ANIMAL MODELS OF HUMAN VIRAL DISEASES: RELEVANCE TO DEVELOPMENTAL THERAPEUTICS

Organizers: John Blasecki, Catherine Laughlin, and John Mc Gowan

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Animal Models of Human Viral Diseases: Relevance to Developmental Therapeutics

Keynote Address

CM 001 ANTIVIRALS: HISTORICAL PERSPECTIVES, CURRENT STATUS AND FUTURE DIRECTIONS, George J. Galasso, National Institutes of Health, 9000 Rockville Pike, Bethesda, MD 20892

The history of antiviral agents research and development is a relatively brief one. The past twenty-five years have seen the major effort in this area. Initially, antiviral agents were a fortuitous outcome of cancer chemotherapy programs. All the agents currently approved by the Food and Drug Administration are a result of screening programs. We are currently at the stage where we can truly pursue the rational approach. There is sufficient information resulting from basic research on viruses to identify ideal targets of viral replication for which specific inhibitors can be developed. A key factor in the development of new agents is the identification and utilization of proper animal models. In vitro systems are not capable of predicting the information needed before a clinical study can be undertaken. Further research is also needed in rapid viral diagnosis and drug delivery to enhance the capability of antiviral agents. This address is intended to provide the background and the perspective for the remainder of the meeting.

Herpes Simplex

CM 002 THE GUINEA PIG MODEL OF GENITAL HERPES, Stanberry L.R., Children's Hospital Research Foundation, University of Cincinnati College of Medicine, Cincinnati, OH, 45229.

Similar to humans, the intravaginal inoculation of guinea pigs with herpes simplex virus (HSV) results in a self-limited infection characterized by genital skin disease (vulvovaginitis) and neurologic and urologic complications. The clinical course of primary infection can be quantified using a skin lesion scoring sytem while the virologic course of infection can be determined by plaque titration of vaginal swab specimens. Experiments examining the pathogenesis of infection have demonstrated that virus is spread from the genital tract to distant sites including sensory ganglia via neural routes. Recovery from primary infection is associated with the establishment of a latent infection in dorsal root ganglia. As with humans, latently infected animals experience both spontaneous and ultraviolet radiation induced recurrent genital infections. Recurrences may be clinically apparent or may be manifested as cervicovaginal HSV shedding. Previous studies have reported that the incidence and severity of both symptomatic recurrences and subclinical viral shedding can be reduced by antiviral therapy.

Studies exploring the ontogeny of immune responses to genital infection has been facilitated by the size of the guinea pig, which is sufficient to permit repetitive collection of large volumes of blood. Investigators have shown that HSV infected guinea pigs, like humans, produce humoral, cytokine and cell-mediated immune responses. The guinea pig has been used to explore the effect of antiviral treatment on the development of host responses to primary genital HSV infection. Further, this model system has been useful in exploring the efficacy and mechanism of action of immune modulators in the treatment of genital herpes.

Because primary, latent and recurrent HSV infections are all observed in the guinea pig, this model has proven useful in exploring the pathophysiology and immunobiology of genital HSV infection. Because genital herpes in the guinea pig is well characterized and quantifiable, this model is ideally suited for examining the efficacy of both putative antiviral drugs and experimental viral vaccines.

Animal Models of Human Viral Diseases: Relevance to Developmental Therapeutics

CM 003 THE RABBIT MODEL FOR THE STUDY OF HERPES ENCEPHALITIS,
William G. Stroop, Veterans Administration Medical Center, Departments of Neurology and Pathology, University of Utah Medical School, Salt Lake City, UT 84148.

Although herpes simplex virus type 1 (HSV-1) is the leading cause of fatal sporadic encephalitis of human adults, the pathogenesis of this disease is unclear. Based on clinical, serologic, and epidemiologic evidence, two theories have been proposed to explain the pathogenesis of HSV-1-induced encephalitis (HSV-E) in the human adult: exogenous reinfection and reactivation of a latent brain infection. We have developed rabbit models of acute and reactivated HSV-1 infections in an effort to not only to test these theories, but to also understand the degree to which the neurovirulence of the infecting strain of HSV-1 affects the outcome of the disease process. We have studied the pathogenesis of the +GC, H129, -GC, KOS and F strains of HSV-1 during acute, latent and reactivated infections using clinical, electrophysiologic, virologic, histologic, immunohistochemical and electron microscopic techniques. We have found that infection by the nasal route appears fundamental to the development of encephalitis, and that the outcome of infection is dependent on the relative neurovirulence possessed by a given HSV-1 strain. Following intranasal inoculation, H129 and +GC are highly virulent, whereas the KOS and -GC strains are completely non-virulent; the F strain displays intermediate virulence. +GC is capable of inducing severe clinical and electroencephalographic (EEG) seizures and death in a high proportion of acutely infected animals. The virulence of +GC appears related to its ability to replicate in specific cortical areas of the brain. Although not highly virulent during acute infections, H129 can be reactivated from latency to produce focal temporal lobe encephalitis accompanied by worsening EEG changes similar in many respects to HSV-E as seen in the human adult. The renewed replication of H129 as it reactivated from latency was documented using *in situ* hybridization and electron microscopic techniques. Although neuroinvasive, the nonvirulent F, -GC, and KOS strains either infected different brain centers than the neurovirulent H129 and +GC strains, or were rapidly cleared from the brain by the immune system. Latent KOS, -GC, and F strain infections could not be reactivated to produce focal encephalitis. Together these results indicate that some strains of HSV-1 (such as H129) can produce temporal lobe encephalitis as a consequence of a reactivation stimulus, whereas other strains cannot. To test whether host immunity and the establishment of preexisting latency could be overcome by exogenous re-infection, groups of rabbits were intranasally infected with the low virulent KOS, -GC and F strains and then superinfected by the nasal route with the virulent +GC or H129 strains. In most cases, previous infection afforded nearly complete protection against the development of clinical signs indicative of encephalitis. This suggests that exogenous reinfection is an unlikely mechanism for the development of HSV-E.

CM 004 THERAPY OF HERPES SIMPLEX VIRUS INFECTIONS OF HUMANS, Richard J. Whitley,
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Herpes simplex virus infections of humans result in a broad spectrum of disease. Over the past decade notable therapeutic successes have been achieved for the management of humans with acute and chronic mucocutaneous herpes simplex virus infections. While progress has been made in the treatment of diseases such as herpes simplex encephalitis and neonatal herpes simplex virus infections, obvious areas for improvement are apparent. The unique pathogenesis of herpes simplex virus infections of the nervous system in newborns warrants particular attention. Virus load, route of spread (bloodstream versus neuronal) immaturity of host defenses, lack of transplacental maternal antibody, among other factors, account for the propensity of severe disease in the newborn. Following neuronal spread of virus to the brain, the agent replicates focally in the brain before spreading to other targeted areas of the brain. Future therapeutic studies will have to take into consideration the necessity for drug delivery to targeted anatomic sites, therapeutics which are active at newly identified molecular targets, and compounds which have a greater propensity to penetrate the central nervous system. These issues will be discussed in detail.

Other Herpesviruses

CM 005 THE GUINEA PIG AS A MODEL OF THE PATHOGENESIS OF VARICELLA-ZOSTER VIRUS INFECTION AND OF THE HOST RESPONSE TO VIRAL PROTEINS. Ann M. Arvin, Celine M. Koropchak, Brandi Watson, and Suzanne Solem, Infectious Diseases Division, Department of Pediatrics, Stanford University School of Medicine, Stanford, CA 94305.

The study of the pathogenesis of varicella-zoster virus (VZV) infection has been complicated by the fact that VZV produces its typical cutaneous manifestations only in the human host and that other stages of VZV pathogenesis have been difficult to document even in the natural host. Nevertheless, since alternatives for animal models of VZV infection are limited, the characteristics of VZV replication have been examined in the guinea pig (1) and an immune response to VZV proteins has been documented (2,3). Our studies of primary VZV infection in guinea pigs have focussed upon VZV viremia as a marker of particular relevance in VZV pathogenesis because viremia is a critical event during the initial infection in human subjects. Peripheral blood mononuclear cells (PBMC) harboring viral gene sequences were detected by in situ hybridization in strain 2 guinea pigs tested at 3 and 5 days after inoculation with guinea pig cell- adapted VZV; the VZV DNA probe used for in situ hybridization was made from HindIII A,B,C and D fragments (4). The frequency of VZV-infected PBMC was 0.001-0.002% which was comparable to that observed in human PBMC from subjects with acute varicella. The detection of VZV viremia and its use as a biological marker to assess the efficacy of immunization strategies or antiviral drugs in protecting against viral challenge has now been accomplished using the polymerase chain reaction (PCR). Oligonucleotide primers corresponding to sequences within the VZV glycoprotein II gene were used to produce an amplified product of 249 bp. Viremia was documented by PCR testing of PBMC from Hartley as well as strain 2 guinea pigs taken 3 days after VZV inoculation. With respect to the immune response, our studies showed that both humoral and cell-mediated immunity were elicited by inoculating strain 2 and Hartley guinea pigs with immunoaffinity purified preparations of VZV glycoprotein I (gp I) and p170; p170 is a nonglycosylated phosphoprotein that we have now identified as the immediate early (IE) protein of VZV, coded for by gene 62. Assessing the immune response to this IE protein has also provided an additional method for investigating VZV replication in the guinea pig model. The production of antibodies to the IE protein, as indirect evidence of viral replication, can be detected using immunoblots prepared with purified IE protein as antigen. With this approach, immunization with VZV gp I, as expressed by a vaccinia recombinant (kindly provided by Drs. I. Hay, W. Ruchyean and P. Kinchington, Bethesda, MD), inhibited VZV replication to the extent that no antibodies were produced against the IE protein.

1. Myers MG, Stanbury LR, Edmond BJ. J Infect Dis 151:106, 1985.
2. Arvin AM, Solem S, Koropchak CM et al. J Gen Virol 68:2449, 1987.
3. Grose C and Friedrichs WE. Virology 118:86, 1982.
4. Koropchak CM, Solem S, Diaz PS, Arvin AM. J Virol 63:2392, 1989.

CM 006 EXPERIMENTAL GUINEA PIG MODELS FOR CYTOMEGALOVIRUS INFECTIONS
Brigitte P. Griffith, Department of Laboratory Medicine, Yale University School of Medicine, New Haven, CT 06510, and Virology Laboratory, Veterans Administration Medical Center, West Haven, CT 06516.

The species specificity of human cytomegalovirus (CMV) prevents the study of this virus in animals and necessitates the use of homologous animal models that mimic the human infection. The similarities in the pathogenicity of human CMV and guinea pig CMV (GPCMV) in their respective hosts are striking. A mononucleosis syndrome in the healthy adult, disseminated CMV infection in the immunocompromised host, and transmission by blood transfusion have been described for both human CMV and GPCMV. Congenital CMV infections occur in both humans and guinea pigs but do not occur in the well defined mouse model of CMV infection. Recent studies on the transplacental transfer of GPCMV have demonstrated that (1) the placenta serves as a reservoir for GPCMV and as a source of virus throughout pregnancy; (2) the placenta acts to limit transmission of GPCMV to the fetus; (3) primary and secondary maternal viremia play a pivotal role in transplacental GPCMV transfer. Evaluation of CMV infection in the central nervous system and ear have recently underlined additional similarities between human CMV and GPCMV infections. Knowledge of the molecular biology of GPCMV has increased in the last few years, and studies of GPCMV gene expression have begun. Furthermore, much needed molecular reagents and monoclonal antibodies are becoming available for use in the GPCMV system. The GPCMV model of self limited mononucleosis has been used for in vivo antiviral testing of a number of antiviral agents; to date, only a few of these compounds have shown efficacy in GPCMV infected guinea pigs. A model of GPCMV infection in immunocompromised guinea pigs, developed in the last 2 years, has more direct clinical relevance for anti-CMV therapy. The usefulness of guinea pig models of GPCMV infections in the development of antiviral therapies is increasing as more is learned about the molecular biology of the virus and the mechanisms controlling the establishment of acute and latent GPCMV infections.

Animal Models of Human Viral Diseases: Relevance to Developmental Therapeutics

CM 007 MURINE MODELS FOR HERPES SIMPLEX VIRUS ENCEPHALITIS AND NEONATAL HERPES, Earl R. Kern, Department of Pediatrics, School of Medicine, University of Alabama at Birmingham, Birmingham, AL 35294

Mice inoculated intranasally with herpes simplex virus type 1 (HSV-1) have been used extensively in our laboratory for evaluation of antiviral agents directed against HSV encephalitis. After HSV-1 inoculation the virus travels from the nasopharynx via olfactory and trigeminal nerve tracts to the brain resulting in death from an acute encephalitis. In this model, treatment with vidarabine (ara-A) initiated within 24-48h after infection resulted in significant protection and correlated with alteration of viral replication in the central nervous system. Treatment with Acyclovir (ACV) was considerably more effective than ara-A in preventing mortality and could be initiated as late as 72h after infection. In most target organs ACV was superior to ara-A in reducing the magnitude of viral replication and promoting a more rapid clearance of HSV-1 in these tissues. ACV therapy was also more effective than ara-A in reducing viral replication in the lung. A new antiviral agent, HPMPC, was also more effective than ara-A and was slightly more active than ACV in protection against mortality and alteration of viral replication in target organs.

Mice inoculated intranasally with HSV-2 provides a model for disseminated neonatal herpes. After viral inoculation, replication is detected initially in lung with subsequent dissemination to other visceral organs. Virus is also transmitted concomitantly by neural routes from the nasopharnyx to olfactory lobe, cerebrum, and cerebellum-brain stem. This animal model provides a severe test of antiviral efficacy and treatment with ara-A failed to provide protection against mortality, however, there was some alteration of virus replication in brain, lung, liver and spleen. In contrast, treatment with ACV was very effective in reducing mortality when therapy was initiated as late as 48-96h after infection and also significantly reduced viral replication in target organs. In this model infection, treatment with HPMPC was much more effective than ara-A and somewhat more active than ACV in preventing mortality. In pathogenesis studies, therapy with HPMPC was superior to ACV in altering virus replication in lung, liver, olfactory lobe, cerebrum, cerebellum and diencephalon. These results indicate that these two model infections have had predictive value for treatment of encephalitis and neonatal herpes in humans and are good models for establishing efficacy of new antiviral coupounds.

CM 008 OCULAR VARICELLA ZOSTER VIRUS INFECTION IN THE RABBIT AFTER INTRASTROMAL AND INTRANASAL INOCULATION, D. Pavan-Langston, M. L. Siegel, and E. C. Dunkel, Molecular Virology, Eye Research Institute and Department of Ophthalmology, Harvard Medical School, Boston, MA 02114.

Animal models of varicella zoster virus (VZV) infections have been difficult to establish and characterize. Ocular infection with VZV (either primary or recurrent) results in dissemination of the virus in the orbit and development of severe corneal stromal and extraocular reactions [1]. The current study describes a rabbit model of human VZV infection that induces both ocular VZV lesions and systemic viral dissemination.

Human VZV was grown on MRC5 cell monolayers, harvested by scraping and sonicated. Cell-free VZV was inoculated either by corneal intrastromal injection (50µl in 20 rabbits) or by intranasal injection (100µl in 20 rabbits). VZV inoculated animals were evaluated daily by slit lamp biomicroscopy and by tear film and nasal mucosal swab virus recovery on days 3, 5, 7, and 9 post inoculation. The spread of the VZV infection to other ocular, neural and systemic sites was evaluated by whole-cell virus recovery and by slot blot and in situ VZV hybridization analysis at sacrifice (day 10, 20 or 45 post inoculation).

Results demonstrated that in corneal intrastromal injected animals, VZV clinical disease (microdendrites and superficial punctate keratitis) developed by day 3-6 PI. Clinically evident disease resolved by day 12-14 and VZV was recovered from tear film cultures on days 3, 5 and 7 PI. In intranasal inoculated animals, VZV-induced disease was slower to develop with peak ocular and nasal mucosal involvement demonstrated on day 7-10 PI. VZV was recovered from these inoculated animals on days 5, 7, and 9 PI. VZV was disseminated to other ocular (iris and retinas), neural (trigeminal ganglia and midbrain) and organ systems (lung, kidney and liver) by both inoculation routes. VZV hybridization results demonstrated persistent ocular colonization as well as detection of the genome in both neuronal and glial cells. Continued use of these models of VZV ocular and systemic infection will allow for complete characterization of VZV gene expression and virus-host cell interactions in both neuronal and extraneuronal sites of infection.

