paper outlines the benefits of studies on the host defense mechanisms of invertebrates and discusses the problems involved in such research. The humoral and cellular defenses and their interactions in invertebrates are then briefly described. Naturally ocurring humoral factors such as agglutinins, prophenoloxidase, lysozyme and other lysins, bactericidins and lysosomal enzymes are found throughout the main invertebrate groups. Induced humoral factors also include agglutinins as well as the highly characterized antimicrobial proteins of certain insects. The cellular defenses are remarkably uniform throughout the higher invertebrates and typified by coagulation/clotting responses following wounding, and phagocytosis and encapsulation reactions towards microbial and macrobial parasites. These host defenses apparently involve co-operative events between the haemostatic and phagocytic cell types during which non-self recognition events occur. The possible role of agglutinins and components of the prophenoloxidase cascade in the recognition of invading parasites is then assessed. Emphasis will be placed on presenting evidence for the functioning of the prophenoloxidase system in arthropod immunoreactivity and on indicating possible research priorities for the future.

CE 006 PROPHENOLOXIDASE ACTIVATION IN INSECT IMMUNITY.
Manickam Sugumaran, Department of Biology, University of Massachusetts at Boston, Harbor Campus, Boston, MA 02125.
Lacking the complicated immunoglobulin system of higher animals, insects have managed to distinguish self from nonself matter and destroy the foreign organisms effectively by a plethora of host defense reactions, of which melanization of the invading organisms is an important reaction. Therefore, melanin and the enzyme causing the synthesis of melanin viz., phenoloxidase, are considered as important constituents of insect's immune system. Phenoloxidase occurs as an inactive proenzyme form in the hemolymph of most insects and is specifically activated in response to the presence of foreign objects. Our laboratory has been engaged in the study of the control mechanisms involved in the activation of prophenoloxidase for the past few years and identified the presence of endogenous proteases and protease inhibitors controlling the prophenoloxidase activation in the hemolymph of Manduca sexta and Sarcophaga bullata larvae. We have purified some of the protease inhibitors and determined their amino acid sequences. Sequence homology studies indicate that these protease inhibitors belong to bovine pancreatic trypsin inhibitor family. In addition, we have recently characterized an enzyme system which destroys the phenoloxidase generated quinones. It is a novel quinone : quinone methide isomerase which converts 4-alkyl substituted quinones to quinone methides. The latter being highly unstable reacts rapidly with water to form nontoxic compounds or gets deposited on foreign objects. Thus apart from helping phenoloxidase to inactivate the nonself matter, the newly discovered isomerase also prevents the destruction of self matter by quinones. The importance of the above described protein factors in controlling the activity of phenoloxidase will be discussed.

Defense Molecules

Non-Immunoglobulin Defense Mechanisms

CE 007 MULTIPLE SOLUBLE β-GALACTOSIDE-BINDING LECTINS FROM RAT INTESTINE, Samuel H. Barondes, Hakon Leffler, Department of Psychiatry, University of California, San Francisco, CA 94143-0984.

Many vertebrate tissues contain soluble β-galactoside-binding lectins. In rat lung 3 have been purified and well characterized. They have subunit molecular weights of 14,500, 18,000 and 29,000 and different relative affinities for a series of mammalian β-galactoside containing glycoconjugates. Because intestine is rich in well-defined glycoconjugates that could act as complementary ligands for endogenous lectins[1], we examined the β-galactoside-binding lectins in extracts of rat intestine. To our surprise we found at least 9 distinct lectins in this tissue. Two, with subunit molecular weights of 14,500 and 18,000, are closely related to or identical with lectins already described in rat lung. The remaining lectins are all different from those previously described. One, with subunit molecular weight of 17,000, appears to be closely related to the rat lung lectin with subunit molecular weight of 29,000 based on limited amino acid sequencing studies. There is also considerable amino acid sequence homology of several other newly identified lectins with those previously sequenced. Limited immunohistochemical studies indicate lectin expression in the mucosal cells of rat intestine. This suggests that some may be secreted and then interact either with endogenous glycoconjugates or those on microorganisms in the intestinal lumen.

[1]Leffler, H., Monogr. Allergy 24, 25-34, 1988.
(Supported by grants from NIH and NSF)

Antibodies

CE 008 PHYLOGENETIC STUDIES WITH REARRANGING IMMUNOGLOBULINS. John J. Marchalonis and Samuel F. Schluter, Microbiology and Immunology, University of Arizona, Tucson AZ 85724. All vertebrates can respond to foreign antigenic challenge by both specific cellular reactions and by production of circulating antibodies. Immunoglobulins (Igs) and the well characterized T cell receptor heterodimers are rearranging members of the Ig superfamily that are specified by gene segments and coding variable (V), joining (J) and constant (C) region segments as well as diversity (D) segments in some instances. Recent studies from our laboratory entail the characterization of a Vβ pseudo-gene fragment from a teolost (goldfish) genomic library; studies of the expression of serological determinants related to J-region synthetic peptide sequences in protochordates (tunicate), cyclostome (sea lamprey) and elasomobranch species; and sequence analysis of gene segments corresponding to C sequences from a cDNA library from sandbar shark spleen. Three major conclusions following from these studies will be reported here: (1), J region segments are the most conserved in evolution, and this probably reflects the essential requirements for these gene segments in the formation of intact Ig genes by rearrangement; (2) the framework segments of V genes are highly conserved in vertebrate evolution as well in comparisons of T cell receptors and classical Igs; and (3) although C region segments of light chains of lower vertebrates are clearly homologous to the mammalian counterparts, the degree of conservation is apparently less than that for V regions and comparison of C region divergence allows reconstruction of our common concepts of phylogeny. (Supported in part by NSF Grants DCB88 02353).

Alloreactivity

CE 009 IN VITRO STUDIES OF ALLOREACTIVITY IN THE FROG, XENOPUS, Nicholas Cohen, Fiona Harding and David Watkins, Department of Microbiology and Immunology, University of Rochester Medical Center, Rochester, NY 14642

Data from previously published studies using thymus/hematopoietic Xenopus chimeras created during embryonic life were consistent with the thymus exerting some in vivo T cell repertoire selection bias for MHC restricted T cell help and cytotoxicity. To further analyze thymic education, we have been attempting to develop in vitro models (e.g., virus-infected self, minor H-antigen disparate stimulators and targets, TNP-modified self) of MHC restricted T cell proliferation and cytotoxicity.

Anti minor H-antigen alloreactive T cell lines have been developed by harvesting splenocytes from skin grafted cloned frogs, reimmunizing them in vitro, and expanding them with homologous TCGF. In some but not all proliferation and cytotoxicity assays, minor H-antigens could be recognized in the context of an MHC background that differed from that to which they were exposed during the immunization processes. In addition, cytotoxicity of fibroblasts that did not express detectable class I antigens at the time of the cytotoxicity assay, has also been seen.

In vivo immunization of cloned frogs with TNP-derivatized syngeneic splenocytes or with TNBS followed by in vitro restimulation with TNP-derivatized self (plus TCGF) has thus far (4 of 4 experiments) resulted in MHC-restricted proliferation. For example, splenocytes from TNP-self-immunized LG-15 frogs proliferated when they were cultured in a 3-day assay with "TNPlated" LG-15, LG-6, and LG-7 (a/c haplotype); they did not proliferate significantly, however, when they were cultured with "TNPlated" LG-3 cells (b/d haplotype) or with "nonTNPlated" a/c-expressing cells.

CE 010 SELF AND NON-SELF RECOGNITION IN SOLITARY ASCIDIAN, HALOCYNTHIA RORETZI, Masako T. Fuke, Department of Biology, Faculty of Science, Kanazawa University, Kanazawa 920, Japan. Blood cells from H. roretzi exibit a nonphagocytic cellular reaction when they are mixed in vitro with such cells from another individuals of the same species(1). The reaction is reciprocal and results in mutual devacuolation or state of incapacitation of both cells involved. As the reaction was only triggered by direct cell contact, it was denoted "contact reaction". The alloreaction occurred in some combination but not all. The pattern of cellular alloreactivity was examined using 30 animals(2). A high level of polymorphism was revealed and no two individuals had identical histocompatibility. In H. roretzi, the allogeneic reaction seems to be triggered by an absense of common self markers rather than by specific reaction to non-self markers as in higher vertebrates. We analyzed the allelic compositions assuming that a non-reactive pair share an allele. Since at most four "alleles" are carried out by each individual, the ascidian must probably has two genes that control alloreactivity. Self and nonself recognition of gametes, that is self sterility of H. roretzi is very strict. None of the animals were self fertile. On the other hand, in all heterologous combinations including those in which somatic cell did not exibit contact reaction, fertilization succeeded(3). The recognition site for self and nonself seems to be chorion of the unfertilized eggs. The relationship between somatic and gamete cell recognition of self and nonself remains to be solved. For identification of the materials which are responsible for the recognition in both somatic and gamete cells, chorion and blood cell membranes were isolated. Using the antisera against both membranes, inhibition test to both contact reaction and fertilization were done. A candidate of materials which were thought to be related to both somatic and gamete alloreactivity was found in both membranes.

1)Biol. Bull. 158:304-315(1980). 2)Biol. Bull. 169:631-637(1985). 3)Roux's Arch. Dev. Biol. 192:347-352(1983).

CE 011 GAMETIC-SOMATIC CELL INTERACTIONS IN PROTOCHORDATES AND MAMMALS, V. Scofield, UCLA, Los Angeles, CA 90024

Among the many unique characteristics of the mammalian major histocompatibility complex (MHC), the extreme polymorphism of some of its loci remains a perplexing evolutionary puzzle. Recent studies in mice suggest that these loci encode or influence olfactory kin recognition markers in ways that affect mate choice and reproductive function. We have explored the possibility that histocompatibility gene products directly influence fertilization events as well. We find that *in vitro* cross-recognition between gametes and blood cells occurs in protochordates and mammals. In both systems, sperm bind and penetrate blood cells after their recognition of cell surface allorecognition structures. In protochordates, which are hermaphroditic, patterns of sperm interaction with blood cells follow mating type-i.e. sperm interact neither with self eggs nor with self blood cells, while they readily bind and penetrate eggs or blood cells of other individuals. Similarly, human sperm bind and penetrate white blood cells through HLA-DR molecules on the leukocyte surfaces. That such interactions may be involved in fertilization is suggested by the presence of HLA-DR epitopes on both on the cumulus cells surrounding the human egg and on the cells of the fallopian tube epithelium. The sperm surface structure involved shares serological epitopes with the T cell surface CD4 molecule, which is the natural ligand for HLA-DR in antigen recognition events. In parallel studies, we have found that the AIDS virus HIV binds readily to normal sperm surfaces, and that this in turn allows subsequent sperm binding and fusion with target cell CD4 molecules via crossbridging with the HIV gp120 envelope glycoprotein. Studies in other laboratories have established that the HIV gp120 molecule is a functional and structural mimic of HLA-DR. We suggest that the biology of HIV cell entry, its sexual mode of transmission and its poisoning effect on the signal transduction systems of T lymphocytes reflect the evolutionary history of the HLA-DR/CD4 recognition axis in both fertilization and histocompatibility discriminations. In this light, co-evolution of the individuality and pathogen defense functions of MHC genes might be understood in terms of the longstanding and dynamic relationship between microbial pathogens and their hosts. Even if pathogens no longer select directly for or against particular MHC alleles, it seems likely that mechanisms maintaining polymorphism for the antigen-presenting MHC proteins were established early in the evolution of immunity in response to microbial selection, by MHC mimicry, for individuality *per se* at these critical loci.

