MICROBIOLOGY AND MOLECULAR BIOLOGY

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ABSTRACTS

The Characterization of Epitopes of a Protective Surface Antigen of the Metacyclic Stage of *Trypanosoma cruzi.* Len M. Archer and Donald G. Dusanic, Department of Life Sciences, Indiana State University, Terre Haute, Indiana 47809.—A monoclonal antibody (4D37) to a surface antigen of *Trypanosoma cruzi* was bound to an Affi-Gel 10 (Bio-Rad) column and used to isolate the surface antigen (4D37 Ag) from crude extracts of *T. cruzi* epimastigotes. Eluates of 4D37 Ag were adjusted to pH 7.0, concentrated, dialyzed and used to immunize BALB/c mice. Spleen cells of the immunized mice were removed asceptically and fused with SP2/0-Ag 14 mouse myeloma cells. Forty-six percent of the wells produced hybridoma colonies of which 6 were positive when screened against crude *T. cruzi* epimastigote antigen by the ELISA. The hybridoma cells were cloned and characterized to determine antibody class and subclass. Epitope mapping studies are presently in progress. Funds for the work were provided by a research grant from the Indiana Academy of Science and NIH Grant AI 14642.

Construction of a Hybrid Plasmid Capable of Transforming Both *Escherichia coli* and *Cyanobacterium, Aphanocapsa* 6803. In Soo Bae and Carolyn N. Vann, Ball State University, Muncie, Indiana 47306.—Cyanobacteria provide an ideal system for studying both the structure and function of photosynthesis. Cyanobacteria carry out oxygenic photosynthesis just as eukaryotic chloroplast do and its simple structure allows for easy gene manipulation. *Aphanocapsa* 6803 is particularly interesting, for it is capable of not only autotrophic growth in light, but also heterotrophic growth on glucose, thus photosynthesis mutations that may be created will not be lethal. A hybrid vector which has a strong temperature sensitive promotor and is capable of controlled gene expression is being constructed. The gene encoding chlorophenicol acetyl-transferase will be inserted into one of the multiple cloning sites of the hybrid vector and the regulated expression of the enzyme will be quantified. Particular proteins involved in the assembly or function of the photosynthetic apparatus may be cloned into this vector to study the effects of site-specific mutagenesis.

Characterization of a Cytochrome P450 Deficient Mutant of *Candida albicans.* M. Bard, N.D. Lees, F.W. Kleinhans, R. Barbuch and D. Sanglard, Departments of Biology and Physics, IUPUI, Indianapolis, Indiana 46223; Merrell Dow Phar-

maceuticals, Inc., Indianapolis, Indiana 46268 and Department of Microbiology and Molecular Genetics, University of Cincinnati, Cincinnati, Ohio 45267.—A previously described *Candida albicans* nystatin resistant mutant blocked in 14α-demethylation of lanosterol was shown to also lack all traces of cytochrome P450 as determined by carbon monoxide difference spectra. This strain does not require ergosterol for growth and reverted to an ergosterol producing cytochrome P450 containing strain indicating no other lesions. The results of electron spin resonance studies indicated that the P450 lesion results in a cytoplasmic membrane that is more rigid than that of the wild type strain. Cytochrome P450 mutants described in *Saccharomyces cerevisiae* are auxotrophic for ergosterol or contain a second mutation in 5,6 desaturation of the sterol B ring. These results suggest that a cytochrome P450 lesion in these yeasts have different phenotypes and may reflect different sterol requirements for the two organisms.

Cloning of the Yeast Sterolmethyltransferase Gene. D.M. Copple, M. Bard and R.F. Gaber, Department of Biology, IUPUI, Indianapolis, Indiana 46223; and Department of Biochemistry, Molecular and Cell Biology, Northwestern University, Evanston, Illinois 60201.—Ergosterol functions as the major membrane sterol in yeast, and also provides a "sparking" function in the cell division cycle, acting as a hormone. In the ergosterol biosynthesis pathway, the methylation of zymosterol by sterol methyl transferase (SMT) is a step unique to yeast. The yeast strain used to clone the SMT gene has a mutation in the SMT gene, but may be "leaky", producing enough SMT gene product to provide the sparking function. By using gene disruption and replacement techniques, any "leakiness" of the gene will be ruled out. Five independent clones containing the SMT gene have been obtained. A restriction map of one clone will be presented. Should this methylation step prove to be required, an inhibitor of SMT could possibly serve as a fungicide.