1. Pavan-Langston D, Dunkel EC. Ocular Varicella-Zoster Virus Infection in the Guinea Pig. Arch Ophthalmol 107:1068-1072, 1989.

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CM 009 SIMIAN VARICELLA VIRUS INFECTION OF AFRICAN GREEN MONKEYS AS A MODEL FOR SEVERE DISSEMINATED VZV INFECTION, Soike, K. F., Delta Regional Primate Research Center, Tulane University, Covington, LA 70433.

Simian varicella virus (SVV) is a nonhuman primate herpesvirus which shares antigens and partial genomic homology with human varicella zoster virus (VZV). Induced infection of African green monkeys has been employed as an animal model for evaluation of antiviral drugs and has been shown to be predictive of activity against human VZV. Intratracheal inoculation of African green monkeys with SVV results in systemic disease where severity of rash, severity of viremia, antibody development and death can be used as parameters to determine efficacy of potential antiviral drugs. Infection of a primate species has served to evaluate the clinical efficacy of several recombinant human cytokines which due to species specificity are minimally effective or ineffective in lower animal species. It has been shown that different cytokines have different influences upon the clinical course of this disease. Alpha, beta, and gamma recombinant human interferons have been shown to inhibit the course of SVV in African green monkeys with varying levels of effectiveness. On the other hand, tumor necrosis factor and granulocyte macrophage-colony stimulating factor have been shown to have an adverse effect upon this infection resulting in high titered viremia, severe systemic disease and death. Studies to determine the mechanisms whereby these differences occur are in progress.

CM 010 CYTOMEGALOVIRUS (CMV) AND VARICELLA-ZOSTER VIRUS (VZV) INFECTIONS: A CLINICAL OVERVIEW, Stephen E. Straus, Medical Virology Section, LCI/NIAID, NIH, Bethesda, MD 20892.

CMV and VZV are common human pathogens, but they do not readily infect or cause illness in laboratory animals. As will be demonstrated in this session, selected animal models of these infections have proven to be of great heuristic value, but much of what is known about CMV and VZV-induced disorders has derived from careful studies of humans.

CMV infects about 50% of adult Americans. The rate is much higher in day-care settings and in the immune-compromised, particularly those with AIDS. In the normal host most infections are subclinical, others appear as hepatitis or mononucleosis-like illnesses. Congenital infections occur in about 1% of births. Some are associated with major hematologic, ocular, and neurologic complications. Transplant recipients and AIDS patients experience profound CMV disease involving the lungs, eyes, liver, or brain. Strategies for preventing CMV infection include careful selection of donor blood and tissues, or CMV immune globulin. Ganciclovir arrests progression of CMV retinitis in AIDS patients.

Chickenpox occurs in over 80% of children and some adults. Severe infection of the lung, brain, liver, etc., occurs in immune-compromised patients. Reactivation infections (zoster) are particularly common in the elderly and in the immune-compromised. Major complications are pain and dissemination to the brain and the viscera. Contact isolation, Varicella-zoster immune globulin, and vaccine are effective strategies for prevention of varicella. Acyclovir is the treatment of choice for patients at risk of complicated infections.

Animal Models of Human Viral Diseases: Relevance to Developmental Therapeutics

Hepatitis Virus

CM 011 HOST CONTROL OF HBV GENE PRODUCT LEVELS IN THE CHRONIC SURFACE ANTIGENEMIC MOUSE MODEL. Robert D. Burk, Depts. of Pediatrics and Microbiology & Immunology and The Marion Bessin Liver Research Center, A. Einstein College of Medicine, Bronx, N.Y. 10461

To investigate the genetic basis and molecular mechanisms of hepatitis B virus liver specificity, genetic regulation, and association with pathologic conditions we have constructed HBV transgenic mice. Our underlying hypothesis is that the HBV genome has evolved mechanisms of gene expression similar to those utilized by native hepatic genes as an adaptation for an efficient symbiotic relationship within host liver cells. It appears that this persistent relationship within the host hepatocyte can, under certain circumstances, lead to the development of pathologic conditions by as yet undetermined mechanisms. We have used this model to test a number of hypotheses concerning the role of host factors on the regulation of HBV gene expression.

Human response to hepatitis B virus (HBV) infection is variable. For example, various ethnic/racial populations have different levels of chronic infection. Moreover, there are differences in HBeAg positivity and circulating viral DNA levels in HBsAg positive individuals from different populations. Delineation of the genetic factors influencing HBV infection in man presents a major challenge for a variety of reasons including the genetic heterogeneity of different individuals even in a given population. To overcome these difficulties, we have developed inbred lines of congenic HBV transgenic mice for the investigation of potential host genes influencing HBV gene expression.

The G26 HBV transgenic locus was bred onto a C57BL/6 (B6) background by repeated backcrossing to inbred B6 mice for approximately 10 generations. The resultant "congenic" B6 transgenic mice were then crossed with a spectrum of different strains of inbred mice. Transgenic heterozygous mice were identified and bled at 2, 3, and 6 months. Serum HBsAg levels were quantitated using the AUSRIA II kit (Abbott Labs). The following results were obtained (ug/ml):

MOUSE CROSS	(N)	MALE	(N)	FEMALE
B6/B6^{HBV+}	(7)	26.8 ± 3.3	(8)	13.6 ± 3.4
C3H/B6^{HBV+}	(3)	26.1 ± 1.4	(3)	10.7 ± 1.5
Balb/c/B6^{HBV+}	(10)	30.4 ± 4.9	(12)	11.3 ± 4.3
CD-1/B6^{HBV+}	(8)	33.3 ± 4.9	(4)	14.0 ± 4.3
DBA/B6^{HBV+}	(4)	65.4 ± 11.2	(10)	42.1 ± 6.2

DBA/B6 transgenic mice were backcrossed to B6 and DBA inbred mice to investigate the genetic basis of the elevated HBsAg levels. Segregation analyses of these crosses suggests the presence of two independently assorting dominant alleles within the DBA mouse genome. To confirm the ability of DBA alleles to influence serum HBsAg levels, "congenic" B6 mice with the G7 HBV transgene were mated with DBA mice. G7-B6/DBA transgenics had 4 fold higher levels of HBsAg than the G7-B6/B6 transgenics. These studies are consistent with the presence of at least two murine loci (Srl 1 & 2) influencing the serum level of HBsAg in transgenic mice. The presence of similar genes in man and their potential influence on the biology of HBV infection awaits further investigation.

CM 013 THE WOODCHUCK MODEL OF EXPERIMENTAL HEPADNAVIRUS INFECTION AND DISEASE: APPLICATION TO ANTIVIRAL THERAPY.

Brent E. Korba[1], Paul J. Cote[1], Bud C. Tennant[2] and John L. Gerin[1]. [1]Georgetown University, Division of Molecular Virology and Immunology, Rockville, MD 20852 and [2]College of Veterinary Medicine, Cornell University, Ithaca, NY 14853.

The Woodchuck hepatitis virus (WHV) and its natural host, the Eastern Woodchuck (*M. monax*) constitute the relevant animal model system for the study of HBV-induced disease in man, especially chronic viral infection hepatocellular carcinoma (HCC) and lymphatic viral infection. Experimental inoculation of colony-bred neonatal woodchucks with standardized virus challenge pools results in uniform kinetics of WHV infection and predictably high rates (60-70%) of chronic viral infections. Based upon predictable responses to WHV infection, we have conducted studies to investigate the pathogenic mechanisms of hepadnaviral-induced disease and the influence of various antiviral compounds on the progress of virus infection and disease. These compounds include nucleoside analogs (adenine arabinoside monophosphate, erythromycin-9 {o-methyl} oxcine, 2'-fluoro-5-ethyl-1-β-D arabinofuranosyluracil) and immune modifiers (thymosin alpha-1, interleukin-2, WHsAg vaccine). Woodchucks were treated at various times (1 to 18 months) following the onset of serologic patterns indicative of chronic WHV infection. Depending upon the specific agent employed, both short (7-10 days) and long term (4-12 weeks) treatment protocols were utilized. In general, the nucleoside analogs examined in these studies produced marked (up to 10,000-fold), but transient, depressions in the levels of circulating WHV virions which returned to pretreatment levels after the cessation of drug administration. The results of the therapeutic application of several antiviral agents will be presented based upon (i) the analysis of serologic markers of WHV infection (e.g. WHV DNA, WHsAg, anti-WHs antibodies) and liver disease (e.g. ALT, SDH), (ii) clinical status of the animals (body weights, hematology, blood chemistries), and (iii) the state of WHV DNA replication, RNA transcription and virus-induced disease in liver tissues.

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CM 014 DUCK AND GROUND SQUIRREL MODELS OF HBV, Patricia L. Marion, Division of Infectious Diseases, Department of Medicine, Stanford University School of Medicine, Stanford, CA 94305.

Duck hepatitis B virus (DHBV) is an HBV-related virus that infects most breeds of domestic ducks. Because of the relative ease of obtaining DHBV-infected tissues and of producing viremic animals following experimental injection, this model of HBV has played a key role in studies elucidating the steps of hepadnaviral replication. Although the genome of DHBV is the most divergent from the HBV genome of the known animal hepadnaviruses, there are no known differences in the steps of viral replication. Chronic DHBV infection can be reliably induced in ducklings by in ovo or post hatch injection of virus or by vertical transmission through eggs laid by infected dams. Antiviral substances can be tested for efficacy either in virus-infected ducklings or in primary hepatocyte cultures prepared from infected ducklings or infected in vitro. Mild to severe hepatic inflammation is associated with the presence of viremia in ducklings infected experimentally, while no hepatitis is observed in congenitally-infected ducks. The level of hepatitis in response to DHBV infection is variable, depending upon the inoculum and the individual host. Development of hepatocellular carcinoma has not been observed in congenitally-infected ducks, and only sporadically in experimentally-infected animals. Therefore, in contrast to the mammalian hepadnavirus models, there is still no firm association of DHBV with primary liver cancer.

Ground squirrel hepatitis virus (GSHV) is very similar genomically, morphologically and antigenically to woodchuck hepatitis virus (WHV). GSHV infects its natural host, Beechey ground squirrels, along with woodchucks and chipmunks, other members of the squirrel family. GSHV has played an important role in studies of viral replication and in defining essential characteristics of the hepadnavirus family. GSHV has a lower level of hepatitis associated with chronic infection than WHV, and a slower rate of development of primary liver carcinoma. The ground squirrel model is as convenient as the woodchuck model for in vivo study of the effect of antivirals, but has been less developed due to the lack of a breeding facility.

CM 015 PRIMATE MODELS FOR HEPATITIS A AND B (AND C AND D AND E), Robert H. Purcell, Head, Hepatitis Viruses Section, Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892

Animals, and especially primates, continue to play an important role in research on the human hepatitis viruses. Although hepatitis A virus (HAV) has been recovered and serially passaged in cell culture, none of the other hepatitis viruses (hepatitis B virus, HBV; blood borne non-A non-B or hepatitis C virus, HCV; hepatitis delta virus, HDV; enterically transmitted non-A non-B or hepatitis E virus, HEV) can be routinely isolated or propagated in cell culture except by transfection. In most cases, primates serve as the only means of biological amplification of the virus outside of its natural human host. Species found to be the most suitable for such studies include, for HAV, chimpanzee, certain marmoset species, owl monkey and, possibly, cynomolgus monkey; for HBV, chimpanzee and gibbon; for HCV, chimpanzee and possibly marmoset; for HDV, HBV-infected chimpanzee; for HEV, chimpanzee, cynomolgus (and possibly other macaque monkeys) and marmoset.

The use of animals, and especially primates, in medical and biological research has been strongly opposed by animal rights groups. Frequently changing regulations for the use of animals in research reflect pressures brought to bear on politicians and regulatory agencies. As a result of these changes, plus the need for adequate biohazard containment and housing for the primates, the cost of such research is very high.

Nevertheless, primates, and especially chimpanzees, remain the best (and in some cases the only) models for studying the pathogenesis, immune response, prophylaxis and therapy for human hepatitis. Such research has led to the discovery of HCV and HDV, new insights into the pathogenesis of HAV, HBV, HCV and HDV, and the development of vaccines for HAV and HBV.

Animal Models of Human Viral Diseases: Relevance to Developmental Therapeutics

AIDS-I

CM 016 SIV INFECTION OF RHESUS MACAQUES: A MODEL FOR VACCINE DEVELOPMENT, Murray B. Gardner[1], Niels C. Pedersen[2], Paul A. Luciw[1], Suganto Sutjipto[3], Marta Marthas[2] and Preston A. Marx[3]. [1]University of California, Davis, Department of Medical Pathology, [2]University of California, Davis, Department of Veterinary Medicine, [3]California Primate Research Center.

SIV infection of rhesus macaques provides a model with many parallels to human AIDS and is an excellent system to test various vaccine strategies. Using a psoralen-UV light inactivated whole SIVmac vaccine with the adjuvant threonyl muramyl dipeptide we attempted to protect rhesus monkeys against challenge infection with 100-1000 animal infectious doses of virulent SIV given intravenously (IV) and 1-4 animal mucosal infectious doses by the genital routes. The monkeys immunized with such a vaccine were not protected against challenge exposure with virulent SIV given by either route. However, 9 months after challenge 5 of 8 unvaccinated controls and 2 of 8 vaccinates have died from simian AIDS (SAIDS). Although the vaccine did not prevent infection, it delayed the onset of clinical disease in some of the IV challenged animals.

We also attempted to protect rhesus monkeys against SIV challenge by preinfecting them with a modified live virus. We used a biologically active molecular clone of SIVmac that causes a transient infection in the monkeys, persistent seroconversion and no disease for one year. Following IV challenge with 100-1000 animal infectious doses of uncloned virulent SIVmac, animals became persistently infected but showed only very low antigenemia and no disease for 7 months. All of the controls, by contrast, developed persistent viremia with high levels of antigenemia after IV challenge and have become sick with or died from SAIDS. Thus, this approach also fails to prevent challenge infection while it does appear to delay or may even prevent disease.

Based on these results and the SIV vaccine data obtained at the New England and Delta Primate Centers showing partial protection of rhesus monkeys against lower challenge doses of SIV we believe that these inactivated whole virus and modified live virus approaches are worth pursuing further and they may guide us towards an eventual effective vaccine for AIDS.

CM 017 THE HIV-1 INFECTED RABBIT AS A MODEL FOR AIDS T.J. Kindt, M.R. Gordon, M.E. Truckenmiller, D.P. Recker, E.A. Kuta, D.R. Dickerson, Lab of Immunogenetics, NIAID, NIH, Bethesda, MD, and H. Kulaga, Neuropsych. Branch, NIMH, St. Elizabeths Hosp., Washington DC.

New Zealand White rabbits may be productively infected with HIV-1 and evidence of infection can be ascertained in several ways. Rabbits injected with the human T-cell line, A3.01, infected with HIV-1 (LAV) produced antibodies against viral proteins p17, p24, gp41, and p55 within 4 weeks. HIV-1 *gag* and *env* sequences were detected by polymerase chain reaction in DNA isolated from PBMC and lymphoid tissues taken from rabbits beginning at 4 weeks after infection. HIV-1 transcripts were detected by *in situ* hybridization in some of these tissues. PCR amplification of RNA isolated from brains of infected rabbits showed transcripts in some, but not all, regions of the brain. Although virus was shown to persist for over a year in the infected rabbits, no signs of illness were observed perhaps because they are bred and maintained under specific pathogen free conditions and therefore are not exposed to the common pathogens for this species. Evidence for suppression of cellular and humoral immune responses has been collected. Immune response to BCG measured by skin reactivity to PPD was depressed in infected as compared to control animals. In addition, lung granulomatous responses to systemic challenge with BCG measured both by lung weight/ body weight ratios and by volume of cells obtained by lung lavage showed marked depression in the BCG immunized/HIV-1 infected rabbits. Humoral responses to tetanus toxoid measured by ELISA were also lowered in infected as compared to control rabbits. The ability to reproducibly infect rabbits with HIV-1 and the possibility of demonstrating immune suppression in infected animals suggests ways in which the rabbit may be used for testing vaccines or pharmaceutical agents directed against HIV-1 infection.