Immunomodulators/Vertebrates

CE 012 THE EVOLUTIONARY CONSERVATION OF IL-2 AND ITS RECEPTOR, Laurens N. Ruben, Lorene Langeberg, Rachel Lee, *Richard H. Clothier, **Arthur Malley, Christopher Holenstein, **Stanley Shiigi,, and *Michael Balls, Department of Biology, Reed College, Portland, OR 97202, *Department of Human Morphology, Queen's Medical Centre, Nottingham, NG7 2UH, U.K. and **Division of Metabolic Diseases, Oregon Regional Primate Research Center, Beaverton, OR 97006. Human IL-2 will selectively modulate *in vivo* immune responsivity in the South African clawed toad, *Xenopus laevis*. It will increase antibody (Ab) levels to the hapten trinitrophenyl, when presented on suboptimal doses of TI-type 2 carrier molecules, e.g. Ficoll or polyvinylpyrrolidone, and it will substitute for carrier primed helper function, when TD hapten carriers are used. TI-type 1 responses, e.g. to TNP-lipopolysaccharide, are uneffected by the cytokine.

To test whether rIL-2 functions in the toad using a specific receptor related to the one in mammals, the binding of FITC-mouse anti-human IL-2 receptor (IL-2R) Ab to uncultured toad spleen, thymus and erythrocytes is monitored by flow cytometry. When Na azide is used for membrane stabilization, some Ab will bind specifically on spleen and thymus cells. More will bind after additional fixation with paraformaldehyde. Erythrocytes fail to bind the Ab. TEM sections of PHA activated spleno- or thymocyte pellets show colloidal-gold conjugated goat anti-mouse Ab (with mouse anti-human IL-2R Ab bound cells) binding cell surfaces. However, some gold particles can be seen within the cytoplasm. A few gold gold particles are seen *only* at the surface, when anti-KLH Ab, of the same isotype and subclass, is the first antibody used. PHA activation *in vitro* increases anti-IL-2R Ab binding onto splenocytes and thymocytes and a carefully controlled acid wash for 60 seconds at pH 4 increases Ab binding further, suggesting the presence of an autologous ligand. Prebinding toad immunocytes with human rIL-2, but not Human serum albumin and Chorionic gonadotropin, specifically reduces anti-IL-2R Ab binding.

Immunoprecipitates of extracts from PHA activated spleen and thymus cells, on an SDS-Page gel, under non-reducing conditions, reveal an anti-IL-2R Ab binding epitope related to a 180 kDa protein band. Internal labelling experiments are currently underway. Moreover, a cDNA probe from the human IL-2 receptor gene, binds mRNA cytoplasmic dot blots made from PHA activated human PBL and Xenopus splenocytes. Northern blots are presently being run. (Partially supported by grants, AI-12846 to LNR, RR 07168 to Reed College and RR 00163 to the O.R.P.R.C. from the N.I.H., Bethesda, Md.)

Immunomodulators/Invertebrates

CE 013 CHARACTERIZATION OF INTERLEUKIN-1 FROM INVERTEBRATES. Gregory Beck and Gail S. Habicht. Department of Pathology, SUNY, Stony Brook, NY 11794 USA.

Interleukin-1 (IL-1), a cytokine with a molecular weight of 17,500 daltons, is released by a variety of cells (e.g., macrophages, Langerhans cells, and astrocytes), and is a major immunoregulatory protein. Equally important are the effects of IL-1 as a mediator of nonspecific host defense mechanisms. These include stimulation of acute phase protein synthesis by the liver, fever, and chemotaxis of neutrophils *in vivo*. Biochemical characterization and studies of the molecular biology of IL-1 from a number of vertebrate species have revealed basic similarities in the structure and biological properties of this crucial molecule. The tremendous importance of IL-1 to host defensive systems along with the striking similarities of its properties and production shared by many different species, suggested to us that it may be protein that has been conserved through evolution. Pioneering work by Metchnikoff demonstrated that echinoderms possess large mononuclear phagocytic cells which participate in host defense. He likened these cells to mammalian macrophages. We asked whether part of the defensive armament of invertebrate leukocytes might include IL-1. We have reported on the characterization of an IL-1-like protein from the starfish *Asterias forbesi*. IL-1 activity was found in the coelomocytes of this invertebrate and was also isolated and purified from the coelomic fluid. Chromatofocusing of the starfish IL-1 revealed three distinct species with pI's of 7.4, 5.4, and 4.8. We have extended these observations by purifying echinoderm IL-1 to homogeneity. When the purified pI 4.8 species was analyzed by SDS-PAGE a single silver staining band containing all the IL-1 actvity was found to have a M_r of 22,000. Amino-terminal sequence analysis was performed on the pI 7.4 species of echinoderm IL-1 using IL-1 that was transferred from the SDS-PAGE gels onto polyvinylidene difluoride membranes by electroblotting. Since echinoderms diverged from animals which gave rise to vertebrates 600 million years b.p. we have sought IL-1 activity in other invertebrates as well. Preliminary characterization of IL-1 from tunicates has been completed. The activity of the tunicate IL-1 in the thymocyte proliferation assay was enhanced by submitogenic amounts of con A. The activity exhibited M_r heterogeneity with the M_r of the most active tunicate fraction being approximately 20,000. Chromatofocusing of the tunicate IL-1 revealed two species with pI values of 7.0 and 5.5. It caused an increase in vascular permeability, a function only recently attributed to human IL-1. And finally, antiserum to human IL-1 inhibited tunicate IL-1 activity just as effectively as human IL-1 when assayed in the thymocyte proliferation assay. These results suggests that all deuterostome animals have IL-1.

Investigations of the evolution of cytokines will help us decipher the complex cellular and humoral interactions of the immune system. Our studies of the invertebrates will shed light on the phylogenetic emergence of inflammatory molecules which may be related to similar molecules in the vertebrate. These studies were supported by NSF grant DCB 8810448.

Complement in Defense

CE 014 ISOLATION FROM HUMAN URINE AND CHARACTERIZATION OF A POLYPEPTIDE INHIBITOR OF THE CLASSICAL COMPLEMENT PATHWAY, Anne Nicholson Weller, M.D., Margarita Lopez-Trascasa, Ph.D., and David H. Bing, Ph.D., Department of Medicine, Beth Israel Hospital and The Center for Blood Research, Boston, MA 02115.

A 20 kd polypeptide inhibitor of the classical complement pathway has been isolated from normal human urine. This protein has been designated Factor J. Factor J acts by dissociating the C1 complex in a manner that is consistent with uncompetitive binding kinetics. There are two known inhibitors of the classical complement pathway, C1 Inhibitor (C1INH) and C1q Inhibitor (C1qINH). Factor J is distinguished from C1INH by having no effect on the esterase activity of C1s or plasmin. It is distinguished from C1qINH by its inability to bind to Sepharose immobilized C1q and by its ability to bind to Heparin Sepharose. Immunoprecipitation competition experiments between ^{125}I labeled Factor J and whole human serum with goat anti-human serum indicates that a cross-reacting antigen to Factor J can be found in normal serum. These results suggest that Factor J is potentially an important inhibitor that can regulate the classical complement pathway at the level of C1.

Supported by NIH grant DK 34028. ANW is a Scholar of the Leukemia Society.

CE 015 VIRAL ENCODED MOLECULES FOR EVASION OF HOST DEFENSE MECHANISMS, Girish J. Kotwal and Bernard Moss, Laboratory of Viral Diseases, National Inst. of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892. Vaccinia virus, the most extensively studied member of the poxvirus family has been widely used for the expression of foreign genes. The medium from vaccinia virus infected cells contains several virus specific proteins (virokines). One of these, VGF has been shown to have similarity to the epidermal growth factor[1] and to stimulate cell proliferation[2]. Recent studies have led to mapping of a major secretory protein of the size of 35K Da[3]. The nucleotide sequence of this secretory protein has the structural feature common to the 60 amino acid repeating unit superfamily which include the Il-2 receptor, beta-2-subunit of clotting factor XIII, and complement binding proteins. Because of a significant identity to the latter group of proteins, the medium from vaccinia virus infected cells was tested and found to inhibit complement mediated hemolysis. In contast, medium from a spontaneous mutant of vaccinia virus which lacks a stretch of 12kbp of DNA including the gene for the 35k Da protein had no inhibitory effect. Furthermore, there was no difference in the extent of hemolysis between the 2 viruses when the standard serum used in the hemolysis assay was replaced by a C4 deficient serum, suggesting that the inhibitory effect is only on the classical pathway of the comlement cascade. Purification of the 35k Da protein revealed that inhibitory activity is associated exclusively with it. These results suggest that vaccinia virus can evade the complement mediated host response by secreting a protein with structural similarity to a complement regulatory protein. The precise event of the cascade blocked by the 35k Da protein is currently being investigated.

1. Reisner, A. H. Nature 313, 801-803 (1985).
2. Brown, J. P., Twardzik, D. R., Marquardt, H. & Todaro, G. J. Nature 313, 491-492 (1985)
3. Kotwal, G. J., and Moss, B. Nature 335, 176-178 (1988).