A Mycological Assessment of Commercial Hot Tubs in Delaware and Madison Counties, Indiana. larry T. Crump, Donald A. Hendrickson, Department of Biology, Ball State University, Muncie, Indiana 47306.—A mycological analysis of several commercial hot tubs in Delaware and Madison Counties was conducted between January and April 1987. This assessment was designed to determine if a statistical relationship existed between the presence of pathogenic fungi isolated from these facilities and coliform or fluorescent pseudomonad bacteria that might be isolated from the same recreational pools. A comparison also was made between various physical parameters and the presence of fungi. Those parameters monitored included pH, temperature and residual chlorine levels. Additionally, bather loads at each facility were recorded for further comparison. Samples were taken from tubs used exclusively by males and females and, in one instance, from a facility used by both sexes concurrently. Male and female patrons used two of the unisex facilities on an alternating schedule. Samples from these tubs were taken in accordance with this schedule. A number of common fungal contaminants were isolated, as were several medically important species, including *Epidermophyton floccosum, Trichophyton rubrum, T. mentagrophytes, Candida tropicalis* and *Phialophora jeanselmei.*

Effects of Ribavirin on Rat Kidney Cells Transformed by Temperature-sensitive Mutants of Rous Sarcoma Virus. K.K. Hoopengardner, G.J. Merkel and Y.C. Chen, Department of Biological Sciences, Indiana University-Purdue University at Fort Wayne, Fort Wayne, Indiana 46805-1499.—The effects of ribavirin (1-B-D-Ribofuranosyl-1,2,4,-Triazole-3-Carboxamide), a synthetic nucleoside, on a series of morphological and growth characteristics of transformed state was studied, using rat kidney cells trans-

formed by temperature-sensitive mutants of Rous sarcoma virus. In the presence of ribavirin at permissive temperature (33°C), cellular morphology changed from transformed phenotype to normal phenotype. This was indicated by reduction in saturation density, decreased colony formation in soft agar, and reduced hexose uptake in less dense cultures. In addition, we have also demonstrated that vimentin, an intermediate filaments protein of the cytoskeleton, is concentrated at peripheral surface of the cell membrane at the permissive temperature (33°C), whereas at nonpermissive temperature (38°C) it appears to be evenly distributed throughout the cell cytoplasm. Ribavirin did not appear to have an effect on vimentin distribution at 38°C but may have caused a more diffuse pattern at 33°C. Currently research is underway in our laboratory to attempt to quantitate the vimentin in transformed and nontransformed cells.

Analysis of Cowpea Mottle Virus RNA. JONG W. KIM, GOPI PODILA AND ROBERT F. BOZARTH. Department of Life Sciences, Indiana State University, Terre Haute, Indiana 47809.—Cowpea Mottle Virus (CMeV) is an isometric plant virus. It has a genomic ssRNA of ($^+$) polarity with mol. wt. of 1.4 x 10° daltons, and one capsid protein with a mol. wt. of 44 K daltons. Three dsRNA segments were obtained from CMeV-infected cowpea leaves. The CMeV, ssRNA and dsRNA were translated *in vitro* in a messenger dependent reticulocyte lysate. Maximum incorporation of ^{35}S-methionine into proteins was obtained at 1.15 mM Mg^{++}, 50.7 mK K^+, and 1 ug of CMeV RNA when incubated 30 min at 32°C. Four major proteins with mol. wts. of 50, 42, 27 and 14 K were produced. The 42 K protein was selectively immunoprecipitated with CMeV antiserum. Comparative peptide mapping confirmed that the 42 K protein is CMeV capsid protein. The function of other products is unknown.

Comparison of Viruses from Healthy and Diseased Mushrooms. YOUNG H. KIM AND ROBERT F. BOZARTH, Department of Life Sciences, Indiana State University, Terre Haute, Indiana 47809.—Virus was extracted from healthy mushrooms and mushrooms of the same clone (M8) showing symptoms of the La France disease. Preprations made at pH 6.0 or 7.0 gave higher virus yields than those made at pH 8.0. Both preparations had 30 nm virus particles. Analysis of the capsid polypeptides of SDS-gels showed that the virus capsids were distinct. Virus extracts from the La France diseased samples contained nine distinct bands of dsRNA on 1.0% agarose gels which ranged from 3.5 to 0.8 kbp, whereas extracts from the VLPs of healthy mushrooms had a single band of 2.3 kbp. This band did not hybridize with the bands from the La France virus. This data clearly shows that the virus found in healthy isolates of this clone is distinctly different from the virus found in diseased sporophores of the same clone.