Animal Models of Human Viral Diseases: Relevance to Developmental Therapeutics

CM 018 SIMIAN IMMUNODEFICIENCY VIRUS MODEL FOR EVALUATION OF EXPERIMENTAL RETROVIRAL THERAPUETIC AGENTS. Preston A. Marx*, Richard Gould*, and Arnold Fridland+, California Primate Research Center, University of California, Davis, CA, 95616*, and St. Jude Children's Research Hospital, Department of Pharmacology, Memphis, TN, 38101+.

Simian immunodeficiency virus (SIV_{MAC}) infection of macaques (monkeys belonging to the genus *Macaca*) is a suitable model for evaluating new anti-viral agents. The virus causes an AIDS-like disease in macaques and the cell tropism and replication of SIV are being similar to human immunodeficiency virus (HIV). Opportunistic infections, central nervous system disease and B cell lymphomas which are relatively common in AIDS also occur in this SIV model.

Twenty-five compounds with therapeutic value against HIV were evaluated in vitro against SIV. The test compounds were added to CEMx174 cells 15 minutes before challenge with 10-20 $TCID_{50}$ of SIV_{MAC}. Cell cultures were incubated for 6 days and tested for inhibition of SIV replication in an MTT and SIV-specific antigen capture assay. The percent protection against cytopathic effect was calculated for each compound. Thirteen compounds (AZT, FdT, Foscarnet, PMEA, DDI, D4T, DDC, D4C, DDA, PMEC, PMPG, PMEMAP, PMEDAP) were found to be have significant therapeutic value against SIV_{MAC}. PMEA (2-Phonyl methoxy ethyl adenine) which gave 74% cell protection in vitro was tested in vivo for its ability to decrease the virus load in healthy SIV-infected macaques. Two animals were treated for 30 days with 30mg/kg/day. In this preliminary experiment, no reduction in virus load was detected. Based on our results, further in vivo studies in this model should involve prophylactic testing of compounds rather than therapy trials. After successful prophlylaxis has been shown, therapeutic compounds can then be tested for their in vivo therapeutic value.

AIDS-II

CM 019 EVALUATING EFFICACY AND TOXICITY OF ANTI-RETROVIRAL AGENTS IN MURINE RETROVIRUS INFECTIONS, Bilello, J.A., MacAuley, C., Yetter, R.A., Shapiro, S.G., Fredrickson, T.N, Bell, M.M and J.L. Eiseman. VA Medical Center, Baltimore, MD 21218. Cancer Center and Department of Microbiology, University of Maryland School of Medicine, Baltimore, MD. 21201 and Department of Pathobiology, University of Connecticut, Storrs, CT 06268. The efficacy and toxicity of AZT was investigated in two retroviral models: the LP-BM5 MuLV induced immunodeficiency disease of adult C57BL/6 mice and the Cas-Br-M MuLV induced encephalopathy in neonatal NFS/N mice. AZT was effective in protecting neonatal NFS/N mice from a single challenge with Cas BrM MuLV when the agent was administered in the drinking water (1 mg/ml ad libitum) to the lactating dams. These AZT-treated, challenged pups were free of virus and disease 6-18 mo. post inoculation. Treatment of adult C57BL/6 mice with 1 mg/ml AZT for 4 weeks, beginning 3 days prior to challenge with LP-BM5 MuLV, suppressed virus replication in 6 of 7 mice. The same treatment regimen for greater than six weeks protected 80% of mice from infection with LP-BM5 MuLV. If AZT treatment was initiated 6 weeks after LP-BM5 MuLV inoculation, virus dissemination and disease progression were not significantly altered. Oral administration of AZT induced aplastic anemia in both NFS/N and C57BL/6 mice. Toxicity was reduced by lowering the dose of AZT or terminating therapy. Continuous infusion of AZT resulted in splenic red pulp hyperplasia and elevations in both bone marrow and splenic BFU-E suggesting that AZT alters hematopoiesis in the absence of overt anemia. These studies support the usefulness of murine models in evaluating antiviral agents for both toxicity and efficacy. (Supported by NIAID Contracts NIH-NIAID-MIDP-1-YO1-60002 and NO1-A1-72666)

Animal Models of Human Viral Diseases: Relevance to Developmental Therapeutics

CM 020 EVALUATION OF EXPERIMENTAL ANTI-RETROVIRAL THERAPEUTIC AGENTS IN THE FeLV-FAIDS MODEL, Edward A. Hoover[1], Nordin S. Zeidner[1], and James I. Mullins[2], [1]Department of Pathology, Colorado State University, Fort Collins, Colorado, 80523, [2]Department of Microbiology and Immunology, Stanford University, Stanford, California 94305.

Immunodeficiency syndrome can be induced experimentally with a molecularly cloned isolate of feline leukemia virus (FeLV-FAIDS). The onset of the clinical disease syndrome is prefigured by the replication of the major pathogenic virus variant in bone marrow and is characterized by persistent antigenemia, gradual CD4 T cell depletion, wasting syndrome, enteropathy, and opportunistic infections. The antigenemia established long before the induction of disease serves as a useful parameter for antiviral studies because it can be quantitated serially, presages the development of clinical immunodeficiency, and provides a reliable indicator of therapeutic efficacy. We have evaluated several nucleoside analogues, including 2',3'dideoxycytidine (ddC), 3'azido 3'deoxythymidine (AZT), dideoxydidehydrothymidine (D4T), and 9(phosphonylmethoxy)ethyl adenine (PMEA) alone and in combination therapy protocols with cytokines in the FeLV-FAIDS model *in vitro* and *in vivo*. Among our findings are that ddC, when administered via controlled release subcutaneous implants, inhibited *de novo* FeLV-FAIDS replication and delayed onset of viremia. When ddC therapy was discontinued after 24 days, however, antigenemia rapidly developed in the treated cats equivalent to that in untreated controls. By contrast, oral administration of AZT, provided an effective prophylactic treatment against FeLV-FAIDS infection throughout a prolonged post-treatment observation period. We also obtained evidence that therapy with interferon-alpha, either alone or in combination with AZT, produced a significant decrease in viral antigenic load in animals with established viremia. Finally, PMEA has proven effective in early post-exposure antiviral chemotherapy and appears to be more potent and less hematotoxic than AZT on a mg/kg basis. Thus the FeLV-FAIDS model has proven useful for rapid assessment of single or combined agent therapies directed against conserved components in the retrovirus replication cycle.

CM 021 NEUROTROPIC RETROVIRUS CAUSES SPONGIFORM CNS DEGENERATION BY ABORTIVE INFECTION OF NEURONS, Arlene Sharpe, John Hunter and Rudolf Jaenisch, Whitehead Institute for Biomedical Research, MIT, Cambridge, MA 02142.

We have studied the interaction of the neurotropic Cas-Br-E virus with cells of the CNA. The virus causes a rapid spongiform degeneration in the brain of infected mice which is associated with progressive hind limb paralysis. Our results identify the postmitotic neuron as the major target for virus infection in the CNS suggesting that the pathological alterations are caused directly by viral gene expression rather than indirectly by infection of support cells. Immunohistochemical and protein analysis demonstrated that the synthesis of the viral env proteins is impaired in neurons but not in non-neuronal cells indicating abortive virus replication. Normal amounts of spliced env mRNA were synthesized in infected neurons suggesting that the lack of detectable env protein was due to a block at the posttranscriptional level.

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CM 022 ANALYSIS OF ANTI-VIRAL AGENTS IN THE SCID-hu MOUSE.

Hideto Kaneshima, Linda Rabin, Chu-Chih Shih, Reiko Namikawa, and J. M. McCune. HIV Group, SyStemix, Inc, Palo Alto, CA 94303. The SCID-hu mouse is a heterochimeric murine model in which interactive human hematolymphoid organs are surgically implanted into the immunodeficient C.B17 scid/scid mouse (1). Standard protocols have been devised in which well-characterized stocks of HIV may be injected intravenously into SCID-hu mice, resulting in infection of 100% of the animals. This process is dose- and time-dependent; two weeks post-infection, viremia is observed in greater than 95% of infected animals. Quantitation of the level of viremia indicates that individual animals show closely comparable amounts of virus in the peripheral circulation with time. As analyzed by in situ hybridization and PCR, HIV infection is only observed in the human organs of the SCID-hu mouse; within these organs, both T and myelomonocytic cells are inrected. This assay thus measures many important variables related to HIV infection in man: entry into the circulation, movement into the parenchyma of lymphoid organs, infection of cells of different lineages, and all stages of intracellular replication. The peripheral viremia also represents a convenient marker by which to analyze the effect of anti-viral agents in various steps of this infectious process. Such agents include organic compounds (AZT, ddI), recombinant proteins (soluble CD4), neutralizing antibodies, and cytotoxic T cells. In this manner, effective anti-viral therapy and systematic vaccine design may be pursued in a small animal, prior to application in lower primates or man.

1. Mc Cune JM, Namikawa R, Kaneshima H, et al. The SCID-hu mouse: murine model for the analysis of human hematolymphoid differentiation and function. **Science** 241: 1632-1639, 1988.

Papillomaviruses

CM 023 IMMUNE RESPONSE TO ANIMAL PAPILLOMAVIRUS, Ghim Singe, Schlegel Richard, Farquhar Carey, Jenson, Bennett, Department of Pathology, Georgetown University School of Medicine, Washington, DC 20007, and Harvard Medical School, Boston, Mass.

Experimental and circumstantial evidence has suggested that the antibody response to papillomavirus virions protects the host against reinfection, and perhaps more importantly, spread of infection. We have collected and analyzed sera from patients with HPV-1-induced plantar warts as well as from cattle, which were immunized or experimentally infected with BPV-1. In both systems, the highest titered sera react with intact virus but not disrupted, denatured virus. This finding suggests that the predominant humoral response is manifested by reactivity with external, conformational viral capsid epitopes. More importantly, bovine sera that react exclusively with conformational, external epitopes can neutralize BPV-1 as assayed by inhibition of in-vitro transformation of C127 mouse cells. In most cases, sera reactive with non-conformational linear epitopes are non-neutralizing. Therefore, we conclude that the antibody response to conformational epitopes on the surface of papillomavirus particles represents the host humoral response which protects against infection/reinfection.

CM 024 TREATMENT OF PAPILLOMAVIRUS INFECTIONS WITH ACYCLIC NUCLEOTIDES. **John W. Kreider, Departments of Pathology, and Microbiology and Immunology, The Milton S. Hershey Medical Center, Hershey, PA 17033** Human papillomavirus genital infections have become a world-wide problem. Current available treatments are primarily surgical and often followed by recurrences. A reservoir of subclinical infections exists in both men and women. There is a need for treatments for a broader region than that obtained by surgical treatment of grossly visible disease. Acyclic nucleotides include a number of compounds which potentially could fulfill this role. The agents have been effective against a number of DNA viruses, but have not been tried before this against papillomaviruses.

The objective of the present studies was to determine the effectiveness of acyclic nucleotides against human and Shope rabbit papillomavirus infections. Human papillomavirus type 11 (HPV-11) infections were induced in an experimental system consisting of HPV-11 infected human foreskin grafts placed beneath the renal capsule of athymic nude mice. The rabbit papillomavirus system used the cottontail rabbit papillomavirus (CRPV) to infect the dorsal epidermis of domestic rabbits. Most of our studies used the acyclic nucleotide 9-(2-phosphonylmethoxy)ethylguanine (PMEG). This agent was highly effective against both HPV-11 and CRPV when treatments were begun on the day of virus infection, and continued during the early papilloma growth period. Papilloma inhibition was dose proportional and associated with significant toxicity (weight loss, epilation, lymphopenia, and death). Subcutaneous PMEG treatments were also applied to Shope papillomas which were previously established for 14, 21, 28, 67, or 138 days. PMEG strongly inhibited papillomas of all durations. Toxicity was greatest for rabbits with older papillomas. Acyclic nucleotides were also given topically, directly upon the papillomas. PMEG was highly effective, completely suppressing treated papillomas in most rabbits. There was no significant systemic toxicity and only moderate erythema and induration at the treated sites. We conclude that acyclic nucleotides are effective against papillomas when given systemically or locally. Systemic treatment is limited by severe toxicity, but local treatment produced no systemic effects.

CM 025 RABBIT AND SIMIAN MODELS FOR THE STUDY OF PAPILLOMAVIRUS BIOLOGY AND POSSIBLE THERAPEUTICS AGENTS, Ronald S. Ostrow, Kristina Forslund, Ronald C. McGlennen, John Schneider, Bruce Kloster and Anthony J. Faras, Institute of Human Genetics, University of Minnesota, Minneapolis, MN 55455; Daniel Houser, Wisconsin Regional Primate Center, Madison, WI 53716. We have been using two animal model systems to study papillomaviruses and their role in neoplastic disease. Using domestic rabbits infected with cottontail rabbit papillomavirus (CRPV) the prophylactic and therapeutic effects of putative antiviral agents to interdict this neoplastic process have been tested. One agent, the nucleoside analogue ribavirin, proved to prevent or drastically reduce the size and time of appearance of warts induced by CRPV. Thus, this model is already being used as an effective and practical primary testing ground for anti-papillomavirus treatment.

On a more basic research level we have described a primate animal model system which closely mimics the natural disease associated with oncogenic human papillomaviruses of the genital tract. We have characterized papillomavirus DNA (RhPV 1) in a metastatic lymph node tumor of a male Rhesus monkey with a primary penile carcinoma. Using *in situ* hybridization, Southern blot hybridization, polymerase chain reaction amplification and cyto- and histopathological analyses, it was found that 71% of the animals within the sexual cohort of this animal had evidence of RhPV 1 infection. In fact, two of the females were also found to have cervical cancer, while other animals exhibited less advanced disease states. *In vitro* studies using RhPV 1 DNA show that this viral species is capable of transforming primary baby rat kidney cells in culture which demonstrate subsequent substrate independent growth. Thus, this model precisely mimics the range of disease, venereal transmission, and biologic activity found in oncogenic human papillomaviruses. We believe that this model system may be used as a more advanced testing ground for agents with putative anti-papillomavirus activity.

Animal Models of Human Viral Diseases: Relevance to Developmental Therapeutics

Respiratory Viruses

CM 026 ANIMAL MODELS FOR THE ACUTE VIRAL RESPIRATORY DISEASES OF MAN, Robert B. Couch, Influenza Research Center, Department of Microbiology and Immunology, Baylor College of Medicine, Houston, Texas 77030
The acute viral respiratory diseases of man are caused by a diverse set of viruses that produce a variety of clinical syndromes, affect all age groups, all populations, and frequently induce reinfections. Although a number of viruses infect and produce symptoms referable to the respiratory tract, those known as the respiratory viruses are the influenza viruses, parainfluenzaviruses, respiratory syncytial virus, the rhinoviruses, coronaviruses, and some of the adenoviruses and enteroviruses. These viruses are primarily infections of the respiratory passages that sometimes produce pneumonia but rarely disseminate. Reproduction of this diversity in animal models has not been achieved. Most human infections are upper respiratory while most animal models are lower respiratory. Most human infections are clinically apparent while most animal model infections are silent. A major proportion of human infections represent reinfections while there are no defined animal models of reinfection. Despite the paucity of animal models that are "like man," considerable information relating to pathogenesis, immunology, and vaccine and antiviral development has been gained from existing models. Notable in this achievement were use of the ferret and mouse for influenza, the cotton rat and hamster for RSV and the parainfluenzaviruses, and the selected use of primates for several viruses. Notably absent are defined models for nasopharyngitis, particularly for rhinoviruses, and bronchiolitis for RSV. Nevertheless, guidance obtained from animal model data has directed definitive studies in man indicating the development of new and improved models would facilitate identification of effective approaches to control of the viral respiratory infections.

CM 027 IMMUNIZATION AGAINST VIRUS INFECTIONS DUE TO ANTIGENIC DRIFT BY INDUCTION OF CROSS-REACTIVE CYTOTOXIC T LYMPHOCYTES. Francis A. Ennis, Koichi Kuwano, Manabu Tamura, Akio Yamada. Department of Medicine, University of Massachusetts Medical Center, Worcester. MA. 01655.