Primordial Immune Mechanisms in Disease and Immunosuppression

CE 016 CELLULAR AND MOLECULAR ASPECTS OF LAK-CELL TUMOR CELL INTERACTION, Evan M. Hersh, William J. Grimes, Anita Chong, Philip Scuderi and Sydney E. Salmon, University of Arizona College of Medicine, Tucson, AZ 85724.
Lymphokine activated killer (LAK) cells derive from peripheral blood lymphocytes stimulated with Interleukin-2 (IL-2). LAK cells are a unique group of cytotoxic lymphocytes with the capacity to kill a wide variety of autologous and allogenic fresh and cultured tumor cells. A major characteristic is that they can kill NK cell resistant target cells. Both Tumor Necrosis Factor (TNFα) and Interferon gamma (IFNγ) play important augmentive roles in LAK cell generation. LAK cells are synergistic with various cytokines in their antitumor activity. Thus, tumor cells treated with IFNα, IFNγ or TNFα show increased sensitivity to killing by LAK cells. LAK cell treated tumor cells also show increased sensitivity to killing by TNFα. We have observed such synergy for the killing of breast cancer, colon cancer and malignant melanoma cell lines. These in vitro synergistic interactions have been confirmed in vivo in animal tumor models such as meth-A sarcoma and B16 melanoma and are now being extended to clinical trials in cancer patients. It was originally thought that LAK cells kill tumor cells only by direct, contact interaction via the release of molecules which lyse target cell membranes. This activity is detected in a 4 hour chromium release assay. We have shown that during LAK cell generation the IL-2 stimulated lymphocytes produce a cytostatic and cytotoxic activity with a MW >50,000. The precise identity of this activity is currently under study. Furthermore upon LAK cell-target cell interaction the LAK cells release cytostatic and cytotoxic quantities of TNFα and IFNγ which mediate their antitumor effects synergistically. These activities are only detected after over 12 hours of exposure of tumor cells to various types of LAK cell supernatants and therefore, are distinct from that detected by chromium release. The signal for the release of TNFα and IFNγ is delivered by tumor membrane molecules and does not require intact tumor cells. Tumor membrane antigen coated beads induce the release of TNFα and IFNγ as effectively as intact tumor cells. Thus, we conclude that LAK cells mediate their activity by at least 3 separate mechanisms and both generation and activity of LAK cells is augmented by other cytokines.

Discussion: Antigen Binding T Cell Products

CE 017 ISOTYPIC VARIATION IN MEMBRANE AND SECRETED NON-MHC RESTRICTED T CELL ANTIGEN BINDING MOLECULES. Robert E. Cone, Robert B. Clark, James Kristie, Robert Wong and Barbara L. Rellahan. Department of Pathology and Medicine, University of Connecticut Health Center, Farmington, CT 06032.

Some T cells produce soluble molecules which bind nominal antigen (TABM) and participate in immunoregulation and/or hypersensitivity-like events. TABM occur as membrane-associated molecules as well as soluble forms and the membrane-associated molecule may act as a surface recognition unit for antigen. We have used a rabbit antiserum to an azobenzene arsonate (ABA) binding TABM produced by an ABA-specific T-cell hybrid and an antiserum to a TNP-specific suppressor factor (TSF) to probe membrane and soluble TABM. We found that TABM in serum, ascites fluid of the ABA-specific hybrid and culture media from T cell clones specific for myelin basic protein are recognized only by an antiserum specific for the ABA-specific TABM while this antiserum did not recognize structurally similar membrane TABM. Conversely, antisera that recognized membrane TABM did not bind some soluble TABM. T cell clones specific for MBP varied in their production of soluble TABM but all expressed membrane TABM in addition to MHC-restricted T cell receptors. The results suggest that membrane and some soluble TABM are isotypically different and that TABM and TCR can be expressed by the same cell.

T Cells in Disease

CE 018 LOW TEMPERATURE-MEDIATED SUPPRESSION OF T CELLS IN ECTOTHERMS AND ENDOTHERMS. L.W. Clem, M.-C. W. Yang, J.E. Bly, C.F. Ellsaesser and N.W. Miller. Department of Microbiology, University of Mississippi Medical Center, Jackson, MS 39216.

It is generally accepted that low environmental temperatures are immunosuppressive for ectothermic vertebrates, such as fish. Furthermore, although endotherms, such as mice, maintain their internal tissues at or near optimal temperatures, indirect evidence suggests that localized temperature changes may also suppress certain aspects of the immune response in such animals. In terms of understanding this phenomenon it has now been shown that low "nonpermissive" temperatures preferentially suppress primary helper T cells, but not memory T cells, B cells or accessory cells, in in vitro antibody responses of both catfish and mice. Furthermore the results of in vitro "shift-down" protocols indicate that the low temperature sensitive "block" likely involves a relatively early step in the generation of functionally active primary helper T cells. Clues to understanding this differential effect on T cells have come from two directions. Firstly, low temperature-mediated suppression of mouse T cells can be obviated ("rescued") by exogenously added IL-2 or IL-4, but not IL-1α or β; suppressed catfish T cell responses can be "rescued" by an illdefined factor(s) secreted by long-term lines of catfish T cells. Secondly, low temperature-mediated suppression of mouse and catfish T cell responses can also be "rescued" by the addition of oleic acid (18:1), a monounsaturated fatty acid that would be expected to increase plasma membrane fluidity at lower temperatures. Hence, the low temperature-sensitive block in generating functionally active primary helper T cells in both mice and catfish clearly involves an activation step(s) which preceeds the secretion (synthesis?) of IL-2 and likely requires the "proper" membrane fluidity. The identification of this seemingly phylogenetically conserved, low temperature-sensitive T cell activation step requires further study. (Supported in part by NIH Grant 1-R37-AI-19530).

Plant Defense Molecules; Lymphocyte Evolution and Development

CE 100 RELEASE AND DISTRIBUTION OF ARACHIDONIC ACID FROM *PHYTOPHTHORA INFESTANS* SPORES IN INOCULATED POTATO TISSUE. K. E. Bretschneider and R. M. Bostock, Dept. of Plant Pathology, University of California, Davis, CA 95616

Arachidonic acid (AA), a component of lipids of *Phytophthora infestans*, induces macroscopic symptoms similar to hypersensitive resistance (HR) in potato (*Solanum tuberosum)* and elicits a number of biochemical responses characteristic of HR, including the accumulation of sesquiterpene phytoalexins. Potato lipoxygenase seems to be involved in the expression of AA elicitor activity, possibly via the formation of cytotoxic lipid hydroperoxides or related metabolites. Spores of *P. infestans* incorporate exogenous ^{14}C-AA, and the sites of incorporation in the spores and in potato tissues inoculated with these spores can be visualized by microautoradiography. We are using this technique to determine release of AA from fungal spores, localization of AA within potato tissues and fungal spores. Lipid analysis of sporangia, and inoculated and non-inoculated potato leaves is used to examine the metabolism of exogenously supplied AA, and to determine if there are differences between compatible and incompatible host pathogen interactions. The release of AA from fungal tissues into host tissues, as well as regulation of this process, might prove to be a critical step in plant defense mechanisms to *P. infestans*.

CE 101 DEFENSE MECHANISMS IN ONION, Alexander P. Dmitriev, Leonid A. Tverskoy and Dmitry M. Grodzinsky, Laboratory of Plant Protection, Department of Biophysics and Radiobiology, Ukrainian Academy of Science, Vasilkovskaya 31/17, 252627 Kiev, USSR

Phytoalexins are known to be antimicrobial agents of plants synthesized in response to stress or pathogen attack. Treatment of Allium cepa bulb scales with elicitor molecules from mycelium of the fungus Fusarium solani resulted in transient gene activation and accumulation of onion phytoalexins which we called tsibulins (from Ukrainian "tsibulya" - onion). The tsibulins are non-volatile fungitoxic substances. The analysis of their mixture and separate preparative fractions by HPLC, plus UV, IR, PMR and chromato-mass spectra revealed a set of common structure features in particular carbohydrate chains with double links, sulfoxide and disulfide units, uncoupled carbonyl and hydroxyl groups. A fungal elicitor from F. solani was purified by gel filtration chromatography. Both lipid and carbohydrate fractions were shown to be associated with elicitor activity. Experimental field studies over 3 years demonstrated that induced resistance by prior treatment of onion seeds or plants with biotic elicitor at a very low concentration (0.002%) resulted in highly effective protection against a downy mildew agent Peronospora destructor. This represents a novel approach to plant immunization based on plant genetic imprinting.

CE 102 BIOSYNTHETIC PRECURSORS OF DEFENSIVE METABOLITES IN *Flaveria linearis*, Lavina J. Faleiro and Kelsey R. Downum, Department of Biological Sciences, Florida International University, University Park, Miami, FL 33199

The plant family Asteraceae (Compositae) is characterized by acetylenes and acetylenic derivatives whose light-activated toxicity against potentially deleterious organisms (i.e., disease-causing microorganisms or herbivores) suggests that they may play an important role in plant defense. Recent studies have shown that these phototoxins are present in healthy tissues but an increase of their concentration can be elicited by fungal infection suggesting that acetylenes are involved in both passive and active plant chemical defense. Although there is considerable information concerning the biological activity of these phototoxins, very little is known about their biosynthesis. Much of our research is focused on the biosynthetic precursors of acetylenic thiophenes (defensive metabolites) in *Flaveria linearis* (Heliantheae). We have detected a conjugated acetylenic compound with the following properties: i) unlike most acetylenes it is polar; ii) hydrolysis with acid or base results in the release of a free acetylene which is non-polar; iii) enzymatic hydrolysis with lipase also releases a free non-polar acetylene; and iv) treatment with β-galactosidase releases an acetylene with intermediate polarity. Based on the above hydrolysis information, we have constructed a working model of a glycolipid: a glycerol molecule containing a sugar moiety plus one or two acetylenic chains. We believe that this unusual acetylenic compound may function as an metabolic intermediate in acetylene biosynthesis. Structural information and the possible biosynthetic role of this compound will be presented.

CE 103 IDENTIFICATION OF AN EVOLUTIONARILY CONSERVED NATURAL KILLER CELL (NK) ANTIGEN RECEPTOR/TARGET ANTIGEN COMPLEX. D.T. Harris, L. Friedmann, R. Devlin, H.S. Koren and D.L. Evans. Univ. of North Carolina, Chapel Hill, NC 27599; Univ. of Georgia, Athens, GA 30602; U.S. EPA, Chapel Hill, NC 27599. Recently, a population of lymphoid cells has been identified in the teleost fish that are similar in phenotype and function to human and murine NK. These effector cells are cytotoxic for naturally occurring fish parasites as well as for a variety of cell lines, including transformed human and murine cells. With the use of monoclonal antibodies (mAbs) made against the fish NK cell and target cell, and screened with the human counterparts, we have identified a receptor/ligand complex conserved across phylogeny (fish, rat and man). This complex appears to be involved in NK function in that the mAbs block NK cytotoxicity in each of these species (i.e., inhibition of fish killing of parasites, rat NK lysis of YAC cells and human NK cytotoxicity against K562 cells). Fluorescence flow cytometry revealed that the receptor seemed to be specific for NK in each species, and that the target antigen was expressed at highest levels on cells most sensitive to NK lysis. Biochemical analyses revealed the receptor to be composed of two polypeptides of 38 and 43 kDaltons (kDa), while the target antigen was a single molecule of 43 kDa. Protein sequencing and cDNA cloning experiments are in progress to determine the similarity of these NK and target cell structures to any other molecules. Finally, both the receptor and the target antigen are involved in the NK-like lysis of antigen-specific CTL, but are distinct from typical T cell antigen receptors and MHC molecules. Thus, it appears that natural cytotoxicity is mediated by this receptor/ligand complex regardless of the species involved, and may have given rise to the antigen-specific T cell receptor system.

This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.