The Phenotypic Rescue of an Interferon Sensitive Mutant of Mengovirus, *is*-1, by Mouse L-cell Lysates. ROBERT W. KING AND EDWARD SIMON, Department of Biological Sciences, Purdue University, West Lafayette, Indiana 47907.—Interferon is an efficient antiviral protein produced by cells in response to virus infection. Viruses differ in their response to interferon. Vesicular stomatitis virus and the enterovirus (rhinovirus, poliovirus and mengovirus) are quite sensitive to the antiviral state induced by interferon whereas herpes virus, vaccinia virus, and adenovirus are resistant. In 1976, Dr. Simon *et al.*, isolated a mutant mengovirus, *is*-1, that under the proper conditions is 100-1000 times more sensitive to the effects of interferon than the wild type, is^+. When interferon-treated mouse L-cells are coinfected with is^+ and *is*-1, is^+ has the ability to increase the yield of *is*-1 to normal titers. We have found that the virus-free cell lysates prepared from is^+ infected L-cells also rescued *is*-1 in

interferon-treated cells. Thus, *is*$^+$ must produce a diffusable agent that actively overcomes the antiviral state induced by interferon and rescues the *is*-1 phenotype.

Phospholipase Production in Morphological Variants of *Candida albicans*. Thomas Lane and J. R. Garcia, Department of Biology, Ball State University, Muncie, Indiana 47306.—The yeast *Candida albicans* is considered a dangerous opportunist in a compromised host. Its pathogenicity is thought to lie primarily in its ability to grow as budding yeasts or hyphae. However, this fact alone does not seem sufficient to account for its success as a pathogen. Recently, Dr. David Soll (Univ. of Iowa) described a spontaneously occurring, high-frequency switching system, in seven morphological variants which may help explain its success. We obtained some of these variants and tested them for their ability to produce extracellular phospholipase, a generally accepted mechanism of pathogenesis in many microorganisms. Our goal was to show a correlation between the amount of enzyme produced and the virulence of the variants. Using egg yolk agar plates, we showed that all variants produced the enzyme. However, one produced significantly more than the others. In all cases we insured that the variant phenotype was still expressed. Although preliminary in nature this suggests that the high-frequency switching system may modulate the virulence of the organism. Virulence tests are being conducted with Balb c mice.

Purification of Argininosuccinate Lyase (AL) by High Pressure Immuno-affinity Chromatography on an Anti-AL-silica Support. Larry R. Massom, Corinne E. Ulbright, Robert L. Shepard and Harry W. Jarrett, III, Department of Biology, Indiana University-Purdue University at Indianapolis, and the Veterans Administration Medical Center, Indianapolis, Indiana 46223.—Argininosuccinate lyase (AL) catalyses a vital step in the urea cycle but is difficult to purify for further study. To facilitate the purification, a monoclonal antibody directed against beef AL (Anti-AL) was isolated from lymphocyte culture media by affinity chromatography on a Protein A-silica column. The purified Anti-AL was then covalently coupled to 500 A pore, 5 μm beaded 3-glycidyloxypropyl-silica and packed in a 4.6 x 30 mm column. This column was used to isolate active AL from crude liver extracts in less than 15 minutes by high pressure (performance) immuno-affinity chromatography. An alternative purification was also carried out: crude liver extract was mixed with anti-AL and applied to the Protein A-silica column. Only the AL/Anti-AL complex was retained by the column and subsequently eluted.

The Use of a Hybrid Cloning Vector for Site Directed Mutagenesis of Cytochrome B559 in *Anacystis nidulans*. Betsy A. Read and Carolyn N. Vann, Ball State University, Muncie, Indiana 47306.—A method for engineering site specific mutagenesis in cyanobacterium *Anacystis nidulans* R2 SPc based on the use of hybrid cloning vector is being applied for the elucidation of the function of a specific PSII protein, cytochrome b559. The shuttle vector pROAN 1 has been constructed which contains an *Escherichia coli* origin of replication, a short portion of the small indigenous plasmid of *A. nidulans* and carries both the left and rightward promoters of lambda, in addition to the gene encoding the CI857 lambda temperature-sensitive repressor protein. During modification to increase the stability of pROAN 1, a specific and unique *Bam* HI cloning site has been created directly behind the lambda promoter. The promoter-less gene for cytochrome b559 has been cloned into pROAN 1 and regulated expression is being examined. Highly conserved sequences of b559 are being altered using oligonucleotide directed metagenesis and the structure and function of the newly constructed protein is being assessed.