Influenza virus infection induces MHC restricted virus-specific cytotoxic T lymphocyte (CTL) responses in humans and mice. The specificity of the viral epitopes involved in CTL responses differ from the epitopes on the HA1 subunit of the hemagglutinin which induce neutralizing antibodies.[1] A CTL site in the transmembrane region of the HA2 subunit is conserved on influenza A viruses of the H1 and H2 subtypes.[2] We have demonstrated cross-reactive protection against antigenic drift of influenza A viruses by adoptive transfer with a clone of T cells which is specific for the conserved CTL site in the transmembrane region of the HA2 subunit.[3] Furthermore, we have demonstrated that active immunization against antigenic drift of influenza A viruses of the H1 and H2 subtypes by inducing cross-reactive CTL to this epitope.[4]

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2. Kuwano, K. Braciale, T.J. and Ennis, F.A. Cytotoxic T lymphocyte recognize in cross-reactive epitope in the transmembrane region of influenza H1 and H2 hemagglutinin. Viral Immunology 2:163-173, 1989.
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4. Kuwano, K., Scott, M., Young, J.F. and Ennis, F.A. Active Immunization against virus infections due to antigenic drift by induction of cross-reactive cytotoxic T lymphocytes. J. Exp. Med. 169:1361-1371, 1989.

Animal Models of Human Viral Diseases: Relevance to Developmental Therapeutics

CM 028 HUMAN PARAINFLUENZA VIRUS TYPE 3 INFECTION PRODUCES PNEUMONIA OR BRONCHIOLITIS IN TWO SPECIES OF COTTON RATS, David D. Porter[1], Gregory A. Prince[2], Val G. Hemming[3], and Helen G. Porter[1], Department of Pathology, University of California School of Medicine, Los Angeles, CA 90024[1], Department of International Health, School of Hygiene and Public Health, Johns Hopkins University, Baltimore, MD, 21205[2] and Department of Pediatrics, Uniformed Services University of the Health Sciences, Bethesda, MD 20814[3]

Human parainfluenza virus type 3 (Strain F518, a plaque purified clone of Strain F367) replicates well in the nose and lung of two species of cotton rats, Sigmodon hispidis and Sigmodon fulviventer. Peak viral titers of about 10^6 PFU/g are reached 2 days after infection in both tissues, are maintained through the fifth day, and are equivalent in the two species. Infectious virus is eliminated by the eighth day after infection. Both species produce a strong neutralizing antibody response with titers of 1:10,000 four weeks after infection. Viral replication in the nasal epithelium results in only minor histological changes, and viral antigen was limited to the luminal surface of the epithelial cells. Infection of S. hispidis causes a bronchiolitis with a peribronchiolar lymphoid cell infiltration which reaches a peak six days after infection, and there is only a minor component of interstitial pneumonia. In contrast, infection of S. fulviventer causes an interstitial pneumonia by 4 days, and this lesion reaches its maximal extent by 6 days after infection. There is minimal peribronchiolar lymphoid cell infiltration in infected S. fulviventer. Lung lesions in both species of cotton rats are largely healed 9 days after infection. These species of cotton rats offer separate models for the two major pulmonary manifestations of human parainfluenza virus type 3 infection. Preliminary experiments indicate that administration of a formalin-inactivated viral vaccine before live virus challenge results in enhanced lung lesions. These models may be useful for studies of the pathogenesis of this infection, and for evaluation of strategies for prevention or treatment of the disease.

CM 029 THE COTTON RAT AS A MODEL FOR HUMAN RESPIRATORY SYNCYTIAL VIRUS INFECTION, Gregory A. Prince, Department of International Health, School of Hygiene and Public Health, Johns Hopkins University, Baltimore, MD 21205

The cotton rat (Sigmodon hispidus and Sigmodon fulviventer) is a semi-permissive host for human respiratory syncytial virus (RSV), supporting viral replication in pulmonary and nasal tissues. The magnitude of replication is independent of the age of the animal, but directly proportional to the challenge dose. Pulmonary disease is directly proportional to challenge dose, and consists of peribronchiolitis in S. hispidus, and interstitial pneumonitis in S. fulviventer.

Potentiated RSV disease seen during vaccine trials in the 1960s has been studied in cotton rats, using formalin-inactivated RSV. Vaccinates challenged with live RSV showed reduced levels of pulmonary viral replication, but developed enhanced pulmonary disease consisting of intraalveolar polymorphonuclear leukocytes and peribronchiolar lymphocytes. Evidence of immune complex disease was seen in pulmonary and renal tissues. Serologic studies showed an increased binding-to-neutralizing antibody ratio.

Parabiotic pairing of immune and non-immune animals showed that pulmonary immunity could be transferred via the blood. Fractionation experiments indicated serum IgG to be the effector of the observed immunity. Adoptive transfer of IgG with high neutralization titer showed that pulmonary RSV replication could be prevented, and that existing infection could be modulated by systemic or topical administration of IgG. Subsequent studies in primates, and current clinical trials in infants suggest an important role for IgG in the prevention and treatment of RSV infection.

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CM 030 INFLUENZA VIRUS VIRULENCE AND STRUCTURE, Robert G. Webster[1], Yoshihiro Kawaoka[1], W. Graeme Laver[2], Gillian M. Air[3], and Peter M. Colman[4].
1) Dept. of Virol./Mol. Biol., St. Jude Child. Res. Hosp., Memphis, TN 38101, 2) John Curtin Med. School, Aust. Natl. Univ., Canberra City, A.C.T. 2601, Australia, 3) Dept. of Microbiol., Univ. Alabama at Birmingham, Birmingham, AL 35294, and 4) Div. of Protein Chemistry, CSIRO, Melbourne, Australia.

The influenza virus hemagglutinin (HA) plays a pivotal role in determining virulence. The HA is synthesized as a single polypeptide chain and must be cleaved in HA1 and HA2 to be infectious. Each of the 13 HA subtypes of influenza A viruses contain avirulent influenza viruses; two subtypes, H5 and H7, also contain virulent viruses. Virulent influenza viruses contain a series of basic amino acids in the connecting peptide, but there is no consensus sequence. Avirulent influenza viruses contain a single arginine at the connecting peptide. Site-directed mutagenesis has established the amino acid requirements for cleaving activation. These are: (i) the sequence of the connecting peptide is crucial for cleavage activation; arginine at the fourth position from the C-terminus of the HA1 is essential, whereas lysine at the third position is not; lysine at the second residue increases susceptibility to cleavage; (ii) structural feature(s) other than at the connecting peptide sequence are also involved in cleavage activation; and (iii) these structural requirement(s) can be abrogated by insertion of basic amino acids in the connecting peptide. The enzymes required for cleavage activation of virulent H5 and H7 viruses are ubiquitous, whereas those required for cleavage of avirulent influenza viruses are found only in the respiratory and intestinal tract.

There is considerable confusion about what constitutes an epitope on a protein. The N9 neuraminidase of influenza virus complexed with monoclonal antibody Fab fragments have been crystallized and analyzed by X-ray crystallography. This has permitted us to define an epitope and establish the interactions involved. We define an "epitope" as the region on the surface of a protein which binds a particular monoclonal antibody molecule. The structure of escape mutants and the mechanism of inactivation of neuraminidase by antibodies will be considered.

CM 031 EVALUATION OF COMPOUNDS AND VACCINES AGAINST PARAMYXOVIRUSES IN COTTON RATS. Philip R. Wyde, Mark W. Ambrose, Heidi Meyer and Brian E. Gilbert. Department of Microbiology and Immunology, Baylor College of Medicine, Houston, Texas 77030. Cotton rats (Sigmoden hispidus or Sigmoden fulviventer) have been shown to be susceptible to respiratory syncytial virus (RSV), parainfluenza virus type 3 (PIV3) and adenovirus type 5 (AV5) pulmonary infections. This susceptibility makes cotton rats most suitable for evaluating compounds with potential activity against these viruses for toxicity and antiviral efficacy, or in the case of vaccines, to assess the vaccine's immunogenicity and protective efficacy against RSV-, PIV3- or AV5-induced disease. Two studies will be presented as examples. The first involves a compound, 89-10, which has been reported to have activity against paramyxoviruses in tissue culture. Using the cotton rat model we have been able to obtain consistent and significant reductions in pulmonary RSV or PIV3 titers in animals given single 1 mg/kg doses of 89-10 on days 1-3 after experimental virus challenge, compared to pulmonary RSV or PIV3 titers in placebo control animals. No significant toxic effects were observed in test cotton rats including those given 20 mg/kg of 89-10 for 8 consecutive days. In the second study, a PIV3 subunit vaccine prepared from the hemagglutinin-neuaminidase (HN) and fusion (F) surface glycoproteins of PIV3 following solubilization and purification by affinity chromatography was tested in cotton rats for immunogenicity and protective efficacy. Animals were immunized twice (i.p.), four weeks apart with graded doses of vaccine mixed with either vehicle or aluminum phosphate (AlPO4). Addition of AlPO4 and/or the administration of a second vaccine dose significantly enhanced all virus-specific antibody responses (i.e., neutralizing, hemagglutination inhibiting and fusion-inhibiting). The minimal protective dose was 1 ug/dose. Immunopathogenicity studies with this vaccine are planned.

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Animal Models for Herpesvirus Infections

CM 100 MCMV OCULAR INFECTION IN IMMUNOCOMPETENT AND IMMUNOSUPPRESSED MICE. JF Bale, Jr., ME O'Neil, S Perlman. Departments of Pediatrics, Neurology and Microbiology. The University of Iowa College of Medicine, Iowa City, IA 55242.

To investigate the pathogenesis of cytomegaloviral ocular infections, we used virologic and in situ nucleic acid hybridization methods to study immunocompetent or immunosuppressed three-week old Balb/c mice inoculated intravitreally with 10^4 plaque-forming units (pfu) of murine cytomegalovirus (MCMV). Immunocompetent mice exhibited a self-limited ocular infection with peak virus titers of $10^{3.5}$ pfu/ml in the retina/choroid fraction on day 4 of infection. Using biotinylated MCMV DNA probes (pAMB25 or HindIII A MCMV DNA fragments), we detected MCMV nucleic acids in cells of the iris, ciliary body and rarely, the retina on days 4 and 7 of infection. Retinal architecture was preserved. By contrast, mice immunosuppressed with cyclophosphamide (200 mg/kg on day 0, 100 mg/kg on days 5 and 11 after MCMV inoculation) developed progressive ocular infection that culminated in retinitis. Virus titers in the retina/choroid fraction rose progressively (10^5 pfu/ml in cyclophosphamide-treated mice on day 7 vs. 10^2 pfu/ml in immunocompetent mice). MCMV nucleic acids were detected in the iris and ciliary body of immunosuppressed mice on days 4 through 11 and in the retina on days 7, 11 and 14. On day 14 we observed abundant MCMV nucleic acids in most retinal layers and extensive retinal disorganization. These studies indicate that immunosuppression potentiates MCMV ocular disease in mice, a finding analogous to HCMV retinitis in immunosuppressed humans.

CM 101 THE OVARIECTOMIZED MOUSE AS A MODEL FOR EVALUATION OF ANTI-HSV-2 DRUGS, A. Braitman and J. M. Clark, The Squibb Institute for Medical Research, Princeton, NJ 08543-4000.

The ability of mice to develop a HSV-2 induced vaginitis is related to the animal's estrous cycle with animals being more susceptible to infection during diestrous. We have employed ovariectomized mice which are in a permanent state of diestrous to study susceptibility to HSV-2 and to evaluate anti-viral agents in these infections. Ovariectomized mice were infected by insertion of a vaginal tampon containing 10^5 PFU of HSV-2. All animals develop vaginal inflammation within 6 days which progresses to hind quarter paralysis and death if untreated. In contrast, sham operated and normal mice show variable susceptibility to intravaginal HSV-2. A 10^5 virus inoculation in both control groups resulted in 50% and 40% death respectively compared to 100% death of ovariectomized mice. The mean day of death for all three groups averaged 8-9 days. Ganciclovir and acyclovir were orally effective in therapy of this infection with both compounds prolonging life of ovariectomized mice when given at 25 to 100 mg/kg/day for 5 days. Only ganciclovir protected the majority of animals from death as indicated by a PD_{50} of 61 compared to >100 for acyclovir. The ovariectomized mouse should prove of use in evaluation of anti-HSV-2 drugs.

CM 102 A MOUSE MODEL OF NEONATAL HSV-1 INFECTION: ROUTES OF VIRUS ENTRY TO THE CNS, Linda S. Crnic and Kenneth L. Vanderslice, Departments of Pediatrics and Psychiatry, University of Colorado School of Medicine, Denver, CO 80262. Neonatal infection of mice with a low virulence mutant HSV-1 produces a specific behavioral syndrome: life-long hyperactivity and difficulty inhibiting behavior (Crnic and Pizer, Neurotoxicol. Teratol. 10:381, 1988). To determine the exact routes by which the virus reaches the CNS, newborn mice were injected subcutaneously in the shoulder with a low virulence strain of HSV-1 which lacked thymidine kinase expression. At 1,1 1/2, 24,48,72,96,168, and 192 hours post infection, whole mice were perfused, paraffin embedded, sectioned and stained for HSV-1 using a polyclonal antibody and a PAP detection system. Infection was apparent in skin at the site of injection, muscle underlying the injection site, and in periscapular brown and white fat. Thus, virus entered the central nervous system via three separate routes. First, the usual route from the skin via sensory nervous system, as stain was seen in neurons and satellite cells in the dorsal root ganglia. Second, virus entered the CNS from the muscle via the alpha motor neurons directly into the ventral horn of the spinal cord. Third, virus entered from brown fat via the sympathetic ganglia to the interomediolateral column of the spinal cord. These three routes of entry correspond to the sites in the brain that were infected. At 1 hr post infection, virus had adsorbed to fat and muscle layers under the skin. At 1 1/2 hrs, virus had entered the cells. By 24 hr, viral antigen was detected in the dorsal root and sympathetic ganglia as well as alpha motor neurons in the spinal cord. At 192 hours, destruction of brown fat and muscle was evident and virus had entered the brainstem and cerebellum. Supported by MH00621 & MH44970.

Animal Models of Human Viral Diseases: Relevance to Developmental Therapeutics

CM 103 EBV-ASSOCIATED LYMPHOMAGENESIS IN SCID MICE. Mary A. Cromwell, Ruthann M. Hesselton and John L. Sullivan. University of Massachusetts Medical Center, Program in Molecular Medicine, Department of Pediatrics, Worcester, MA 01655. It has been reported by Mosier, et al. (Nature, 1988) that immunodeficient scid mice reconstituted with 50-90 x 10^6 human peripheral blood mononuclear cells (PBMC) from EBV seropositive donors develop with high frequency (>80%) human B-cell lymphomas. We have performed similar reconstitutions and confirmed the occurence of EBV-associated lymphomas. Human immunoglobulin levels in the serum of reconstituted mice increased over time and approached normal human adult levels (12-17 mg/ml). We have successfully established cell lines from tumors and lymphoid tissue of a number of reconstituted mice. Surface antigen expression demonstrated B-cell lineage with phenotypic characteristics of both EBV lymphoblastoid cell lines and Burkitt's lymphoma. We are currently generating EBV-specific cytotoxic T-cells and have initiated studies to determine the effect of CTL administration on the development of EBV-associated lymphomas in reconstituted scid mice. The scid mouse model of EBV-induced lymphomas appears to be an excellent model for studying mechanisms of immunopathogenesis.

CM 104 A MURINE MODEL OF HUMAN CYTOMEGALOVIRUS INFECTION

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Human cytomegalovirus (HCMV), a member of the herpes virus family is known to be a major pathogen in immunocompromised patients.
The lack of animal model for HCMV infection is a major handicap to test new potential treatment.
Recent studies have led to the hypothesis that human β2 microglobulin (hβ2m) could contribute to the binding of HCMV to the cell membrane (1).
To test this hypothesis, hβ2m transfected murine fibroblasts were infected with HCMV and their susceptibility for infection were compared with non-transfected murine fibroblasts. Human fibroblasts were used as reference target-cells.
The results show an infecting titer of about 75 % for transfected murine fibroblasts and only 3 % for non transfected murine fibroblasts compared with 100 % for reference human fibroblasts, as revealed by immediate early antigen immunoenzymatic detection.
The establishment of an inbred animal model of HCMV carrier state is essential to examine the molecular mecanism of the development of HCMV related diseases. Transgenic lines were established expressing detectable levels of hβ2m. This mice were challenged for their potential to develop CMV infection and acute disease after immunosuppression. The results obtained with this animal model will be discussed.