CE 104 HUMAN MILK-DERIVED LIPID-DEPENDENT ANTIVIRAL AND ANTIBACTERIAL ACTIVITY, Charles E. Isaacs[1] and Halldor Thormar[2], Department of Developmental Biochemistry, N.Y.S. Institute for Basic Research in Developmental Disabilities, Staten Island, NY 10314[1], and Institute of Biology, University of Iceland, Reykjavik, Iceland[2]

The lipid fraction of human milk is both a source of essential nutrients and of antimicrobial activity. Milk itself does not contain lipid-dependent antimicrobial activity but the milk lipid fraction becomes antimicrobial in the stomachs of milk-fed infants. Milk lipids inactivate enveloped viruses including herpes simplex virus-1, cytomegalovirus, measles virus and visna virus. Viral inactivation is consistently $\geq$ 4 $\log_{10}$ and results from the destruction of the viral envelope by certain medium chain saturated and long-chain unsaturated fatty acids and monoglycerides. Both gram positive (Staphylococcus epidermidis and Group B streptococcus) and gram negative (Hemophilus influenzae and Salmonella enteritidis) bacteria have also been shown to be inactivated by antimicrobial human milk lipids. All enveloped viruses tested were inactivated equally at the same concentration of antimicrobial lipid. When mixtures of antimicrobial monoglycerides and fatty acids were made containing each lipid at a concentration too low to be active against enveloped viruses by itself, the mixtures were found to be antiviral if the total lipid concentration was $\geq$ 7 - 8 mM. In contrast to enveloped viruses, bacterial inactivation depended on both the particular bacterium and the lipid incubated with it. Inactivation of viruses and bacteria is rapid having been shown to take place in less than 30 seconds. Antimicrobial lipids are therefore present not only in plants but also at the mucosal surfaces of milk-fed human infants.

CE 105 CLONING OF THE cDNA OF A HUMAN NEUTROPHIL BACTERICIDAL PROTEIN, Steven R. Leong, Patrick W. Gray, Jerrold Weiss, Chean Eng Ooi and Peter Elsbach, Department of Developmental Biology, Genentech Inc., South San Francisco, CA 94080, and Department of Medicine, New York University School of Medicine, New York, NY 10016. One of the most potent antimicrobial proteins in granules of polymorphonuclear neutrophils is the Bactericidal Permeability-increasing protein (BPI). BPI is a 50-60 kD membrane-associated protein which specifically kills gram-negative bacteria. This specificity may be due to the strong affinity of the cationic BPI for the negatively charged lippopolysaccharides (LPS) unique to the envelope of gram-negative bacteria. Binding of BPI to susceptible bacteria results in discrete envelope alterations and subsequent cessation of growth. We have isolated a full length cDNA clone encoding human BPI and the derived amino acid sequence reveals a structure that is consistent with its previously determined biological properties. BPI is organized into two functional domains: an amino terminal 25 kD fragment which contains all of the bactericidal activity of the holoprotein and a hydrophobic carboxy terminal fragment which may be important for retaining the holoprotein in the granule.

CE 106 INDUCTION OF PEROXIDASES IN CUCUMBER HYPOCOTYLS BY WOUNDING AND FUNGAL INFECTION. Børre Robertsen and Øystein Svalheim, Department of Plant Physiology and Microbiology, University of Tromsø, 9000 Tromsø, Norway. Lignification appear to be an important defense mechanism against fungal pathogens in cucumber. Peroxidases (E.C. 1.11.1.7) have been shown to catalyze the polymerization of phenolic compounds into lignin. We have studied the induction of peroxidases in cucumber hypocotyl segments during incubation with α-1,4 linked oligogalacturonides, elicitors of lignification. The experiments showed that mere slicing of the hypocotyls induced a 19-fold increase in peroxidase activity during the following 72 hours. Isoelectric focusing gel electrophoresis showed that the increase was partially due to increase in activity of a constitutive enzyme (pI 8.9) and partially due to the expression of new peroxidase isozymes (pIs 3.8, 5.4, 6.2, 8.4, 9.1 and 9.4). The lignin elicitors did not appear to induce peroxidase activity in addition to that induced by wounding. Induction of peroxidases by wounding was inhibited by cycloheximide, but not by actinomycin D, indicating that the process is regulated at the level of translation. Peroxidase activity increased more rapidly in resistant than in susceptible cucumber hypocotyls after inoculation with the fungal pathogen Cladosporium cucumerinum. The pattern of peroxidase isozymes which is induced by fungal infection of resistant hypocotyls is similar to the pattern of isozymes induced by wounding.

CE 107 CHARACTERISATION OF SUPPRESSIVE MONOCYTE FACTORS FROM LEPROSY PATIENTS. M.Selvakumaran, I. Nath, Deptt. of Pathology, All India Institute of Medical Sciences, New Delhi - 110 029.

Earlier studies from our laboratory have documented that factors derived from lepromatous monocytes [MoF(s)] abrogated antigen specific lymphoproliferation and the production of T cell growth factors (IL-2). The present study indicates that such factors (i) contain arachidonic acid metabolites such as PGE_2 (evaluated by radioimmunoassay) thromboxane and leukotrienes (identified on HPLC). Whereas nonsuppressive MoF(s) from 5 tuberculoid patients had PGE_2 levels ranging from 0.8 to 2.2 ng/ml, the suppressive factors from 13 lepromatous patients showed levels of 1.8 to 5.8 ng/ml. (ii) Biologically, suppressive factors showed antigenic hierarchy at the effector levels of in vitro immune responses. Lymphoproliferation to M.leprae was abrogated maximally followed in order of importance by M.vaccae, M.smegmatis and H37 Ra. Mitogen induced proliferation were not affected by the addition of suppressive MoF(s).

Invertebrate Defense Molecules

CE 200 RESISTANCE OF LEIOPHRON UNIFORMIS TO THE IMMUNE RESPONSE OF LYGUS LINEOLARIS: NON-RECOGNITION OR IMMUNOSUPPRESSION, Jack W. Debolt, Honey Bee and Insect Biological Control, USDA, ARS, 2000 E. Allen Road, Tucson, AZ 85719. The encapsulation of the parasitoid Leiophron uniformis (Hymenoptera: Braconidae) eggs by the host, Lygus lineolaris (Heteroptera: Miridae), is the only immune response of this type reported in this order of insects. Variation exists in the level of immune response between strains and ages of L. lineolaris. The parasitoid uses internal cues to adjust its oviposition in inverse relationship to the level of immune response of the host. This study reports the existence of L. uniformis strains that are completely resistant to the host's immune reaction and readily oviposit in hosts with even the highest level of immunity. The immune resistance is transmitted by both male and female parasitoids to their offspring. The overall immune response of the host is not suppressed during oviposition by resistant wasps and subsequent eggs from susceptible wasps are encapsulated. This suggests that surface properties of the resistant eggs or a coating applied to the eggs prevent recognition of the egg as non-self by the host.

CE 201 PROPERTIES OF HUMORAL LECTINS AND HUMORAL HEMOLYSINS FROM PENAEUS AZTECUS (BROWN SHRIMP) HEMOLYMPH, B. L. Middlebrooks, R. D. Ellender, and Yeuk-Mui Lee, Department of Biological Sciences, University of Southern Mississippi, Hattiesburg, MS 39406. At least one lectin in the hemolymph of wild Penaeus aztecus (brown shrimp) was detected by hemagglutination of 14 out of 16 erythrocyte species tested, with highest titers observed against rabbit, rat, and human type A erythrocytes. Lectin activity is apparently naturally occurring, as the titer is not induced or increased by injury or injection of foreign antigens. Binding specificity of the lectins was determined by hemagglutination inhibition tests against over 30 sugars and sugar derivatives. The predominant lectin was determined to have binding specificity for N-acetyl-neuraminic acid (NANA) and related compounds. Neuraminidase treatment of erythrocytes reduced, but did not completely eliminate hemagglutinability. Hemolytic activity was detected in one pool of hemolymph collected in December, 1987, immediately after the first incidence of sub-freezing atmospheric temperatures. Hemolytic activity was found to be asssociated with two protein fractions obtained from the hemolymph by HPLC using a P-E Isopure DEAE 500 ion exchange column. No hemagglutinating activity could be detected in any fraction of the sample showing hemolytic activity, indicating either that the hemolysin may have replaced or inactivated the hemagglutinin, or that in the particular shrimp sample from which the hemolymph was taken, the lectin possessed hemolytic activity (which would preclude detection of any agglutinating activity it might possess).

CE 202 IMMUNE SUPPRESSION IN INSECT PARASITOID-HOST INTERACTION. Otto Schmidt, Institut für Biologie III, Schänzlestr. 1, D-7800 Freiburg, WEST-GERMANY. In a hymenopteran parasitoid Venturia canescens virus-like particles are found on the egg surface, which are responsible for the protection of the parasitoid against the encapsulation reaction of the host Ephestia kühniella. Some of the particle proteins are structurally and probably functionally related to a host protein (p42), which appears to play an important role in insect immune system. Ephestia p42-protein appears to interfere with the encapsulation reaction. Several observations indicate that the protein inhibits coagulation of hemolymph proteins and aggregation of hemocytes to form a capsule. This can explain the protective function of virus-like particles on the egg surface of the wasp, provided that the corresponding proteins in the particle actually display a similar effect.

CE 203 AMYLOID DEPOSITS IN THE REJECTION LESIONS OF *Botryllolides leachii*, J.M. Watson and V.L. Scofield Department of Microbiology and Immunology, U.C.L.A., Los Angeles, CA. 90024.
As a result of fusion incompatibility between individuals of the colonial ascidian *Botryllolides leachii*, dense fibers are deposited within the initial region of contact now termed the rejection lesion. Upon staining with congo red, the rejection lesion exhibits characteristic red-green birefringence under polarized light suggestive of amyloid fiber proteins. Further analysis by electron microscopy has revealed the presence of condensed, stacked, hollow helical fibers of 100A diameter. These fibers are composed of proteins identical to those isolated and precipitated from the hemolymph. Amino acid composition analysis of these purified blood proteins shows significant composition homology to the pentraxin molecules of higher organisms C-reactive protein and serum amyloid protein P component. Thus, the deposition of SAP and/or CRP-like molecules in the rejection lesions of *B. leachii* suggests that amyloid formation is a primary primitive immune encapsulation response.

Major Histocompatibility Complex; Immunoglobulins

CE 300 A MONOCLONAL ANTIBODY SPECIFIC FOR GANGLIOSIDE-LACTONE, Bernadette Bouchon, Henrik Clausen and Sen-itiroh Hakomori, The Biomembrane Institute and University of Washington, 201 Elliott Avenue West, Seattle, WA 98119

A mouse IgM monoclonal antibody was established after immunization of mice with polysialoganglioside-lactones. Lactones which are inner esters naturally occur in free carbohydrates, as well as in gangliosides. The antibody reacts strongly with GD1b and GD3 ganglioside-lactones, but not with the native gangliosides or with GD1a ganglioside-lactone or monosialoganglioside lactones. These results suggested the monoclonal antibody requires two adjacent sialic acid residues for binding, with an ester ring between them. Native gangliosides are poorly immunogenic, but the modification of the ionic charge by lactonization increases the immunogenic response, as it has been shown for GM3 ganglioside. Such an antibody could be useful to detect and localize in situ these ganglioside-lactones.