The Effects of Liposome Encapsulated Ethidium Bromide on the *in vitro* Growth of *Trypanosoma musculi*. SRI S. SRISKANDA AND DONALD G. DUSANIC, Department of Life Sciences, Indiana State University, Terre Haute, Indiana 47809.—Liposomes are artificial lipid vesicles that could be made in the laboratory using relatively simple techniques. They are useful in encapsulating many chemical substances for delivery to specific cells. In the present study ethidium bromide (EtBr) was encapsulated in phosphatidyl choline (lecithin-egg yolk derived) vesicles, also containing phosphatidyl ethanolamine and cholesterol. These liposomes along with control liposomes containing phosphate buffered saline were incubated with blood stream form trypomastigotes growing *in vitro* in tissue culture medium at 37°C. The growth pattern of the trypomastigotes was monitored in order to evaluate the effectiveness of liposome-drug preparations *in vivo*. At a concentration of 0.045 mg/ml EtBr the encapsulated drug killed all the trypanosomes within 72 h compared to 120 h for the unencapsulated drug. These studies were supported in part by PHS Grant AI 14642.

Differential Effects of Anacardic Acid on *in vivo* Antibody Response, Concanavalin A and Lipopolysaccharide Mitogenesis. DIANA STORHOFF AND NANCY BEHFOROUZ, Ball State University, Muncie, Indiana 47306.—Anacardic acid, the primary component of cashew nut oil extracted from the fruit shell of the Anacardium occidentale tree, and/or its alkali salts possess antipyretic, bactericidal, vermicidal, anthelminthic, antiparasitic, larvicidal, antiprotozoic activities. This study illustrates the effects of anacardic acid on the mitogenic stimulation of murine T and B cell populations by Concanavalin A (Con A) and lipopolysaccharide (LPS), respectively, and the *in vivo* antibody response of murine lymphocytes. C57/black mice subcutaneously injected with anacardic acid for 16 days exhibited enhanced mitogenic stimulation of lymphocytes by both Con A and LPS. C57/black mice subcutaneously injected for 16 days with anacardic acid and stimulated by antigen on day 15 exhibited a dose related suppression of antibody plaque formation.

Inhibition of an *in vitro* Antibody Response by Cyclosporine A. DIANA STORHOFF AND NANCY BEHFOROUZ, Ball State University, Muncie, Indiana 47306.—Cyclosporine A (CsA) is a relatively new immunosuppressive drug which has novel, poorly understood, effects on the immune system. This study attempts to elucidate the effect Cyclosporine A has on the *in vitro* antibody response of mouse lymphocytes towards sheep red blood cells (SRBC). CsA was shown to inhibit antibody plaque formation of naive spleenocytes or T and B cell mixtures when added to cultures on day 0, 1 and 2 following antigenic stimulation but not on day 3. Lymphocytes taken from animals pre-stimulated with sub-immunogenic doses of SRBC could be inhibited completely by CsA only on day 0 of culture. IL-2 rich Concanavalin A stimulated rat spleen supernatants were not able to restore the ability of CsA inhibited lymphocytes to produce antibody to SRBC antigen.

Immunological Parameters in Cyclosporine Protected Balb/c Mice Infected with *Leishmania major*. WILLIAM WHITE AND NANCY BEHFOROUZ, Ball State University, Muncie, Indiana 47306.—Paradoxically, the highly susceptible Balb/c mouse strain can be made to become resistant to the parasite pathogen *Leishmania major* by prophylactic treatment with Cyclosporine A, an immunosuppressive undecapeptide. In order to better understand the apparent immuno-modulatory effect which Cyclosporine A has on the outcome of this infection in Balb/c mice, several immune parameters were followed during infection with *L. major* in animals either treated or not treated with the drug. Mitogen responsiveness, antigen-specific antibody levels and lymphokine

production was assessed at various time intervals during infection in both groups. Results indicate that overall the immune system is suppressed only minimally early after treatment but this minimal alteration apparently leads to a profound difference in disease outcome.