(1) J.E. GRUNDY et al. J. Gen.Virol. 1987, 68 : 793-803

CM 105 T CELL RESPONSE TO VARICELLA ZOSTER VIRUS IN GUINEA PIGS, Anthony R. Hayward & Ann Arvin. Departments of Pediatrics, University of Colorado Health Sciences Center, Denver CO 80262 and Stanford University Medical Center, Stanford, CA, 94305.
Varicella zoster virus, adapted to grow in guinea pig fibroblasts, was injected subcutaneously into Hartley, strain 2 and strain 13 guinea pigs. Serum IgG antibodies were detected 2 weeks later and T cell proliferative responses by blood lymphocytes were found 3 weeks after injection. The proliferating cells bound the 155 antibody, which defines a CD4-like subset of guinea pig T lymphocytes. VZV-infected fibroblasts of human, Hartley and strain 13 origin elicited equivalent amounts of proliferation, which was quantitatively greater than that obtained with an extracted VZV antigen. Uninfected (control) human or guinea pig fibroblasts did not elicit T cell proliferation. The proliferative response to VZV required the presence of autologous (strain 2 or 13) antigen presenting cells and was blocked by addition of an anti-class II MHC antibody. Cytotoxicity experiments in which *in vitro* stimulated lymphocytes were cultured with ^{51}Cr labelled MHC class I-matched and mismatched fibroblasts, either VZV-infected or control, were negative. The data indicate that guinea pigs resemble man in making predominantly class II restricted T cell responses to VZV.

CM 106 THE MURINE ZOSTERIFORM MODEL OF HERPES SIMPLEX VIRUS INFECTION FOR THE EVALUATION OF TOPICAL ANTIVIRAL THERAPIES, D.C. Lobe, T. Spector and M.N. Ellis, Wellcome Research Laboratories, Research Triangle Park, NC 27709
HSV inoculated intradermally on the flank of mice produces a zosteriform like rash that extends from the midventral to the middorsal line. The disease first appears as discrete lesions at the site of inoculation on about day 3. The involvement subsequently includes the entire dermatome served by the affected nerve and results in the development of the zosteriform rash. In the past, this system has been used to examine the replication of virus at both the inoculation site and distal areas infected via nerve endings. The former is similar to primary infection and the latter is suggestive of the infection cycle of reactivated virus. We have adapted this model to examine the antiviral effects of topically applied compounds which may have potential for the treatment of cutaneous herpes disease. The model results in reproducible lesions that are easily scored and treated. Lesion scores can be confirmed by doing virus titrations of infected skin homogenates. In a typical experiment with HSV-1, untreated mice succumbed to the infection by day 7 or 8. Treatment with 5% acyclovir (ACV) resulted in lesion scores of about 4 on day 12 (0 = no infection and 6 = death and/or complete necrotic rash) while a combination therapy of ACV and BW A1110U, an inhibitor of the HSV ribonucleotide reductase, had a score of 1.5. Data will be presented illustrating the many infection and treatment parameters that may be measured.

CM 107 MUTAGENESIS OF MURINE CYTOMEGALOVIRUS: IDENTIFICATION OF GENES REGULATING GROWTH IN THE SALIVARY GLAND AND REACTIVATION FROM LATENCY, William C. Manning, Gerardo B. Abenes and Edward S. Mocarski, Department of Microbiology and Immunology, Stanford University School of Medicine, Stanford, CA 94305 The genetic control of CMV tissue tropism and latency is not well understood. We have made mutants in murine CMV that do not affect growth in cell culture and have studied these mutants in mice.To examine the role of MCMV immediate-early gene 2 (ie2) in pathogenesis, we constructed a recombinant virus with a mutation in ie2 (RM408.) We also isolated an ie2 mutant, RM427,which has a deletion in an early gene, sgg1 (salivary gland growth.) RM427 was partially rescued to recover a virus containing the sgg1 deletion alone, (RM401.) RM408 (ie2-,sgg1+), RM401 (ie2+,sgg1-), and RM427 (ie2-,sgg1-) replicate as well as parental wild type MCMV in cell culture indicating the dispensability of ie2 and sgg1 in vitro. In the mouse, MCMV replicates to high titer in the salivary gland where it persists until 30 dpi . In mice infected with RM401 or RM408, there is a marked reduction in salivary gland replication. Salivary gland replication in mice infected with RM427 is reduced 4 to 6 orders of magnitude compared to parental virus. Growth in other organs is similar to wild type. In addition to its role in salivary gland growth, our data suggest that ie2 is also involved in reactivation from latency in the spleen. The sgg1 mutants fail to synthesize two abundant early proteins when analyzed by immune precipitation, indicating that these two proteins are not required for growth in vitro or in organs other than the salivary gland.

CM 108 **Analysis of cytokine gene transcription in $CD4^+$ and $CD8^+$ T cells following sublethal infection of mice with murine cytomegalovirus**, Murray, L. and Martens, C. DNAX Research Institute, Palo Alto, CA 94304. Consequences of murine cytomegalovirus (MCMV) infection include both suppression of hemopoiesis and induction of $CD8^+$ virus-specific cytotoxic T cells. These effects may be due to up- or down-regulation of transcription of various cytokine genes. $CD4^+$ and $CD8^+$ T cells were highly purified by FACS-sorting from spleens of mice at different times post-infection with a sublethal dose of MCMV. RNA was prepared from these cells without further stimulation, and was reverse-transcribed and amplified using polymerase chain reaction primers specific for several cytokine genes. Both $CD4^+$ and $CD8^+$ T cell subsets from Balb/c mice transcribed easily detectable levels of interferon-γ and tumor necrosis factors-α and -β, with no apparent change in message levels following infection. Spontaneous transcription of the IL-2 gene was easily detectable in $CD4^+$ T cells, but was barely detectable in the $CD8^+$ T cell subset, at times up to 5 days post-infection. These results suggest that $CD8^+$ T cells in vivo produce little IL-2 even after virus infection, and if they are in fact IL-2-dependent, may depend on $CD4^+$ T cells as a source of this growth factor. The cytokine responses of the genetically resistant C3H mice and susceptible Balb/c mice are being compared to determine if any of the known cytokines may contribute to the resistant phenotype of C3H mice.

Animal Models of Human Viral Diseases: Relevance to Developmental Therapeutics

CM 109 **Therapy of a fatal MCMV infection with Thymic Humoral Factor (THF-γ2) treated immune spleen cells,**.B. Rager-Zisman[1], F. Zuckerman[1], D. Benharroch[2], M. Pecht[3], Y. Burstein[4], and N. Trainin[3], [1]Department of Microbiology and Immunology, [2]Institute of Pathology, Ben Gurion University of the Negev, Beer-Sheva, and Depts. of [3]Cell Biology and [4]Organic Chemistry, The Weizmann Institute of Science, Rehovot, Israel. We performed adoptive transfer experiments to evaluate the prospects for enhancing the antiviral potential of murine cytomegalovirus (MCMV) immune spleen cells by THF-γ2. Adult Balb/c mice resistant to MCMV, become highly susceptible following immunosuppression by cyclophosphamide (CY). Recipient mice were injected with MCMV and CY concomitantly and 24 hours later adoptive transfers of syngeneic immune spleen cells were performed. We showed that passive transfers of MCMV immune spleen cells prevented the development of a fatal disease in 38% of the recipient mice. Daily injections of MCMV immune donor mice with THF-γ2 enhanced considerably (93%) the therapeutic potential of virus specific immune cells. These experiments provide direct evidence for the antiviral capacity of THF-γ2 through its immunomodulatory effect on immune T cells.

CM 110 VIRAL IDENTIFICATION IN SITU WITH DIRECT-LABELED DNA PROBES, Floyd Taub, Christine King, Thomas Higgs, Digene Diagnostics, Silver Spring, MD 20904.

In situ hybridization is extremely useful in the precise localization and tracing of the natural history of viral infections in animal models and human disease. It allows histological, cellular, and sub-cellular localization of the virus; determination of tissue damage; a semi-quantitative analysis of the virus amounts in individual cells; and a quantitative analysis of the percentage of cells infected with the virus.

In the past, tritium-labeled probes have yielded high resolution but have been difficult and time-consuming to work with. S^{35}-labeled probes have reduced exposure time to several days; however, morphologic resolution suffers due to the high energy of the ß particles and the presence of emulsion on the specimen. Biotin-labeled probes detected with alkaline phosphatase-avidin conjugate have been used effectively in in situ hybridization; however, they generally provide a more diffuse signal than probes directly labeled with horseradish peroxidase (HRP).

Probes directly labeled with HRP have been found to give highly resolved signals in assays as short as 1½ hours, with high sensitivity (50 copies per cell). HRP direct-labeled probes have been found to detect approximately twice as many specimens infected with virus as standard histological methods for detection of CMV. In situations where type specificity is of paramount importance--for instance, in distinguishing HPV types--direct-labeled probes have been found to be much better than biotinylated probe kits. A new silver amplification technique has been applied with HRP direct-labeled probes to make readily observable, dark black spots where signals were previously invisible or barely visible.

CM 111 IMMUNE REGULATION IN MURINE HERPES SIMPLEX VIRUS-INDUCED RETINITIS, Judith A. Whittum-Hudson, The Wilmer Institute, The Johns Hopkins University School of Medicine, Baltimore, MD 21205

Herpesviruses have been implicated in retinal infections in several immunodeficient patient populations, including AIDS patients. Injection of Herpes simplex virus Type 1 (KOS strain) into the anterior chambers of both eyes of BALB/c mice results in ocular infection and inflammation of the anterior portions of both eyes. However, the retina and optic nerve remain free of disease unless mice are immunosuppressed. In contrast, unilateral infection of the anterior chamber results in destruction of the opposite retina, with preservation of only the retina of the virus-injected eye. Although virus spreads to the brain in both models, animals remain healthy and survive indefinitely. Retinal preservation and survival in these models is dependent upon intact T cell-mediated immunity. Recently we have treated mice with anti-interferonγ antibody prior to, and after, bilateral or unilateral ocular infection with HSV-1. This treatment abrogates the retinal preservation observed in untreated mice, although mice survive their infections: 18/18 virus-injected eyes developed irreversible retinitis or retinal necrosis, whereas only 1/10 virus-injected eyes from untreated mice exhibited retinitis. These results provide evidence that γinterferon is important in controlling herpes simplex virus-induced retinal destruction in this mouse model, and may provide clues to successful immune intervention in human retinal infections.

CM 112 HERPES SIMPLEX VIRUS LATENCY IN NEURONAL CULTURES: CHARACTERIZATION AND REGULATION OF LATENCY, Christine L. Wilcox, Christian Doerig, and Lewis I. Pizer, Department of Microbiology and Immunology, University of Colorado Health Sciences Center, Denver, CO 80262. Herpes simplex virus (HSV) establishes latent infections in neural crest-derived sympathetic and sensory neruons in humans. The mechanisms regulating the establishment, maintenance, and reactivation of latent HSV remain poorly understood. To better understand the HSV infection in the neuron, we have studied the virus infection *in vitro*. Previously we showed that latent HSV infections are established in sympathetic rat neuronal cultures, and that nerve growth factor (NGF) deprivation results in reactivation of latent HSV (J. Virol. 62:393, 1988). Recently we have investigated HSV infection in rat dorsal root ganglia neurons (sensory) in culture and found that latent HSV infections reactivated after NGF deprivation. We have examined the sensory neuronal cultures for viral gene expression during latency and after NGF deprivation. By *in situ* hybridization, Northern blot anlaysis, and RNase protection assays expression from the region of the genome corresponding with the latency associated transcripts was detected only during the latent infection. Expression of the gD gene was not detected during the latent infection, but was detected 24 h after NGF deprivation.

Animal Models for Hepatitis B Virus Infections

CM 200 SEROLOGICAL ANALYSIS OF DHBV INFECTION, Christopher J Burrell, Ming Qiao, Eric J Gowans, and Allison R Jilbert, Division of Medical Virology, Institute of Medical and Veterinary Science, Frome Road, Adelaide South Australia 5000.

A radioimmunoassay to detect duck hepatitis B virus surface antigen and antibody was developed and used to examine the appearance of these markers after intravenous infection of Pekin-Aylesbury cross bred ducks. Viraemia (DHBV DNA or DHBsAg) was detected in all ducks inoculated within 3 weeks post-hatch, and persistent infection invariably developed in this group. In contrast, only 80% and 60% of ducks inoculated 4 weeks and 6 weeks post-hatch respectively developed viraemia, and approximately 70% of the viraemic ducks became carriers. Markers of viraemia were undetected in ducks inoculated 8 weeks post-hatch and in uninfected controls.

Anti-DHBs developed subsequently in two of four birds that showed transient viraemia, thus demonstrating typical seroconversion, and in three of seven ducks inoculated 4-8 weeks post-hatch that showed no prior markers of viraemia. It is likely that these latter ducks had undergone transient acute infection without detectable viraemia.

These results demonstrate progressive development of increasing resistance to infection with increasing age that may be related to several factors including the development of host immunity. This model may help elucidate similar age-related features of human hepatitis B infections.

CM 201 MODULATION OF HEPATITIS B VIRUS SURFACE ANTIGEN EXPRESSION DURING THE ACUTE PHASE RESPONSE IN TRANSGENIC MICE. P.N. Gilles, C. Pasquinelli, T. Moriyama, G. Fey, F.V. Chisari. Departments of Molecular and Experimental Medicine, and Immunology, Research Institute of Scripps Clinic, La Jolla, CA 92037. Since hepatitis B virus (HBV) infected liver is typically inflamed, it is possible that HBV gene expression might be influenced by mediators of the acute phase response. We have analyzed HBV major surface antigen (HBsAg) gene expression in transgenic mice following the administration of bacterial LPS under conditions previously shown to induce an acute phase response. We injected graded doses of LPS into 6 week old mice of the albumin-HBsAg (50-4) and the metallothionein-HBsAg (23-3) lineages and monitored the differential effects of LPS on expression of the transgenes and on the simultaneous expression of cellular genes known to be modulated during the acute phase response by northern blot analysis of total liver RNA from treated and untreated control mice. The transgene transcripts were substantially decreased at 16 hours after LPS administration in both lineages in contrast to an increase in transcripts corresponding to the acute phase proteins serum amyloid A (SAA) and alpha-2 macroglobulin. Thus it appears that either the activity of the endogenous HBV promoter and the upstream exogenous promoters are down-regulated or the stability of these transcripts is decreased by one or more of the mediators of the acute phase response. We intend to further characterize this phenomenon by examining HBsAg expression in transgenic mice following treatment with individual inflammatory cytokines and other agents known to be induced by LPS.

Animal Models of Human Viral Diseases: Relevance to Developmental Therapeutics

CM 202 USE OF THE WOODCHUCK HEPATITIS VIRUS AS A MODEL FOR THE DEVELOPMENT OF ANTIVIRAL THERAPIES AGAINST HBV. Brent E. Korba[1], Paul J. Cote[1], Bud C. Tennant[2] and John L. Gerin[1]. [1]Georgetown University, Division of Molecular Virology and Immunology, Rockville, MD 20852 and [2]College of Veterinary Medicine, Cornell University, Ithaca, NY 14853.