CE 301 S107 V_H CHARACTERIZATION OF A MOUSE EXHIBITING LOW-RESPONSIVENESS TO PHOSPHORYLCHOLINE, Stacy E. Ferguson*, Barbara A. Osborne*+ and Michael P. Cancro~. *Program in Molecular and Cellular Biology and + Dept. of Veterinary and Animal Sciences, Univ. of Massachusetts, Amherst, MA 01003; ~Dept. of Pathology and Laboratory Medicine, Univ.of Pennsylvania, Philadelphia, PA 19104. A recently derived inbred strain of Mus musculus domesticus, CLA-2/Cn, responds poorly to the hapten, phosphorylcholine (PC). Usage of the S107 V_H V1 family member predominates in the response to PC in most strains examined to date. The germline S107 V_H segments are characterized in the present study to 1) determine if the poor responsiveness to PC exhibited by CLA-2/Cn mice is attributable to an alteration in the appropriate germline V_H segment (V1) and 2) to further evaluate the potential contribution of gene conversion to the sequence diversity evident among S107 genes of different inbred strains. Preliminary evidence suggests that poor PC-responsiveness is not due to a defect in the V1 gene segment. Accumulated data indicates that there are particular nucleotide positions that have undergone multiple, independent, conversion events.

CE 302 POST-TRANSCRIPTIONAL REGULATION OF HUMAN GLUTATHIONE PEROXIDASE GENE EXPRESSION BY SELENIUM. *Sunil Chada and Peter Newburger. *University of Massachusetts Medical Center, Worcester, MA 01605. ^Viagene Inc. 11075 Roselle St., San Diego, CA 92121.

Selenium is an essential element in the diet, yet the only well characterized mammalian selenoprotein is Glutathione Peroxidase (GPx). This enzyme serves as an important component of the cellular anti-oxidant defence system by detoxifying peroxides and hydroperoxides which would otherwise cause damage to cell membranes and DNA. We have isolated cDNA clones for human GPx and found that the selenocysteine in the active site of the enzyme is encoded by a UGA terminator codon. We have examined human GPx enzyme activity and gene expression in the presence and absence of selenium using the human HL-60 cell line. This cell line may be grown in a defined medium with selenium added as an optional supplement.

When the HL-60 cell line is grown in a selenium-free medium, GPx enzymatic activity falls 30-fold compared to selenium-replete cells. Upon return to selenium-containing medium, GPx activity in the cells starts to rise within forty-eight hours and reaches maximal (selenium-replete) levels at seven days. However, GPx mRNA levels and the rate of transcription of the human GPx gene change very little and thus appear independent of the selenium supply. Cycloheximide studies indicate that protein synthesis is required for the increase in enzymatic activity observed upon selenium replenishment. Steady-state immunoreactive protein levels correlate with enzymatic activity. Thus the human GPx gene appears to be regulated post-transcriptionally, probably co-translationally, in response to selenium availibility.

CE 303 RESISTANCE TO COMPLEMENT-MEDIATED BACTERIAL KILLING ENCODED BY THE VIRULENCE PLASMIDS OF SALMONELLA, Donald G. Guiney, E.J. Heffernan, Joshua Fierer and Chantal Roudier, Department of Medicine, UCSD School of Medicine, San Diego, CA 92103 Plasmids have been shown to encode traits essential for mouse virulence in Salmonella dublin, typhimurium, enteritidis, and choleraesuis. A 4kb homologous EcoRI fragment is present in the essential virulence region of plasmids from each of these serotypes. Recently, Hackett, et.al., (J. Infect. Dis. 155, 540, 1987) cloned a 2.1kb fragment (pADE016) from the S. typhimurium plasmid that expresses complement resistance in strains of E. coli and S. typhimurium possessing rough LPS. We have used Southern hybridization to show that the virulence plasmids of S. dublin (pSDL2) and S. enteritidis (pSE1) each contain a region homologous to the complement resistance locus (rck) in pADE016; S. choleraesuis lacks homology. The rck homology in pSDL2 and pSEI is not located on the 4kb EcoRI fragment from the virulence region. Elimination of the virulence plasmid from a smooth S. dublin strain had no effect on complement resistance. Furthermore, an S. dublin plasmid deleted for rck still conferred a fully virulent phentoype. pADE016 expressed a high level of serum resistance in rough LPS strains of Salmonella (Ra mutant of S. minnesota) and E. coli (K12) as well as in the complement sensitive smooth E. coli strain 0111. We conlcude that pADE016 encodes serum-resistance independent of the LPS-structure of the host strain. However, in smooth LPS strains such as S. dublin that are inherently resistant to complement, the plasmid rck locus is not required for either serum resistance or virulence.

CE 304 A MONOCLONAL ANTIGEN-BINDING T CELL IMMUNOPROTEIN. Roger A. Hubbard and John J. Marchalonis. Microbiology and Immunology, University of Arizona, Tucson, AZ 85724. The monoclonal murine T cell hybridoma 51H7D was previously shown to bind the arsazobenzene hapten and to produce a soluble antigen-binding molecule. The 51H7D cell expresses a protein of subunit size approximately 31kDa that reacts antigenically with affinity purified antibodies directed against synthetic first framework and joining segment peptides corresponding to the gene sequence of the T cell receptor β chain YT35. This molecule does not react with affinity purified antibodies directed against murine immunoglobulin, framework 1 sequences of α and γ T cell receptors or with antibodies against synthetic heavy chain joining segments. The 31kDa subunit can form higher aggregates,notably in the range 60-70 kDa dependent upon extraction conditions. The soluble form of the antigen-binding molecule bears the Jβ cross-reactive determinant and occurs predominantly as a charge restricted molecular species of approximately 60-70 kDa. The purified molecule has a blocked N-terminus, but quantitative statistical analysis of its amino acid composition indicates a closer relatedness to T cell receptor β chains and other antigen-binding T cell products than it has to α, γ or σ TCR chains. No evidence for more than one type of polypeptide chain was found and the polymerization is not dependent upon the formation of disulfide bonds. These studies raise the possibility that antigen-binding soluble T cell molecules might belong to a new family of immunoproteins that is related to but distinct from classical immunoglobulins and α/β or γ/δ heterodimers. (Supported by grant NCI CA 42049).

CE 305 CLASS SPECIFIC SERUM IMMUNOGLOBULINS AND ANTIBODIES TO MYCOBACTERIAL SONICATES AND AUTOANTIGENS IN LEPROSY PATIENTS AND CONTACTS., M.M.James and VR. Muthukkaruppan, Department of Immunology, School of Biological Sciences, Madurai Kamaraj University, Madurai - 625 021, India. A comprehensive analysis of the humoral immune response in lepromatous (LL) and tuberculoid (TT/BT) leprosy patients and contacts was undertaken. In this report the level of class specific total serum immunoglobulins and antibodies to mycobacterial sonicates, autoantigens and haptens, as estimated by ELISA, are presented. The salient findings were: 1] The IgM levels, total and antigen specific, were not different among different groups of patients and normals. 2] The level of serum IgG was signficantly higher in both LL BI positive and negative patients than in TT/BT and controls. 3] Anti-mycobacterial IgG antibodies occurred in LL patients at a very high level, as much as 20 times more than that in the non-contacts. 4] However, the level of these antibodies was significantly reduced , when the LL patients became bacteriologically negative as a result of effective chemotherapy. 5] Considerable amount of anti-mycobacterial IgG was also detected in TT/BT patients. 6] As in the case of total serum IgG , the auto-antibodies of IgG isotype occurred at significantly higher levels in both LL positive and negative patients. 7] Anti-hapten antibodies were not detected in any of the patients and controls studied. These results indicated that, the amount of IgG antibodies is directly correlated with the antigen load in the system, be it infectious agents or auto-antigens. Further, there may not be any polyclonal activation of B cells. Based on these and other studies, it may be speculated that the antibody production in leprosy may be induced and sustained by the normal immunological processes.

CE 306 POSSIBLE ROLE OF FOLLICLE CELLS IN THE MAINTENANCE OF SELF-STERILITY IN THE SOLITARY TUNICATE *Ciona intestinalis*, C.J. Lahti and V.L. Scofield, Department of Microbiology and Immunology, U.C.L.A. School of Medicine, Los Angeles, CA 90024

The solitary ascidian *Ciona intestinalis* is hermaphroditic and fertilization takes place pelagically. Individuals exhibit incomplete self-sterility, with 10-15% occurence of self-fertility in most populations. This suggests a transient or labile factor contributes to self-recognition in *Ciona* fertilization. For self-fertile animals, we find that sperm incubated in a suspension of follicle cells removed from self eggs or non-self eggs are subsequently unable to fertilize self whole intact eggs. The same sperm are able to fertilize whole intact nonself eggs. Identical treatment of gametes from self-sterile animals has no effect on self-sterility or cross-fertility. This suggests that some element(s) released by follicle cells restores self-sterility.

CE 307 AVIAN IMMUNOGLOBULIN LIGHT CHAIN GENE ORGANIZATION AND REARRANGEMENT IN THE BURSA, Wayne T. McCormack, Louise M. Carlson, Larry W. Tjoelker, and Craig B. Thompson, Howard Hughes Medical Institute, Ann Arbor, MI 48109.

Single functional VL and JL gene segments are joined in all chicken B cells and subsequently diversified by gene conversion from pseudogene donor sequences. We investigated whether the chicken IgL gene organization and rearrangement pattern is a general feature of avian IgL gene families. Southern blot analysis was performed on rearranged bursal lymphocyte DNA and germline erythrocyte DNA from four species, including quail, turkey, pigeon, and mallard duck, using chicken VL and CL probes. We observed a single major rearrangement in bursal lymphocyte DNA of all four species, and the presence of a family of VL-hybridizing elements. Molecular analysis of the Muscovy duck IgL locus revealed IgL gene organization similar to the chicken. Nucleotide sequencing of the VL-hybridizing elements revealed at least two functional VL genes and Ig recombination signal sequences associated with some pseudogenes. Southern blot analysis confirmed that several VL elements rearrange in the Muscovy duck bursa. Our results suggest that the basic IgL gene organization and rearrangement pattern are similar in most avian species, with some variation in the number and structure of the functional and pseudogene VL segments.

CE 308 THE B-G ANTIGENS OF THE CHICKEN MAJOR HISTOCOMPATIBILITY COMPLEX, Marcia M. Miller, Ronald Goto, Sharon Young, and Garry Miyada, Department of Molecular Biochemistry, Beckman Research Institute of the City of Hope, Duarte, CA 91010-0269.

From the viewpoint of major histocompatibility complex (MHC) function and structural evolution it is of interest to understand more about the nature of the B-G antigens of the chicken MHC. The B-G antigens are highly polymorphic molecules expressed apparently only on chicken erythrocytes. There are presently no known mammalian counterparts. It is not clear whether the B-G antigens should be considered immune function molecules or molecules encoded by genes only coincidentally located within the B system gene complex. In order to better understand the structure of the B-G antigens, complementary and genomic DNA clones from the B-G subregion have been isolated and are being sequenced. Sequence and hybridization data have provided evidence that there are two classes of B-G genes, suggesting that the antigens may be heteropolymers, and that at least one class contains sequences encoding immunoglobulin-like domains. Ongoing sequence analyses should help to clarify the polymeric nature of the B-G antigens as well as their structural similarity to other Ig-superfamily molecules.

Supported in part by grants from NIH (AI21736) and NSF (DCB-8609632).

CE 309 A SOLUBLE MONOCLONAL T CELL V_β RELATED MOLECULE. C. Richard Ross, Roger A. Hubbard, John J. Marchalonis and Samuel F. Schulter. Department of Microbiology and Immunology, University of Arizona, Tucson, AZ 85724.
The murine T cell hybridoma 51H7D has previously been shown to bind arsonate hapten on various carriers. Western blot analysis of both 51H7D membrane extracts and serum-free media in which 51H7D were grown have shown a protein which reacts strongly with antibodies to synthetic peptides corresponding to consensus sequences of the first framework (FR1) and J region of TCR β. However, no molecules having the properties of the α/β heterodimer were found. The serological reactions indicate that the 51H7D protein is Ig-like and is therefore capable of possessing a variable region binding site. The protein has an apparent subunit mass of 31kDA under reducing conditions on SDS PAGE and is seen at 60kDa and 140kDa under conditions using lesser amounts of detergent indicating dimer-and tetramerzation. The protein is also excreted in culture and appears in the 140kDa form when run on PAGE under native conditions. A protein of similar subunit mass which also reacts positively with anti-peptide antibodies described above is seen in membrane extracts of both mouse spleen and mouse thymus. This protein is visibly distinct from Ig light chain (spleen extract) and the α/β heterodimer which also reacts with the anti-peptide antibodies. Subunit mass as well as abundance in thymus extracts as compared to α/β indicate that the 31kDa protein is also not γ or δ. Attempts are now under way in the purification of this protein from the above-mentioned serum-free media with the goal of obtaining its amino acid sequence. (Supported by Grant CA-42029).

CE 310 Ig LIGHT CHAIN GENES CLONED FROM SANDBAR SHARK ARE HOMOLOGOUS TO λ CHAIN. Samuel F. Schulter and John J. Marchalonis. Microbiology and Immunology, University of Arizona, Tucson, AZ 85724.
We prepared a cDNA library from mRNA from sandbar shark (*Carcharinus plumbeus*) spleen in the expression vector λgt11, and used rabbit antisera against shark light chain to isolate four clones. We also obtained limited peptide sequence using peptide fragments prepared from pooled light chain preparations. Several peptide sequences were identical to segments in the cloned gene sequences, thereby unequivocally proving that our cloned genes were shark light chain genes. Computer search indicated that the best matches were with λconstant regions and >40% homology was obtained. Certain stretches were remarkably conserved, whereas others varied in a manner consistent with accepted concepts of speciation. One pentapeptide (131-135) TLVCL was identical in sequences of shark, man, mouse, rabbit, pig and chicken. There was also universal conservation of certain half-cysteins, phenylalanines, tryptophans and glycines and strong conservation in the block of residues from serine 176 to tryptophan 186. Light chains of a primitive vertebrate thus show homology to mammalian chains and evolutionary divergence of the Cλdomain reconstructs phylogeny. (Supported by NSF Grant DCB88 02353).

CE 311 DEVELOPMENT OF AN OPTIMAL ANIMAL MODEL FOR MOLECULAR STUDIES OF PROTOCHORDATE ALLORECOGNITION. K.A. Svoboda and V.L. Scofield, Department of Microbiology and Immunology, UCLA School of Medicine, Los Angeles, CA 90024. Fusibility in the colonial tunicate *Botryllus* is controlled by a single genetic locus with multiple alleles. Upon contact, two colonies that share an allele at this locus fuse, whereas colonies that do not share an allele reject. Biochemical characterization of the molecules controlling this self/nonself recognition has been extremely difficult due to the small size (2-3 cm) of individual *Botryllus* colonies. We therefore sought to identify a more suitable species for these studies. Here we present genetic evidence that fusibility in the larger colonial tunicate *Botrylloides leachii* (10-20 cm) is also controlled by a single genetic locus with multiple alleles. Allorecognition in *Botrylloides leachii* is now the model system for our ongoing molecular studies of protochordate allorecognition.

In vivo toluidine blue staining of the mast cell-like tunic cells (test cells) of interacting *Botrylloides* oozoids has allowed us to characterize further the various allorecognition processes in this species. Stained test cells from one oozoid do not migrate into the test of an oozoid to which it has fused. Additionally, observed movement patterns of test cells in oozoids undergoing resorption suggest a specific role for these cells in this aspect of *Botrylloides* allorecognition.

Immunomodulators

CE 400 REGULATION OF THE OXIDATIVE BURST IN MURINE MACROPHAGE VARIANTS: A POSSIBLE in vitro MODEL FOR A HUMAN GENETIC DISORDER. Linda S. Belkowski, Michael Goldberg, and Barry R. Bloom. Department of Microbiology and Immunology, Albert Einstein College of Medicine, Bronx, New York 10461 USA. We have isolated variants of a murine macrophage cell line (J774.16) which fail to produce superoxide (O_2^-) upon stimulation with phorbol esters (PMA). These variants can be divided into two classes. The first class produces O_2^- if pretreated with interferon-gamma (IFN-γ) prior to PMA stimulation, in contrast to J774.16 which does not require IFN-γ treatment in order to produce O_2^-. The second class of variants fails to produce O_2^- upon stimulation with PMA even if pretreated with IFN-γ. The gene encoding the heavy chain of the cytochrome b_{558} involved in the oxidative burst is expressed constituitively in J774.16 cells and is induced by IFN-γ in the first class of variants. Even high doses of IFN-γ fail to induce the expression of this gene in the second class of variants. Southern analysis of this gene locus in all of the variants fails to show any structural rearrangements relative to J774.16. Thus these variants appear to have anomalies in the regulation of this gene relative to the parental line. These variants may prove useful as models for chronic granulomatous disease, in which this transcript is also deficient and in which IFN-γ has been recently shown to be useful in some cases as a therapeutic agent.

CE 401 CHARACTERIZATION OF REGULATORY REGIONS IN THE PROMOTER OF THE HUMAN INTERFERON β 2 / IL6 / HEPATOCYTE GROWTH FACTOR GENE. Yuti Chernajovsky and Hattie Kirby, Department of Immunology, University of Texas, M.D. Anderson Cancer Center, Houston TX 77030.

We have sequenced 1.2 kilobases of 5' flanking DNA of the interferon (IFN) β2 gene. The promoter sequence contains regions of homology with already known phorbol ester, serum and cAMP regulatory elements. Some of the constitutive (CCAAT and TATA boxes) and the cAMP regulatory elements are repeated twice and there is a strong homology with the promoter region of the mouse IL1β gene. We cloned this 1.2 kb DNA fragment in front of the bacterial reporter gene chloramphenicol acetyl transferase (CAT). The fused IFN β 2-CAT construct named plasmid A47 was cotransfected with the HSV thymidine kinase (tk) gene into mouse LTK$^-$ cells and stable transfectants were selected in HAT medium. The selected population of cells showed increased gene expression of the chimeric A47 plasmid upon treatment with the following inducers: cycloheximide, dsRNA, cAMP, PDGF, IL1α and TNF. In order to locate regulatory regions and dissect this promoter we subcloned promoter regions in front of a reporter plasmid containing the CAT gene under regulation by the HSV tk promoter. The IFN β 2 promoter fragment from -309 to -203 was sufficient to confer inducibility by cAMP, iL1α, cycloheximide, TNF and PDGF but not to by dsRNA. Moreover this DNA fragment showed specific binding to nuclear proteins in-vitro as measured by gel retardation assays. Analysis of other promoter regions is under investigation.

CE 402 MODULATION OF T-CELL GROWTH BY GANGLIOSIDES AND BY THE INHIBITION OF GLYCOLIPID BIOSYNTHESIS, *Felding-Habermann B., *Handa K., *Hakomori S., +Park L. and #Radin N., *The Biomembrane Institute and University of Washington, Seattle, WA 98119; +Immunex Corp., Seattle, WA 98101; #Mental Health Institute, University of Michigan, Ann Arbor, MI 48109 The incorporation of exogenous gangliosides (acidic glycolipids) and their derivatives into the plasma membrane of murine T-cells enhanced their responsiveness to alloantigen (MLR), mitogen (Con A, PHA) and IL-2 (IL-2 dependent growth of CTL cells). The inhibition of glycolipid biosynthesis, induced by 1-phenyl-2-decanoyl-amino-3-morpholino-1-propanol which blocks ceramide: glucosylation resulted in the loss of T-cell proliferative responses. Very early steps in T-cell activation are not affected by these modulators: the production of IL-2 upon mitogenic stimulation and the affinity of the IL-2 receptor, as well as the internalization of the receptor-ligand complexes, display normal functions following both, ganglioside- and the inhibitor-induced lymphocyte state. Thus, the modulatory effects observed favor or, respectively, suppress a step in T-cell activation subsequent to the arrival of IL-2 inside the cell through an as yet unidentified mechanism. B. Felding-Habermann's support is by a fellowship from the Deutsche Forschungsgemainschaft; S. Hakomori is supported by a National Cancer Institute grant, OIG CA42505.

CE 403 LYSOZYME AND OTHER NON-SPECIFIC DEFENSE MOLECULES IN RAINBOW TROUT AND ATLANTIC SALMON. Viggo M. Fosse, Mohasina Syed, Knut Røed and Øystein Lie. Department of Animal Genetics, National Veterinary Institute, PO Box 8156 Dep.,N-0033 Oslo 1, Norway.
In marine fishes non-specific defense mechanisms appear to play an important role in conferring disease resistance. We have purified and characterized lysozyme from rainbow trout kidney which contain the highest activity of the enzyme in this species. Two, or possibly three, lysozyme variants were identified. They had similar molecular weights (14.4 kDa) but different isoelectric points, i.e. 9.5 and 9.65. Their amino acid sequences as well as activity profiles indicate that rainbow trout lysozymes are of the c-type. The intraspecies variation of lysozyme activity is being determined in large family groups to assess the heritability of lysozyme levels as well as the influence of lysozyme activity on disease resistance. Similar studies are conducted with regard to other non-specific defense mechanisms in relation to disease resistance. We are currently also engaged in isolating the lysozyme gene(s) from rainbow trout and atlantic salmon by classical gene cloning strategies as well as by the novel PCR (polymerase chain reaction) technique. The aim is to sequence the gene(s) and to identify adjacent regulatory elements. We also aim to identify factors which interact with these elements and hence control transcription of the lysozyme gene. Results of this work will be presented and discussed.