The Woodchuck hepatitis virus [WHV] and its natural host, the Eastern Woodchuck (*M. monax*) constitute the relevant animal model system for the study of hepatitis B virus [HBV]-induced infection and disease in man, including chronic hepatitis and hepatocellular carcinoma. Based upon predictable responses to experimental WHV infection, it is possible to investigate the effects of various antiviral compounds (e.g. nucleoside analogs) and immune modifiers on the pathogenic mechanisms of hepadnaviral infections. The purpose of the current studies was to examine the therapeutic application of several potential antiviral agents in WHV carrier animals. Woodchucks were treated at various times (1 to 18 months) following the onset of serologic patterns indicative of chronic WHV infection. Depending upon the specific agent employed, both short (7-10 days) and long term (4-12 months) treatment protocols were utilized. In general, the nucleoside analogs examined in these studies produced marked (up to 10,000-fold), but transient, depressions in the levels of circulating WHV virions which returned to pretreatment levels after the cessation of drug administration. The relative efficacy of these compounds will be discussed based upon (i) the analysis of serologic markers of WHV infection (e.g. WHV DNA, WHsAg, anti-WHs) and liver disease (e.g. ALT, SDH), (ii) clinical status of the animals (body weights, hematology, blood chemistries), and (iii) the state of WHV DNA replication, RNA transcription and virus-induced disease in liver tissues.

CM 203 INHIBITION OF DUCK HEPATITIS B VIRAL SUPERCOILED DNA BY TOPOISOMERASE AND DNA GYRASE INHIBITORS, Stephen Locarnini, Gilda Civitico, Yanyan Wang, Gilda Tachedjian and Ian Gust, Macfarlane Burnet Centre for Medical Research, Fairfield Hospital, Fairfield, Victoria 3078,AUSTRALIA.

Hepatitis B virus supercoiled DNA(or covalently closed circular DNA) is the main transcriptional template for hepadnaviral replication. Recent investigations have shown that the supercoiled (SC) form of the viral DNA is one of the major targets in attempts to treat chronic carriers of hepatitis B. The other forms of the hepatitis B virus (HBV) genome replication, relaxed circular, linear and single-stranded DNA, are sensitive to conventional DNA replication inhibitors such as interferons, phosphonoformate, adenine arabinoside and ganciclovir, whilst the SC DNA form is resistant to such therapy. In an attempt to understand more about SC DNA generation and processing we examined the effects of a range of supercoil-active compounds on duck HBV SC DNA. These compounds included topoisomerase I and II inhibitors, DNA gyrase inhibitors, and compounds targeted directly at SC DNA. We found that topoisomerase II inhibitors (amsacrine and ellipticine) in vitro and DNA gyrase inhibitors (coumermycin A1 and nalidixic acid) in vitro and in vivo significantly inhibited DHBV DNA replication. The level of inhibition was shown to be DHBV SC DNA generation and subsequent processing. This investigation has defined a new class of antiviral agents which can act alone or in combination with other agents to significantly inhibit DHBV. The application of the results of this study to the management of the human hepatitis B chronic carrier will be discussed.

Animal Models of HIV Infections

CM 300 ANTIVIRAL EFFICACY OF THE HETEROPOLYANIONS AVS 2575 AND AVS 2576 IN MURINE AIDS MODELS, Paul L. Black, Michael A. Ussery, James T. Rankin, Jr., and Michael A. Chirigos. Southern Research Institute-Frederick Research Center and USAMRIID, Ft. Detrick, Frederick, MD 21701 and FDA, Rockville, MD 20857.

We have employed two murine retrovirus models of AIDS, *viz.* the Rauscher leukemia virus (RLV) as a primary model and the LP-BM-5 murine AIDS (MAIDS) virus, to screen antiviral agents and biological response modifiers (BRM) for potential therapeutic activity against AIDS. RLV rapidly produced intense splenomegaly and viremia, both of which served as measures of disease progression. We have tested the heteropolyanions AVS 2575 [$K_7(BVW_{11}O_{40})$] and the closely related AVS 2576, which have previously shown activity *in vitro* against HIV, in these models. When given prophylactically at 20 mg/kg every day from day -1 through day 13, AVS 2575 reduced splenomegaly and serum reverse transcriptase levels in mice sacrificed on day 14 or day 21 after infection. Lower doses of AVS 2575 on the same schedule were not effective. Results with AVS 2576 were similar. However, the efficacious treatment regimens produced toxicity, as evidenced by loss in body weight. Therefore, alternative treatment schedules with less frequent administration were examined. A single dose of AVS 2575 or AVS 2576 at 30 mg/kg was effective when given one day before infection, but not one day after infection. A single administration of AVS 2575 or AVS 2576 at 100 mg/kg was effective either before or after infection. These findings suggest that the heteropolyanions AVS 2575 and AVS 2576 may be useful agents in treating retroviral infection.

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Animal Models of Human Viral Diseases: Relevance to Developmental Therapeutics

CM 301 IDENTIFICATION OF A HYPERVARIABLE REGION IN EQUINE INFECTIOUS ANEMIA VIRUS LTR. Susan Carpenter[1], Mary Jane Long[1] and Sylvia Perryman[2]. [1]Iowa State University, Ames, IA 50011 and [2]Rocky Mountain Laboratories, Hamilton, MT 59840. Genetic heterogeneity is a hallmark feature of all lentiviruses thus far characterized. One consequence of this heterogeneity is the presence of viral antigenic variants which evade the host immune system and contribute to viral persistence. It is also possible that genetic heterogeneity is important in biological variation and viral pathogenesis. To better define genetic structures associated with biological variation of equine infectious anemia virus (EIAV), an avirulent field isolate, designated MA-1, was molecularly cloned and sequenced. The sequence of MA-1 was compared with the published sequences of the prototype Wyoming isolate of EIAV and with antigenic and biologic variants of EIAV isolated from ponies following in vivo passage of EIAV-Wyoming. These comparisons identified a hypervariable region in the U3 regions of viral LTR. Sequence divergence in a 65 bp stretch between the CAAT and TATAA boxes was found to be as high as 37%. In addition, the presence and location of several enhancer elements, including direct repeats, core enhancer sequences and SP-1 binding sites, varied among these antigenic and biologic variants of EIAV. The heterogeneity in the viral LTR was in marked contrast to the 1-2% divergence found in the viral env gene. These results suggest that variation in viral regulatory sequences may be important in EIAV variant selection and pathogenesis.

CM 302 EXPERIMENTAL INFECTION OF RABBITS WITH HUMAN T-CELL LEUKEMIA VIRUS TYPE II. Gary L. Cockerell*, Michael D. Lairmore**, Aimee Post**, Beverly Roberts**, Joel Rovnak* and M. Glade Weiser*. *Department of Pathology, Colorado State University, Fort Collins, CO 80523; and **Retrovirus Diseases Branch, Centers for Disease Control, Atlanta, GA 30333.
To develop an animal model of infection with human T-cell leukemia virus type II (HTLV-II), weanling rabbits (n=4) were inoculated intravenously with $1x10^8$ lethally irradiated Mo-T cells, a cell line derived from an HTLV-II-infected patient with a T-cell variant of hairy cell leukemia. Control rabbits (n=2) were inoculated similarly with irradiated, uninfected Hut 78 human T-cells. Persistent seroconversion to HTLV-II p24, as determined by western immunoblot, occurred in all virus-inoculated rabbits by 2-weeks post-inoculation; seroconversion to other HTLV-II *gag* or *env* antigens also occurred, but not as early or not in all virus-inoculated rabbits. Serological cross-reactivity to HTLV-I was limited to the p24 antigen. Confirmatory evidence of *in vivo* infection in HTLV-II-inoculated rabbits included the presence of HTLV-II p24, determined by antigen capture ELISA, in culture supernatants of peripheral blood mononuclear cells (PBMC) in 2 out of 4 rabbits, and HTLV-II *pol* sequences in PBMC following amplification by polymerase chain reaction in 3 out of 4 rabbits tested over a period of 6-months post-inoculation. All control rabbits remained negative for evidence of HTLV-II infection by each of these criteria during the same time period. No clinical or hematological abnormalities have occurred in infected rabbits to date. These results demonstrate that rabbits are susceptible to experimental infection with HTLV-II, and that the host-viral relationship in rabbits is similar to that which occurs in naturally occurring HTLV-II infections in humans.

CM 303 HEMATOPOIETIC TARGET CELLS OF APLASTIC ANEMIA-INDUCING FELINE LEUKEMIA VIRUS, Gregg A. Dean*, James I. Mullins§ and Edward A. Hoover*, *Department of Pathology, Colorado State University, Fort Collins, CO 80523, §Department of Microbiology and Immunology, Stanford University, Stanford, CA 94305.

Bone marrow suppression resulting in fatal aplastic anemia can be induced in outbred cats by a molecularly cloned feline leukemia virus [FeLV-C-Sarma (FSC)]. FeLV-FAIDS clone 61E is a molecularly cloned prototype subgroup A FeLV that is nonanemogenic. Superinfection interference of subgroup A viruses by subgroup C viruses but not the reverse suggests that the C-type viruses use a receptor in addition to the receptor used by A-type viruses. Moreover, previous studies employing chimeras constructed between these two FeLV clones demonstrated that the FSC genomic determinant of pathogenicity is contained within the 5' end of the envelope gene domain which codes for the major extracellular glycoprotein (gp70). In FSC infected cats, a decrease in bone marrow erythroid burst forming units (BFU-e) and erythroid colony forming units (CFU-e) coincide with the onset of viremia and precede a decline in hematocrit. In the terminal stages of anemia, granulocyte-macrophage colony forming units (CFU-GM) also decline. Both FSC and 61E are known to infect bone marrow hematopoietic cells yet lineage specificity in the distribution of virus receptors has not been demonstrated. Using flow cytometry and immunogold staining we have demonstrated that while FSC and 61E bind to high percentages of cells of all hematopoietic lineages, in virus infected cats, the erythroid lineage is infected first and to highest levels. Subsequently, the myeloid lineage also becomes infected. These results indicate that the erythrocytopathicity of FSC may result in interference of an erythroid-specific receptor.

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CM 304 FELINE NEUTROPHIL CHEMILUMINESCENCE SUPPRESSION BY THE SYNTHETIC PEPTIDE CSK-17, Charlene S. Dezzutti, J. Michael Marr and Richard G. Olsen, Center for Retrovirus Research, The Ohio State University, 1925 Coffey Road, Columbus, OH 43210 Experimental feline leukemia virus (FeLV) infection of cats still remains an efficient model to study retrovirus infections. This model allows researchers to determine the efficacy of new drugs against aquired immunodeficiency syndrome and adult T-cell leukemia. Further, researchers are able to dissect the retrovirus/immune system interactions. Previous work from this laboratory has demonstrated a neutrophil chemiluminescence (CL) suppression in FeLV-infected cats (viremic and nonviremic). Additional work demonstrated that this suppression can be induced by exogenously applied whole FeLV and the envelope protein, p15E. The current study provides data which show neutrophil CL suppression after exposure to the synthetic peptide CSK-17. This peptide has homologus regions corresponding to the transmembrane proteins from murine, feline and human leukemia/lymphotropic viruses. Complete suppression was recorded for 100 μg of CSK-17 while 20 μg produced partial but significant suppression and 10 μg of peptide had no suppressive effect on naive cat neutrophil CL responses. The data presented here imply a commonality observed in retrovirus-induced suppression which may aid in retrovirus vaccine and drug development.

This work was supported in part by the NIH grant CA 40714 and The Center for Retrovirus Research, Columbus, OH 43210.

CM 305 NEUROTROPISM OF FELINE IMMUNODEFICIENCY VIRUS, Steven W. Dow, Mary L. Poss, Edward A. Hoover, Department of Pathology, Colorado State University, Fort Collins, CO 80523.

The potential neurotropism of feline immunodeficiency virus (FIV), a lentivirus associated with development of immunodeficiency diseases in domestic cats, was investigated in naturally and experimentally infected cats. FIV-specific antibodies were detected in CSF from 7 of 9 naturally infected cats by immunoprecipitation; FIV was also recovered by co-culture of CSF from 6 of these cats with primary cultures of feline glial cells and/or feline PBL. After experimental FIV inoculation of 4 cats (via either the intrathecal or intra-bone marrow routes), CSF antibodies appeared within 16 weeks, together with CSF pleocytosis, increased IgG concentration, and high CSF IgG index. Brain lesions consisting of perivascular mononuclear cell infiltrates and glial nodules were observed in the midbrain and thalamus of 2 cats 7 months after inoculation. Virus was recovered by primary culture from several brain regions of these cats, but not from choroid plexus. FIV-specific antibodies increased in CSF of 2 cats evaluated through one year. Parallel in vitro studies of FIV neurotropism indicated that FIV infected primary cultures of astrocytes and brain macrophages. Astrocyte infection was productive and cytopathic (syncytium formation and eventual cell death), whereas macrophage infection was persistent and non-cytopathic. Oligodendrocytes and neurons were not productively infected. FIV persistently and noncytopathically infected human astrocytoma cells (cell line U-373). These results indicate that FIV is a neurotropic lentivirus and suggest that FIV infection of feline brain may be a useful model for study of HIV infection of the human CNS.

CM 306 DEVELOPMENT OF CLINICAL RESISTANCE TO AZIDOTHYMIDINE IN RAUSCHER MURINE LEUKEMIA VIRUS INFECTED MICE RECEIVING ORAL THERAPY, M.N. Ellis and D.W. Selleseth, Wellcome Research Laboratories, Research Triangle Park, NC 27709

Recent reports indicate that prolonged treatment of AIDS patients with azidothymidine (AZT) can lead to the emergence of drug-resistant strains of HIV. In our studies, we have attempted to generate AZT-resistant strains of Rauscher murine leukemia virus (RLV) by sequential passages of virus in AZT-treated mice. Groups of ten BALB/c mice were treated with either 1.0 or 0.1 mg AZT/ml in the drinking water for 21 days. On day 21, mice were sacrificed and spleens were weighed for assessment of splenomegaly. Only the enlarged spleens were homogenized and used to inoculate the next passage of mice. Mice treated with the high AZT dose (1.0 mg AZT/ml) exhibited insignificant splenomegaly through passage 6 (126 days of treatment). In contrast, mice receiving the suboptimal dose (0.1 mg AZT/ml) had increasing numbers of enlarged spleens in passages 4, 5, and 6. The mean spleen weights of passage 2 and 3 were 408 and 422 mg, while passages 4, 5, and 6 had means of 740, 1074, and 1071 mg, respectively. While our results suggest the development of clinical resistance, both the original virus and the isolates from passages 3 and 6 had similar *in vitro* sensitivities to AZT (IC_{50} = 2.9, 3.7, and 3.9 nM). We are continuing sequential mouse passages in hope of isolating an AZT-resistant strain of RLV to be used in studying retroviral resistance to antiviral drugs.

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CM 307 BOVINE LEUKEMIA VIRUS PATHOGENESIS AND CONTROL, J.F. Evermann[1], R.F. DiGiacomo[2], S.G. Hopkins[2], D. Derse[3], and P.L. Dorn-Williams[3], [1]Department of Veterinary Clinical Medicine and Surgery, Washington State University, Pullman, WA 99164, [2]Department of Comparative Medicine and Epidemiology, University of Washington, Seattle, WA 98195, and [3]Laboratory of Viral Carcinogenesis, National Institutes of Health, Frederick, MD 21701. Bovine leukemia virus (BLV) is an exogenous oncornavirus of cattle, which was initially reported in 1969 by Miller et al from dairy cattle with the adult form of lymphosarcoma. While BLV has been shown to be highly cell-associated with B lymphocytes, more recent studies by Williams et al (1988) have detected functional effects upon both the B and T cell populations. BLV is a transactivating retrovirus, which places it in the same group as HTLV-I and HTLV-II (Rice et al, 1984). While there may be several mechanisms whereby the BLV genome is expressed, our studies to be reported (using a BLV-LTR plasmid linked to a CAT gene) on the enhancement of BLV LTR function by bovine herpesvirus type 1 infection, indicate that this mechanism should be considered when studying the expression of BLV gene functions. Further investigations to be reported, have concentrated on understanding the epidemiology of BLV in populations of dairy cattle under natural conditions. The majority of BLV spread occurs horizontally by blood transfer using contaminated instruments (gouge dehorners, ear tagging and ear tattooing devices). Our studies have also demonstrated that rectal paipation using common obstetrical sleeves facilitates the spread of BLV between cattle. We propose that the transmission of BLV, in particular rectal deposition of virus-infected cells may serve as a potential model for the spread of retroviruses in nature.

CM 308 TREATMENT OF OPPORTUNISTIC CYTOMEGALOVIRUS AND HERPES SIMPLEX VIRUS INFECTIONS IN MURINE AIDS (MAIDS): Therapy with 9-(2-PHOSPHONYLMETHOXYETHYL) ADENINE (PMEA).