CE 404 RESTORATION OF ANTIGEN-SPECIFIC ANTIBODY RESPONSES *IN VITRO* IN COMMON VARIABLE IMMUNODEFICIENCY, Michael G. Goodman, Sudhir Gupta, M.D.*, Linda F. Thompson and William O. Weigle, Department of Immunology, Research Institute of Scripps Clinic, La Jolla, CA 92037 and *Department of Medicine, University of California, Irvine, CA 92717
Common variable immunodeficiency (CVI) is a primary immunodeficiency disease characterized by defective B cell function resulting in hypogammaglobulinemia, failure to generate specific antibody responses, and an increased incidence of infection and malignancy. This disease includes variants attributable to intrinsic defects of the B cell itself, to immunoregulatory T cell imbalances or to autoantibodies to B or T cells. The inability to generate responses to nominal antigen was confirmed in nine patients with diagnosed CVI. Recent studies in mice have provided evidence that these guanosine derivatives transmit a T cell-like differentiative signal directly to B cells, enabling them to respond to T-dependent antigens in the absence of T cells. In addition, they markedly increase the efficacy of helper T cells. In cultured lymphocytes from 8 of the 9 CVI patients studied, normal level or greater responses were elicited by the intracellular immunostimulatory nucleoside, 7-methyl-8-oxoguanosine. Restoration of specific immune responses was antigen-dependent, with responsiveness increasing directly with increasing concentrations of nucleoside. This restorative activity is apparently attributable to the ability of these compounds to transmit alternate T cell-like signals to the patients' B cells subsequent to antigen activation, analogous to their action in cultures of normal human PBL. Substituted guanine ribonucleosides are novel and potentially very useful immunotherapeutic agents for patients with CVI and possible for those with other immunodeficiency syndromes.

CE 405 A SYNTHETIC RETROVIRAL p15E PEPTIDE INHIBITS NATURAL KILLER CELL (NK), CYTOLYTIC T LYMPHOCYTE (CTL) AND LECTIN-MEDIATED CYTOTOXICITY. H. Koren, G. Cianciolo, K. McKinnon and D. Harris. U.S. EPA, Chapel Hill, NC 27599; Genentech, Inc., South San Francisco, CA 94080 and Univ. of North Carolina, Chapel Hill, NC 27599. The retroviral envelope protein p15E has been implicated in suppression of various immune functions. We reported that CKS-17, a hectadecapeptide homologous to a conserved region of the p15e molecule common to many retroviruses; 1.) suppressed NK and LAK cytotoxicity and 2.) inhibited augmentation of NK lysis by IFN-alpha and -gamma but not by IL-2. The inhibitory activity was at the post-binding stage of lysis. We further investigated the peptide's effects on other effector cell populations. It was observed that the peptide not only inhibited the direct lytic activity of NK and LAK but also inhibited ADCC and LDCC lysis by these populations. The peptide also inhibited antigen-specific and NK-like lysis by antigen-speicific CTL as well as inhibiting the generation of such CTL. These effects were seen at both the clonal and population levels. CKS-17 had no effect on LAK generation. It appeared that NK-like lysis was more susceptible to inhibition than antigen-specific lysis. Studies to identify the suppressive mechanism revealed that inhibition of lysis was not caused by a block at the recognition step of lysis. Neither did the peptide inhibit the synthesis and secretion of soluble cytolytic factors. The peptide did, however inhibit the reorientation of effector cell cytoplasmic granules towards the point of contact with the target cell. Since these granules contain lytic moieties,and since this process is necessary for lysis by both NK and CTL, inhibition at this stage of lysis may be responsible for the inhibition of various types of lysis (NK, CTL, ADCC and LDCC). Thus, CKS-17 appears to inhibit lysis by inhibiting the intracellular processes necessary for lysis.
This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.

CE 406 REGULATION OF THE BINDING AND INTERNALIZATION OF INTERLEUKIN-2 BY GLUTATHIONE, Shu-Mei Liang, Nancy Lee, Debra Cattell and Chi-Ming Liang, Food and Drug Administration, Bethesda, MD 20892. Interleukin-2 (IL-2) stimulates the proliferation of activated T-cells. In this study, we have evaluated whether the cellular activity of interleukin-2 (IL-2) is affected by glutathione (GSH), an important tripeptide existing in most cells. Cell culture and thymidine incorporation assay showed that addition of GSH enhanced the effect of IL-2 on proliferation and thymidine incorporation of IL-2-dependent cells such as CTLL-2. This effect of GSH was time-dependent and was accompanied by an increase in the intracellular level of GSH. Binding studies with radiolabeled IL-2 showed that GSH treatment did not affect the number of receptors available for binding, it, however, increased the affinity of IL-2 for IL-2 receptors. Evaluation of internalization of IL-2 demonstrated that treatment of CTLL-2 with GSH enhanced the rate and the amount of IL-2 internalized. Analysis of IL-2 receptors showed that the turn-over rate of the receptors was potentiated by GSH treatment. These results suggest that GSH potentiates the effect of IL-2 on IL-2 dependent cells by facilitating the binding and the internalization of IL-2; variations in the GSH level may play an important role in the response and dependence of these cells to IL-2.

CE 407 INDUCTION OF CYTOKINE GENE EXPRESSION IN VIVO BY FLAVONE ACETIC ACID, Kenneth F. Mace, Robert H. Wiltrout and Howard A. Young, Biological Response Modifiers Program, NCI-FCRF, Frederick, MD 21701 Flavone Acetic Acid (FAA) is undergoing clinical investigation because it has demonstrated some anti-tumor activity against murine solid tumors in vivo. FAA also augments natural killer (NK) cell activity in both humans and rodents after in vivo administration and synergizes with Interleukin-2 for treatment of murine renal cancer. One possible mechanism for these effects is through induction of immunoactive cytokines. Studies were therefore intiated to define the kinetics of cytokine m-RNA induction after in vivo administration of FAA. Spleen cells from untreated and drug-treated mice were assayed for NK activity or used as a source of RNA for Northern blot analysis. NK activity was augmented at 18 hr. after FAA treatment and this level of activity was maintained for 3 days. Serum Interferon-α,β (IFN) and Tumor Necrosis Factor (TNF) were detectable by bioassay following FAA (200mg/kg,i.v.) administration with peak levels occuring by 3 hr. post injection. IFN m-RNA in the spleen was upregulated within 1.5-2.0 h after FAA, while an upregulation of TNF m-RNA was detected by 1 hr. after treatment. The upregulation by FAA of m-RNA and the corresponding serum protein was also observed to be dose dependent. In summary, these results demonstrate that FAA can act as a potent inducer of at least two cytokines in vivo, and suggest that the immunomodulatory and immunotherapeutic effects of FAA may be partially mediated by cytokines.

CE 408 PLASMA FIBRONECTIN IS A MAJOR HOST DEFENSE PROTEIN, Janos Molnar[1] and Pierson J. Van Alten[2], Department of Biochemistry[1] and Anatomy[2], University of Illinois at Chicago, Chicago, IL 60612.
Even though the host defense role of plasma fibronectin has been documented by many laboratories the importance of these findings has not been fully recognized. This fact is illustrated by the program of this symposium on defense molecules which doesn't have any representation of fibronectin. In our presentation we want to use data from our laboratories and also from the literature to demonstrate the essential role of plasma fibronectin (PFN) in a mammalian host defense system. The opsonic role of PFN was first demonstrated with the use of gelatinized particles. It was shown later that PFN is required for phagocytosis of effete tissue and thus essential for wound healing. The collaboration between the complement, or antigen/antibody system and PFN for efficient phagocytosis by monocyte has been documented by many laboratories. Plasma fibronectin is also involved in the binding, and in some instances phagocytosis of microorganisms by phagocytes. Evidence shows that these reactions require a specific fibronectin receptor which rapidly recycles during phagocytosis but does not activate the bacteriocidal mechanism.

CE 409 AUTOREGULATION OF INTERLEUKIN-1 IN PULMONARY TUBERCULOSIS PATIENTS, Padma, R.S. and Vaidya, M.C., Cellular Immunology Laboratory, Department of Anatomy, All India Institute of Medical Sciences, New Delhi 110029, India

The production of Prostaglandin E, a major immunesuppressor secreted by the macrophages was inhibited by the addition of 0.1 M indomethacin to the cultures of monocytes harvested from patients suffering from pulmonary tuberculosis and those from equal number of normal controls. The IL-1 activity was estimated in the supernatants of these cultures by their ability to proliferate mice thymocytes. It was found that the supernatants from cultures with indomethacin showed a greater IL-I activity than the ones without it(44% P 0.001). This indicates the possibility of PGE offering a negative feedback control over IL-I production. The defective cell mediated immunity in patients with pulmonary tuberculosis may be explained through the inhibition on IL-I production by PGE whose enhanced production is reported in our earlier studies. The results and our hypothesis on the autoregulation of IL-I production will be presented and discussed.

CE 410 INTERLEUKIN 2 CONFERS STRESS RESISTANCE UPON HUMAN NK ACTIVITY, Bernice Schacter and Susan Hansal, Bristol-Myers Company, Wallingford, CT 06492

Natural killer activity is a non-MHC restricted cellular cytotoxicity demonstrated in vitro, mediated by several non-B lymphoid cells including large granular lymphocytes (LGL) and implicated in the control of viral and fungal infections. NK activity of resting LGL of humans is sensitive to radiation (30 greys of γ-irradiation or UV). Such sensitivity is polymorphic under the control of an X-linked gene. Interleukin 2, but not IFN-γ, renders NK activity radioresistant. DNA damage and its consequences are implicated in the inhibition of NK by radiation by 1) the radioprotective ability of 3-aminobenzamide, a specific inhibitor of adenosine diphosphoribosyl polymerase, a nuclear enzyme activated by DNA strand breakage and 2) a UV action spectrum for inhibition consistent with a nucleic acid chromophore. Heat shock (>10 minutes @ 42°C) inhibits fresh NK activity, but incubation with IL-2, which moves these cells from G_0 into the cell cycle, confers resistance to heat shock and radiation to the NK activity prior to DNA synthesis. These results suggest that IL-2 by stimulation of synthesis of cell cycle dependent proteins may modulate the sensitivity of this effector mechanism to environmental stress.

CE 411 A FAMILY OF LIPOPOLYSACCHARIDE BINDING PROTEINS, P.S.Tobias, J.C. Mathison, and R.J.Ulevitch, Dept. of Immunology, Research Institute of Scripps Clinic, La Jolla, CA 92037. The lipopolysaccharides (LPS) of gram negative bacteria are known for their ability to initiate cellular responses that lead to irreversible cellular and organ injury. Endotoxemia results in an acute phase response; we have recently discovered an acute phase serum LPS binding protein protein (LBP) that is a 60 kDa glycoprotein synthesized in hepatocytes. LBP rises to a blood concentration of up to 50 ug/ml from a normal level of <100 ng/ml upon acute phase induction with a variety of inducing agents. LBP binds to rough and smooth forms of LPS with a high affinity (Kd <5x10^-9 M) for the lipid A moiety of LPS. N-terminal amino acid sequence data for LBP shows >50% sequence identity with BPI, a bactericidal/permeability increasing protein of neutrophil primary granules (JBC, 262:14891 (1987)), suggesting that these two proteins are the first two members of a family of LPS binding proteins to be recognized (JBC, 263:13479, (1988)). Although LBP and BPI both bind isolated LPS via lipid A and are immunologically cross reactive, BPI is synthesized in PMNs, is tightly bound to PMN primary granule membranes, and is strongly bactericidal while LBP circulates in the blood and is not bactericidal. LBP and BPI are the first two well-characterized lipid A binding proteins and may serve as models for LPS receptors. In vitro, LBP lowers the threshold dose of LPS that is recognized by macrophages, PMNs and endothelial cells. We hypothesize that enabling an organism to recognize and respond to the presence of LPS at as low a concentration as possible is an adaptive response to the multiple threats of gram negative sepsis and endotoxemia.

CE 412 PEPTIDES OF THE CORTICOLIBERIN SUPERFAMILY EXERT ANTI-INFLAMMATORY ACTIONS ON EPITHELIAL TISSUES, E.T. Wei, School of Public Health, University of California, Berkeley, CA 94720

Injury to epithelial tissues produce inflammatory responses which are manifested initially as swelling, increased vascular permeability and protein extravasation. Peptides belonging to the corticoliberin superfamily have the unusual property of making epithelial tissues resistant to these indices of injury. For example, the 41-residue human/rat corticotropin-releasing factor (CRF, 28 μg/kg), sauvagine (2.5 μg/kg) or sucker fish urotensin I (10 μg/kg) injected iv 10 min before immersion of the anesthetized rat's paw in 58°C water for 5 min, reduced by 50 to 60% the heat-induced edema and Evans blue dye extravasation into the pawskin. Using CRF as the prototype agent, the time-course of CRF's actions was studied. Pretreatment with CRF, 28 μg/kg sc, was effective for up to 4 hr in reducing the swelling produced by heat (58°C for 30 sec, observation period of 1 hr). The same dose of CRF injected iv 0, 10, 20 min after heat exposure immediately inhibited the progressive development of swelling. The pharmacological specificity of CRF's actions was revealed by the α-helical CRF(9-41) antagonist. The antagonist administered alone, 92 μg/kg iv, did not affect heat injury; but it both prevented and reversed the inhibitory effects of CRF on the swelling produced by heat. α-Helical CRF(9-41) also antagonized the anti-inflammatory effects of sauvagine and urotensin I. The results described here may provide clues for interpreting the mysterious homology among peptides of the corticoliberin superfamily which are distributed in cells of the hypothalamus, the amphibian skin and the teleostean urophysis. Perhaps, these peptides have common ancestry, not only as hypophysiotropic and vasodilatory agents, but also as peptide agonists designed to defend and limit the inflammatory response of epithelial tissues to severe injuries. Their protective effects would be akin to the anti-inflammatory actions of the corticosteroids.

CE 413 Race specific resistance protein of cotton Fusarium wilt, Yishen Zeng, Liangpeng Yang, Guangyu Zhou, Shanghai Institute of Biochemistry, Academia Sinica, Shanghai, China. A specific resistance protein(protein R) of cotton Fusarium wilt was identified and purified from resistant cotton cultivars. It inhibits the growth of mycelia and germinations of spores of incompatibles but not compatible race of Fusarium oxysporum f.sp.vasinfectum(ATK)Snyder & Hansen. On the other hand, a protein S from wilt susceptible cultivar was also purified. Protein S is very similar to protein R in electrophoresis behavious of PAGE and IEF but without the inhibitory effects. The molecular similarity between the two proteins agrees well with the fact that the resistance was selected in the field by spontaneous mutation from the susceptible one. Both of protein R and protein S are constitutive.Some biochemical characteristics of the two proteins have been clarified.

Hypersensitivity; T Cells

CE 500 THE ABSENCE OF DIRECT ANTIMICROBIAL ACTIVITY IN EXTRACTS OF CYTOTOXIC LYMPHOCYTES. Sanjay Joag and J.D.-E Young. Laboratory of Cellular Physiology and Immunology. The Rockefeller University, 1230 York Avenue, New York, NY 10021.

Cytotoxic lymphocytes are recognized to play an important role in battling infections caused by intracellular pathogens, by destroying the host cell. It is unclear, however, whether more directly antimicrobial mechanisms are present. In an effort to answer this question we have tested extracts, prepared by cell fractionation, from murine cytotoxic T cell lines and from a rat natural killer cell line, against Listeria monocytogenes, Salmonella typhimurium, and Escherichia coli. The extracts were also tested for antiviral activity against a DNA virus (pseudorabies virus) and an RNA virus(vesicular stomatitis virus). All extracts showed no antimicrobial activity under the conditions tested.

CE 501 A MEMBRANE-BOUND CYTOTOXIN OF MURINE CYTOTOXIC T LYMPHOCYTES RELATED TO TUMOR NECROSIS FACTOR/CACHECTIN, Chau-Ching Liu, Patricia A. Detmers, Shibo Jiang, and John Ding-E Young, Laboratory of Cellular Physiology and Immunology, The Rockefeller University, 1230 York Ave., New York, NY, 10021.
Cytotoxic T lymphocytes (CTLs) are known to kill their targets by a contact-dependent mechanism. The possibility that the CTL membranes themselves could exert direct cytotoxicity was investigated here. Both murine CTLs that have been fixed with paraformaldehyde and the membrane fractions of CTLs display a slow cytotoxic activity towards various target cells that are sensitive to tumor necrosis factor/cachectin. Immunoblot analysis reveals a membrane protein band of 50-60 kD that reacts with anti-TNF antibodies. This membrane-bound cytotoxic activity can also be neutralized by anti-TNF antibodies. The surface localization of this cytotoxin was further ascertained by fluorescence activated cell sorter and immunoelectron microscopy studies. This cytotoxin appears to an integral membrane protein because it can be removed from membranes only by treatment with detergent but not with high salt buffers. The role of this membrane-bound cytokine in CTL-mediated killing as well as in other physiological functions of CTLs remains to be explored.

CE 502 ACTIVATION IN VIVO OF THE PERFORIN- AND GRANZYME A-GENE IN INFILTRATING CELLS DURING A LCMV-INFECTION IN THE MOUSE, Christoph Mueller[+], David Kaegi[*], Eckhard Podack[$], Rolf M. Zinkernagel[*], Werner Held[+], Max W. Hess[+], and Hans Hengartner[*], [+]Dept. of Pathology, University of Bern/ Switzerland, [*]Dept. for Experimental Pathology, University of Zurich/ Switzerland, [$]Dept. of Microbiology and Immunology, University of Miami, FL 33101
The role of various putative effector molecules in cell mediated cytotoxicity in vivo is still controversial. The recent cloning of genes preferentially expressed in cytotoxic cells, including the genes for perforin and several serine proteases, allows the detection of cells containing transcripts of these genes directly in tissue sections by in situ hybridization.
We followed the cellular and molecular events during an infection with lymphocytic choriomeningitis virus (LCMV) in C57Bl/6 mice by immunostaining and in situ hybridization with radiolabeled RNA-probes of the perforin- and granzyme A-gene. These experiments revealed a close histological association of LCMV-infected cells with infiltrating cells containing transcripts of the perforin- and the granzyme A-gene. The frequency of perforin-positive cells in the liver was maximal 6 days after intravenous infection with the hepatotropic strain LCMV-WE, i.e. 2 days before LCMV-specific cytolysis was maximal in lymphoid cells isolated from the infected organs. These results are a strong indication for an involvement of a perforin-mediated mechanism in the elimination of virally infected cells in vivo.

CE 503 IMMUNOTOXIN MODULATION OF IgE TITERS, R. Blake Whitaker, C. Brad Shuster, and Lynn G. Hannum, Department of Biology, Bates College, Lewiston, Maine 04240.
An immunotoxin comprised of goat anti-mouse IgE antibody covalently coupled to pokeweed antiviral protein specifically suppresses murine IgE antibody titers. Anti-ovalbumin IgE titers in appropriately immunized BDF1 high-responder mice were reduced greater than 94% by intravenous administration of 100 ug of pokeweed antiviral protein immunotoxin (PIT). Control mice received 100 ul of PBS and exhibited peak IgE titers greater than 1 / 4096. Antibody of IgM and IgG isotypes is not modulated by immunotoxin treatment. Furthermore, PIT treatment has no effect on T lymphocyte responses as assessed by delayed-type hypersensitivity or T lymphocyte proliferation assays. These results suggest that immunotoxins may be employed as highly potent, isotype-specific immunosuppressants.

CE 504 IDENTIFICATION OF PFP/PERFORIN AND A TNF-RELATED PROTEIN IN A HIGHLY PURIFIED PREPARATION OF MURINE LYMPHOKINE ACTIVATED KILLER (LAK) CELLS. Zychlinsky A. and J. D.-E. Young. Department of Cellular Physiology and Immunology, The Rockefeller University, 1230 York Avenue, New York NY 10021.

A highly purified preparation of LAK cells was obtained through culture of Nylon wool non-adherent mouse splenocytes in high concentrations of interleukin 2 (IL-2; 1000 U/ml). More than 95% of the cell population was positive for the natural killer (NK) cell marker GM-asialo and for Thy 1, but the cells were negative for the T cell related markers Lyt-1 (CD5) and Lyt-2 (CD8). These LAK cells were shown to kill in a wide range of tumor cell lines in a calcium dependent fashion. In an effort to identify the molecules involved in killing by LAK cells we showed by immune blotting and in functional assays the presence of PFP/perforin and of a Tumor Necrosis Factor (TNF)-related protein. On reducing SDS-gels, PFP/perforin of LAK cells migrated with an apparent molecular mass of 70 KDa while the TNF-like protein had a Mr of 50-60 KDa. By immunofluorescence, both proteins were shown to be localized in the cytoplasmic granules of LAK cells. The role of each one of these factors in LAK cell-mediated killing is currently being assessed.