J.D. Gangemi, L. DeCastro, A. Ghaffar, E.P.Mayer, and *E.R. Kern, Dept. of Micro. and Immuno., Univ. So. Carolina Sch. Med., Cola. S.C., 29208 and *Dept. of Pediatrics, UAB Medical Center, Birmingham, AL 35254.

The nucleoside analog, PMEA, and a recombinant human interferon alpha B/D hybrid which is biologically active in murine cells, were evaluated in immunocompromised mice superinfected with a lethal dose of murine cytomegalovirus (MCMV) or herpes simplex type 1 virus (HSV-1). Murine AIDS was induced in C57BL/6 mice following infection with the LP-BM5 retrovirus complex. Infected mice developed splenomegaly, generalized lymphadenopathy and suppression of B and T cell responses 100 days post infection. Intraperitoneal inoculation of 100 days post LP-BM5 infected mice with murine CMV (2.0×10^6 pfu) or HSV-1 (1.5×10^6 pfu) resulted in death (8-12 days), while age-matched, immunocompetent mice were fully resistant. Mice treated with PMEA (100 mg/kg, i.p.) on the day of infection and on days 2, 4, 6, 8 and 10 following HSV-1 or MCMV infection were protected. In contrast, alpha interferon (10^6 international units per mouse) given subcutaneously on the same schedule had no effect on MCMV but did show some effect on HSV-1 infections.

CM 309 SEQUENTIAL STUDIES OF T- & B-CELLS IN MICE WITH A RETROVIRUS-ASSOCIATED IMMUNODEFICIENCY SYNDROME (IDS) RESEMBLING PEDIATRIC AIDS. Richard C. Hard, Jr., Malvin Stern & Bruce A. Fuchs, Medical College of Virginia/VCU, Richmond, VA 23298

RFM mice, perinatally inoculated with (T6xRFM)F1 spleen cells, develop a lethal IDS with many features of pediatric AIDS. The semiallogenic donor cells are vectors of retrovirus(es) and induce an allogenic Host Versus Graft reaction. Like pediatric AIDS, the murine IDS is manifested by lympho-splenomegaly, T cell depletion and polyclonal hyperglobulinemia despite poor primary responses to antigens.

In the present studies, progressive changes in the numbers of splenic and nodal T- and B-cells were measured by flow cytometry and proliferation assays. The severe progressive loss of responses to Con A exceeded the moderate declines in Th and Ts cells. The Th:Ts ratio of 0.76 in the nodes was less than controls (1.2), but was near normal (1.1) in the spleen. The cells of the chimeras responded less to new alloantigens (A/J) than to (T6xRFM)F1 donor antigens in MLR. There was also a progressive decline in responses to the B-cell mitogen, LPS.

These results have revealed a severe defect in the ability of T-cells to proliferate. This may indicate impairment or destruction of the IL-2 secreting Th1 subset. The loss of LPS reactivity may reflect the activation and maturation of B-cells known to occur in this model.

Supported by monies from the Carnes & Privat Memorial Funds.

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CM 310 TESTING OF AN EXPERIMENTAL HIV PARTICLE VACCINE IN SMALL LABORATORY ANIMALS AND CHIMPANZEES, Joachim Hilfenhaus, Jens-Peter Gregersen, Patricia N. Fultz[1], Research Laboratorys of Behringwerke AG, D-3550 Marburg, Fed. Rep. Germany, [1]Yerkes Primate Research Center, Emory University, Atlanta, USA. Purified and inactivated whole virus preparations of HIV-1 were produced from supernatants of infected cells. These 500-fold concentrates contained 3-4 mg/ml protein, 2-3 x 10^{11} physical viral particles and 10^{8} - $10^{8.6}$ $TCID_{50}$/ml before inactivation. Immunization trials in rabbits and guinea pigs were performed to find optimal immunization conditions with respect to dose responses, booster intervals and adjuvants. Subsequently, these HIV preparations were combined with three different adjuvants and used to immunize 9 chimpanzees. The chimpanzees were monitored for humoral and cellular immune responses by ELISA, western blot, virus neutralization, delayed type hypersensitivity, antibody and complement-dependent cytotoxicity, lymphocyte proliferation and ADCC assays. Weak and inconsistent responses were observed with $Al(OH)_3$ as adjuvant whereas Freund's incomplete adjuvant and an experimental adjuvant induced stronger humoral and cellular immune responses. Challenge infection of selected chimpanzees with infectious HIV-1 are planned to determine whether the induced immune responses against from an infection.

CM 311 LOCALIZATION OF SIMIAN IMMUNODEFICIENCY VIRUS IN SERIALLY SACRIFICED RHESUS MONKEY TISSUES BY *IN SITU* HYBRIDIZATION, F-S HU*, G. B. Baskin, M. Murphey-Corb, D. Kuebler, L.N. Martin, and B. Davison-Fairburn, Delta Regional Primate Research Center, Tulane University, Covington, La. 70433.

By using in situ hybridization methodology, we have directly localized simian immunodeficiency virus (SIV) in the tissues of rhesus monkeys that were sacrificed at intervals of 2,4, 8 and 24 weeks after inoculation Tissue sections were hybridized with ^{35}S-labeled gag-specific RNA probe and exposed to autoradiographic emulsion for 2 to 7 days. Numerous infected cells were found in lymph nodes, spleen, thymus and brain. In lymph nodes, most infected cells were in the paracortex, however, large amounts of viral RNA were also detected in the follicular dendritic cells of germinal centers. In the thymus, intense viral signal was shown in rare macrophages in the cortex, while numerous cells in the medulla had a low level signal. In brain, cells expressing viral RNA were in inflammatory lesions in the meninges and around vessels in the parenchyma. In lymph nodes no obvious relationship was found between numbers of infected cells and serum antigenemia, while such a relationship was apparent between the number of infected cells in the thymus and serum antigenemia.

CM 312 CHARACTERISTICS OF B CELL ACTIVATION AND PROLIFERATION IN MURINE AIDS. Dennis M. Klinman, Division of Virology, CBER, FDA, Bethesda Md, 20892.
A syndrome characterized by lymphadenopathy, hypergammaglobulinemia and immunodeficiency develops in C57Bl/6 mice inoculated with LP-BM5 murine leukemia viruses. A series of reproducible changes in the humoral immune system were detected through studies of the number and antigenic specificity of B cells activated in the course of this disease, The rate of B cell proliferation and the proportion of B cells activated to secrete Ig increased 10-fold within 4 weeks of inoculation. B cells reactive with a panel of 3 foreign antigens and 5 autoantigens were stimulated simultaneously and proportionally to secrete, demonstrating that such activation was polyclonal in nature. IgM secretion was disproportionately elevated during the initial phase of disease. From 4 - 12 weeks post inoculation, production of IgG antibodies (especially IgG2a) was more significantly affected, rising 30 fold. This isotype switch affected B cells of many different antigenic specificities, suggesting that an isotype switch inducing factor was being systemically overproduced in these mice. Consistent with such an interpretation, serum from infected mice induced in vitro LPS-activated B cells from normal animals to produce IgG2a. At 12 weeks post inoculation, infected mice began to express B cells repertoires which diverge markedly from those of uninfected animals ($p < .01$). This was accompanied by a 5 - 15 fold decline in serum Ig level and Ig secreting B cell number. These changes are comparable to those found in some human patients infected with HIV-1 and provide a useful model to study the association between retrovirus infection and the appearance of regulatory abnormalities in the humoral immune system.

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CM 313 IMMUNE RESPONSES TO RETROVIRAL INFECTIONS WITHIN THE MURINE CENTRAL NERVOUS SYSTEM
Jonathan Korostoff, Kenneth J. Blank*, and Glen N. Gaulton. Division of Immunobiology, Department of Pathology and Laboratory Medicine, Philadelphia, PA 19104 and *Department of Microbiology and Immunology, Temple University School of Medicine, Philadelphia, PA 19140. We have previously shown that exposure of neonatal BALB.B mice to a variant of Gross Murine Leukemia Virus, WB91-GV, within the first 24 hours after birth, resulted in a selective white matter infection within the CNS. Immunohistochemical analysis of both frozen sections prepared from the brains of animals immunized in this manner and of highly enriched glial cell primary cultures for viral gp70 expression indicated that oligodendrocytes and possibly a minor subset of astrocytes were the targets of this viral infection. Further, light microscopic analysis of brain tissue failed to reveal any overt signs of gross pathologic change associated with the infection. More recent data demonstrates the lack of expresssion of MHC Class I and II gene products in the brains of infected animals as well as the absence of virus specific antibody in the serum of these mice, suggesting a state of virus specific immunologic tolerance. We believe this system represents a novel model for studying the effects of persistent retroviral infection of the CNS, similar to situations which may precede the development of human neuropathologic disorders.

CM 314 DEMONSTRATION OF THE BIOLOGICAL HETEROGENEITY OF HTLV-I INFECTION IN A RABBIT MODEL.
Michael D. Lairmore*, Gary L. Cockerell**, Joel Rovnak**, Aimee Post*, Beverly Roberts* & M. Glade Weiser**. Retrovirus Diseases Branch*, Centers For Disease Control, Atlanta, GA; Department of Pathology**, Colorado State University, Fort Collins, CO.

To compare the biological effects of HTLV-I infection weanling rabbits were inoculated by systemic (intraperitoneal) or neural (intracisternal) routes with irradiated HTLV-I cell lines derived from patients with adult T-cell lymphoma (ATL) or from patients with HTLV-I-associated myelopathy/tropical spastic paraparesis (HAM/TSP). Control rabbits were inoculated with uninfected rabbit or human lymphoid cells. Infectivity of the viral cell inocula was demonstrated by the in vitro transfer of infection following co-culture with normal rabbit peripheral blood mononuclear cells (PBMC). In vivo infection of rabbits was demonstrated by the presence of antibodies to HTLV-I gag (p19, p24, p53) and env (gp46 or gp68) antigens in serum and/or cerebrospinal fluid, the detection of HTLV-I antigen (p19 and p24) in culture supernatants of PBMC and lymphoid tissues, and amplification of HTLV-I gag and pol sequences in tissues by polymerase chain reaction. To date, no clinical disease, hematological or pathophysiological abnormalities have been detected in the infected rabbits. However, differences in the patterns and intensity of antibody reactivities in serum and cerebrospinal fluid, in the level of HTLV replication from cultured tissues and the tissue distribution of HTLV occurred within and between groups of infected rabbits. In particular, virus isolation data indicated that a HTLV-I isolate derived from a patient with acute onset transfusion-associated myelopathy replicated much more efficiently and had a broader tissue distribution than the ATL isolate in rabbits.

CM 315 ELEVATED LEVELS OF CIRCULATING INTERLEUKIN-6 ARE ASSOCIATED WITH INFECTION WITH AN ACUTELY FATAL SIMIAN IMMUNODEFICIENCY VIRUS ISOLATE (SIVsm/pbj).
Mark G. Lewis[1], Deborah L. Birx[2], Philip M. Zack[3], Mary Ann Vahey[2], Robert R. Redfield[2], Donald S. Burke[2] and Peter B. Jahrling[3], [1]Southern Research Inst., [1,3]Disease Assessment Division, USAMRIID, Fort Detrick, MD 21701. [2]Division of Retrovirology, WRAIR, Suite 201, 13 Taft Ct, Rockville, MD, 20850.

Interleukin-6 (IL-6) is an important mediator of inflammatory responses, usually associated with acute infections and tissue damage. IL-6 is released by numerous cell types and one of its roles is induction of acute-phase reactants in the liver. We measured the levels of the cytokines IL-1 alpha, IL-6 and TNF alpha in serum samples of macaques experimentally infected with SIVsm/pbj, an acutely lethal SIV strain that causes death within 2 weeks of inoculation. Three different species of macaques (rhesus, cynomolgus and pigtailed) were used in this study, and are susceptible to SIVsm/pbj infection and acute death. No significant increase in circulating levels of IL-1a or TNFa were observed. The highest levels of circulating IL-6 were observed in pigtailed macaques dying acutely (>10,000 units/ml), a 1000 fold increase over normal. Cynomolgus macaques dying acutely developed between 100 to 1000 fold increases of IL-6 (1000 to 10000 units/ml) and rhesus macaques had between 10 to 100 fold increases (100 to 1000 units/ml). Rhesus that survived the acute disease associated with SIVsm/pbj had an initial rise of circulating IL-6 (>100 units/ml) which returned to near-normal levels by 10 to 15 days post challenge. Elevated levels of IL-6 message were observed in spleen and mesenteric lymph nodes of animals dying acutely. Levels of IL-6 correlate directly with virus load as determined by immunohistochemistry and serum p24, and were associated with elevated levels of acute-phase reactants. Supported in part by NCDDG IUO1A125619 and the US Army Medical Research and Development Command.

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CM 316 THE ROLE OF ADJUVANT IN SIV VACCINE INDUCTION OF PROTECTIVE IMMUNE RESPONSES, Thomas P. McGraw, James C. Carlson, Benjamin R. Vowels, Paul A Luciw, and Murray B. Gardner, Dept. of Medical Pathology, Univ. of California, Davis, School of Medicine, Davis, CA 95616. Rhesus monkeys were given a series of five immunizations with 100 ug of beta-propiolactone inactivated simian immunodeficiency virus (SIV_{mac}) delivered intramuscularly (IM) in a 1.0 mL volume of aqueous solution or in adjuvant. The adjuvant was either 250 ug of threonyl muramyl dipeptide (MDP) in 0.5 mL of Syntex emulsion formulation M (SAF-M)(Syntex Research, Palo Alto, CA) or incomplete Freund's adjuvant (IFA)(Gibco, Inc., Grand Island, NY). The adjuvants were mixed with 0.5 mL of an aqueous solution of SIV immediately prior to immunization. Immunization with aqueous SIV resulted in low ELISA antibody responses (O.D. = 1.0), whereas, high ELISA responses were observed in MDP and IFA immunized monkeys (O.D. $\geq$3.5) using HIV-2 plates (Genetic Systems Corp., Seattle, WA). Immunoblots using SIV antigen indicate that the MDP immunized animals produced antibodies to the major SIV proteins including gp120, whereas, IFA did not elicit responses to gp120. Monkeys immunized with aqueous SIV produced responses primarily to p66 and p55. Three weeks following the final immunization, the monkeys were challenged with live virus IM. Virus cultures were positive at 2 weeks post-challenge in 2/2 non-immunized, 2/2 adjuvant only control animals, 2/3 SIV aqueous solution and 1/2 SIV-IFA immunized animals. However, 3/3 SIV-MDP immunized animals remain virus culture negative at six weeks post-challenge suggesting that MDP adjuvant elicits immune responses that may significantly delay or prevent SIV infection. Investigations to determine neutralizing antibody, ADCC, and T cell proliferative responses are in progress. Further study is need to confirm protection from SIV.

CM 317 A MOUSE GENETICS MODEL FOR EVALUATION OF BIOLOGICAL RESPONSE MODIFIERS FOR THE TREATMENT OF RETROVIRAL INFECTION, John D. Morrey*, Reed P. Warren*, Kevin M. Okleberry*, Roger A. Burger*, Margaret I. Johnston¶, Robert W. Sidwell*. *AIDS Research Program, Utah State University, Logan, Utah 84322-5600, and ¶Division of AIDS, NIAID, Bethesda, Maryland 20892. Biological response modifiers (BRM's) have been the subject of considerable research efforts as an approach for enhancing current anti-AIDS therapies. A genetics mouse model using hybrid strains possessing genes that specifically regulate immune responses to Friend virus complex (FV) were used that have analogies to that seen in AIDS. The production and persistence of FV-specific neutralizing antibodies, not observed in Balb/c mice, are coupled with a reduction of infected cells and viremia. Regardless of the viral immune response, the disease is still fatal. Disease parameters evaluated in BRM-treated mice included infectious centers in the spleen, plasma virus, viral RNA, survival of infected animals and splenomegaly. Immune parameters included FV-specific neutralizing antibody, mitogen-induced blastogenesis, interleukin-1 production, interferon production and enumeration of splenic lymphocyte subpopulations. Infection was significantly inhibited in order of AZT (3'-azido-3'-deoxythymidine) > MVE-2 (maleic anhydride divinyl ether copolymer) > imexon (4-imino-1,4-diazobicyclo-[3.1.0]-hexan-2-one) > oxamisole. Bropirimine (2-imino-5-bromo-6-phenyl-4-pyrimidinone) appeared to enhance the infection as seen especially by increased splenomegaly and recoverable virus.

CM 318 FELINE LENTIVIRUS MODEL FOR CHEMOTHERAPY OF AIDS, Thomas W. North, Richard C. Cronn, Kathryn Martin Remington and Rolf T. Tandberg, Division of Biological Sciences, University of Montana, Missoula, MT. 59812. Feline immunodeficiency virus (FIV) causes on immune deficiency in cats that is very similar to the acquired immune deficiency syndrome (AIDS) in humans. The goal of this work is to determine whether the reverse transcriptase from FIV is similar enough to the RT from human immunodeficiency virus (HIV) to enable its use as a model for RT-targeted chemotherapy. The FIV RT has been purified and compared to the HIV RT (obtained from Dr. S. Hughes, NCI). These two enzymes are similar in physical properties, catalytic activities and sensitivities to inhibitors. The FIV RT has a MW of 67,000 as determined by SDS-PAGE; it is similar to the HIV RT in requirement for $MgCl_2$ and in template specificity. These two enzymes are also similar in their sensitivities to the 5'-triphosphates of 3'-azido-3'-deoxythymidine (AZT) and dideoxynucleosides, and to phosphonoformate. The sensitivity to AZT of FIV replication in cell culture is also similar to that of HIV. FIV replication is inhibited greater than 95% by 1 mM AZT. FIV should provide a useful model for coordination of in vitro and in vivo approaches for design of strategies for therapy of AIDS. (Supported by PHS Grant AI 28189 from NIAID; AZT and its 5'-triphosphate were provided by Burroughs-Wellcome.)

CM 319 NEUROTROPISM OF LENTIVIRUSES - AN ULTRASTRUCTURE STUDY, Kalman Perk, Department of Veterinary Biology, Koret School of Veterinary Medicine, Hebrew University of Jerusalem, Rehovot, Israel.
There is much evidence to indicate neurotropism by the lentiviruses. It is, however, unclear if or which neural cell type harbors the virus. This initiated the search for virus particles in neural tissue, by thin section electronmicroscopy in arthritis encephalitis (CAE) diseased goats. Different examined ganglion cells of the diseased goats showed clusters of typical intracytoplasmic A type retrovirus particles. The particles consist of two electron dense shells, of which the inner is more dense. These infected ganglia showed their normal and typical ultrastructure in spite of the fact that in some cells an abundance of A particles were present. Budding particles from cell plasma membranes, which are present in lentivirus infected permissible cell cultures, were not seen in any of the neural tissue examined. Since HIV and CAEV have many morphological and biological parallels, this will be comparatively discussed and presented.

CM 320 SEQUENCE VARIABILITY BETWEEN TWO DISTINCT MOLECULAR CLONES OF FELINE IMMUNODEFICIENCY VIRUS, Tommy R. Phillips, Randy L. Talbott, Christina Lamont, Susie Muir, Kathleen Lovelace, and John H. Elder, Department of Molecular Biology, Scripps Clinic and Research Foundation, La Jolla, CA 92037. The complete nucleotide sequences of two distinct clones of feline immunodeficiency virus (FIV) have been determined. The first clone, 34TF10, was from a Petaluma, California isolate; the second (PPR) was isolated from a cat in the San Diego area. The cats from which these viruses were obtained both showed signs of severe immunodeficiency. Comparison of the nucleotide sequences of the 34TF10 and PPR isolates revealed an overall sequence similarity of 91%. As expected, the percentage of similarity differed dramatically from one genomic region to another with the greatest degree of conservation in GAG (95%) and POL (93%) and the greatest diversity in ENV (86%). It was observed that the putative TAT exon 1 of the 34TF10 isolate prematurely terminated, producing a truncated TAT product relative to PPR. Interestingly, this truncation did not prevent either the *in vitro* or *in vivo* replication of this clone. Analysis of the LTRs indicated a 7% divergence between the two clones and a lack of conservation within the putative NF-kappa B site. However, the putative binding site for the enhancers AP1, AP4, and ATF were highly conserved as were the promoter and polyadenylation sites of the LTR.

CM 321 BOVINE IMMUNODEFICIENCY-LIKE VIRUS (BIV) INFECTION OF RABBITS: A SMALL ANIMAL MODEL FOR LENTIVIRUS-INDUCED DISEASE, Dominique Y. Pifat*, Willis H. Ennis*, Jeffrey L. Rossio*, Jerrold M. Ward†, and Matthew A. Gonda*, PRI* and NCI†, NCI-FCRF, P.O. Box B, Frederick, MD 21701.
BIV is a unique member of the Lentivirus subgroup of retroviruses. The BIV genome is organizationally most similar to that of primate lentiviruses in that it encompasses *gag*, *pol* and *env* structural genes as well as several non-structural/regulatory genes which are believed to play a role in the pathogenesis of the virus. BIV, which was first isolated from cattle, causes in these animals a disease syndrome which includes lymphadenopathy, lymphocytosis, central nervous system lesions, progressive weakness, and emaciation. We now report the experimental infection of rabbits with BIV. These infections have resulted in a persistent serologic response which was accompanied by pathologic lesions in organs of the immune system to include spleen and lymph nodes. In addition, infectious BIV was recovered from lymphoid organs in 100% of infected animals as early as one month and as late as one year post-inoculation. BIV-infected animals also showed signs of immune impairment based on mitogen stimulation assays. Immunoperoxidase staining for BIV *gag* antigens in affected organs demonstrated viral protein expression in macrophage-like cells, but not mesothelium. The infection of rabbits with BIV may prove to be a useful model system in which to study the biology of lentivirus-induced disease and to test novel experimental vaccines and antiviral compounds.

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CM 322 DISTINGUISHING PROPERTIES OF FELV-FAIDS ENVELOPE GLYCOPROTEINS, Mary L. Poss*, James I. Mullins**, Edward A. Hoover*, *Department of Pathology, Colorado State University, Ft. Collins, CO 80523; **Department of Microbiology and Immunology, Stanford University Medical School, Stanford, CA 94305.

FeLV-FAIDS virus induces an acute and fatal immunodeficiency disease in inoculated weanling cats. Two viral genomes, a major pathogenic variant (clone 61C) and a closely related but apathogenic genome (clone 61E), are identified by acquisition of 2 new restriction sites in *env* of 61C. A viral construct (EECC) created by fusing the 5' LTR, *gag* and *pol* of 61E with the *env*-3' LTR of 61C demonstrates in vivo pathogenicity similar to 61C, indicating that the pathogenic determinants of this virus reside in the *env* gene.

Several properties distinguished the envelope glycoproteins of 61C (or EECC) and 61E. Both the precursor glycoprotein, gp80 and the mature extracellular glycoprotein, gp70, from EECC were larger than those of 61E; a property conferred by differences in post translational modifications of the proteins. Post translational changes were also responsible for antigenic differences between these viral glycoproteins. Furthermore, the rate of post translational processing of the EECC gp80 was significantly slower than that for the 61E gp80 resulting in the intracellular accumulation of gp80 in EECC infected cells. The steps in the glycosylation pathway responsible for the processing delay were removal of the first and second glucoses from the nascent oligosaccharide. Experiments conducted in susceptible target cells, (3201 cells, a T-lymphoblastoid cell line that are killed following infection with EECC) indicated that the delayed processing of precursor envelope glycoproteins contributed significantly to the cytopathic effects of the FeLV-FAIDS virus.

CM 323 LYMPHOCYTE SUBSET ALTERATIONS IN CATS INFECTED WITH IMMUNODEFICIENCY DISEASE INDUCING CHIMERAS OF FeLV-FAIDS, Sandra L. Quackenbush*, Gregg A. Dean*, Christopher D. Ackley**, Peter R. Donahue***, Diane Pardi****, Gerald N. Callahan*, Max D. Cooper**, James I. Mullins****, Edward A. Hoover*. *Department of Pathology, Colorado State University, Ft. Collins, CO 80523, **Department of Pediatrics and Microbiology, University of Alabama at Birmingham, Birmingham, AL 35294, ***Biomedical Research Institute, St. Paul, MN 55102, ****Department of Microbiology and Immunology, Stanford University Medical School, Stanford, CA 94305.

To further elucidate both the immunopathogenesis and viral genetic determinants responsible for immunodeficiency syndrome induced by FeLV-FAIDS, cats were inoculated with three viral chimeras constructed between the two molecularly cloned components of FeLV-FAIDS: the low pathogenicity "common form" virus (61E) and the pathogenic variant (61C).

Beginning 14 weeks after inoculation significant decreases of CD4 lymphocytes developed in 11 of 12 cats of the FeLV-FAIDS chimera infected groups when compared with normal control cats. No significant concomitant changes were present in either CD8 or B lymphocytes. Progressive decline in CD4 cells occurred throughout the course of infection and development of immunodeficiency syndrome. Prior to the initial perceptible decline in CD4+ cells, the capacity of all FeLV-FAIDS chimera-infected animals to produce a T cell dependent antibody response to (KLH immunization) was markedly impaired whereas both 61E-infected and uninfected control cats produced normal antibody responses. During this same period, the blastogenic responses of the FAIDS chimera-infected cats to the phytomitogens were within normal limits.

Our results imply that depletion of a CD4 cell subset important in antibody production may be one of the earliest events in the pathogenesis of immunodeficiency caused by FeLV-FAIDS.

CM 324 SYNTHESIS AND PROCESSING OF THE TRANSMEMBRANE ENVELOPE PROTEIN OF EQUINE INFECTIOUS ANEMIA VIRUS. Nancy R. Rice[1], Louis E. Henderson[1], Raymond C. Sowder[1], Terry D. Copeland[1], Stephen Oroszlan[1], and John Edwards[2], [1]Laboratory of Molecular Virology and Carcinogenesis, BRI-Basic Research Program, NCI-Frederick Cancer Research Facility, Frederick, MD 21701-1013 and [2]Department of Veterinary Pathology, College of Veterinary Medicine, Texas A & M University, College Station, TX 77843

In contrast to the type C retroviral transmembrane proteins (TMP), whose C termini occur only slightly downstream from the membrane-spanning domain, lentiviral TMPs have long C-terminal extensions. The equine infectious anemia virus (EIAV) TMP, for example, contains more than 200 amino acids after the membrane-spanning region. We have studied the synthesis and processing of the EIAV TMP in tissue culture adapted virus as well as in the virulent Wyoming strain, using immune precipitation and immunoblotting experiments. We observed that the EIAV TMP is cleaved, presumably in the virus, into the membrane-spanning glycosylated gp 32-35 and the downstream nonglycosylated p20. Fractionation of EIAV proteins by high pressure liquid chromatography led to the isolation of p20 and the determination of the precise cleavage point. We have also observed that in some cell lines producing attenuated virus two sizes of envelope precursor polyprotein are synthesized. The larger represents the entire *env* gene while the smaller is missing about 180 amino acids at the C terminus of the TMP. As in the simian immunodeficiency virus, virus with the truncated envelope is selected for in culture. (This research was sponsored in part by the NCI, DHHS, under contract NO. NO1-CO-74101 with BRI.)

Animal Models of Human Viral Diseases: Relevance to Developmental Therapeutics

CM 325 IN VIVO INFECTION OF SHEEP WITH MOLECULARLY CLONED BOVINE LEUKEMIA VIRUS. Joel Rovnak*, James W. Casey**, Wayne A. Jensen*, Ann L. Boyd***, M. Glade Weiser* and Gary L. Cockerell*. *Department of Pathology, Colorado State University, Ft. Collins, CO 80523, ** Department of Veterinary Microbiology and Immunology, Cornell University, Ithaca, NY 14853, ***Department of Biology, Hood College, Frederick, MD 21701.

The objective of this study was to determine the *in vivo* infectivity and pathogenicity of a molecular clone of bovine leukemia virus, BLV-913, previously derived from BLV-infected fetal lamb kidney (FLK) cells. FLK cells contain 4 full length proviral copies per cell; all 4 proviruses have been cloned and 3 out of the 4 clones have been shown to be infectious *in vitro* following microinjection into bovine embryonic spleen (BESP) cells, as evidenced by syncytia induction, expression of BLV RNA and proteins, and reverse transcriptase activity in culture supernatants. BLV-913 is a construct of the large 5' Eco RI fragment of infectious proviral clone 9 and the small 3' Eco RI fragment of infectious proviral clone 13, and is also infectious for BESP cells *in vitro*. Two 3-month old lambs were inoculated intraperitoneally with $1x10^7$ BLV-913-infected BESP cells. Both lambs seroconverted to BLV by 4-weeks post-inoculation (p.i.), and remained persistently seropositive during an 18-month period of observation. BLV *pol* sequences, amplified by polymerase chain reaction, were first detected in peripheral blood mononuclear cells (PBMC) at 5- and 9-months p.i. in the 2 lambs. At 15-months p.i., $1x10^8$ PBMC from each BLV-913-infected lamb were transferred intravenously to each of 2 additional 3-month old lambs. One out of each set of 2 recipient lambs seroconverted to BLV by 5-weeks p.i. To date, no clinicopathological abnormalities have been observed in any BLV-913-infected lambs. These results demonstrate the *in vivo* infectivity of molecularly cloned BLV in sheep; this system will provide a valuable model for future pathogenetic studies of BLV infection.

CM 326 AZIDOTHYMIDINE AND BONE MARROW TRANSPLANTATION AS A TREATMENT FOR RAUSCHER MURINE LEUKEMIA VIRUS INFECTION OF MICE, D.W. Selleseth, D.W. Barry and M.N. Ellis, Wellcome Research Laboratories, Research Triangle Park, NC 27709

BALB/c mice infected with Rauscher murine leukemia virus complex (RLV) develop massive splenomegaly in two to three weeks and then go on to develop lymphocytic leukemia. Bone marrow transplants (BMT) have been done by other investigators in an attempt to eradicate RLV-induced leukemias. Previous studies have also shown (azidothymidine AZT) to have antiviral activity against RLV. In our studies, oral AZT therapy was combined with a BMT in an attempt to completely eradicate RLV from infected mice. Mice were inoculated intraperitoneally with a homogenate from RLV-infected mouse spleens. Fourteen days later the mice were irradiated with 946 R, followed by intravenous injection of bone marrow from syngeneic donors. Mice were given AZT in the drinking water at the time of infection (2 weeks before BMT), one week before BMT, or at the time of BMT. Animals receiving AZT therapy two weeks before BMT had longer survival, less splenomegaly, lower WBC counts, and lower serum titers of virus at 14 and 27 days after transplant than mice receiving AZT treatment only one week before or at the time of BMT. Even though the combination was unable to completely eradicate RLV from infected mice, two weeks of oral AZT before BMT appears to slow the progress of the disease. Subsequent studies included combined oral AZT with intraperitoneal doses of mouse interferon as treatment.

CM 327 USE OF THE PUNTA TORO VIRUS MURINE PHLEBOVIRUS MODEL FOR THE EVALUATION OF IMMUNOMODULATING AGENTS ALONE AND IN COMBINATION WITH ANTIVIRALS. R.W. Sidwell, J.H. Huffman, V.K. Singh, R.P. Warren, J. Coombs, R. Burger, M. Kende, and J.H. Huggins. Antiviral Research Program, Utah State University, Logan, UT; and U.S. Army Medical Res. Inst. for Infectious Diseases, Ft. Detrick, Frederick, MD.

Subcutaneous inoculation of C57BL/6 mice with Punta Toro virus (PTV), a *Phlebovirus* closely related to the serious African pathogen, Rift Valley fever virus, results in a lethal infection associated with profound immunosuppression in the host. The total white blood cells, peripheral blood lymphocytes, T cells, and T-suppressor/cytotoxic cells all decline dramatically 2 to 3 days after virus inoculation. The liver becomes severely infected, with maximal icterus and serum transaminase levels occurring by day 5. Virus is recovered from serum, liver, and white blood cells. Percentages of splenic T and B cells, mitogen-induced T and B cell responses and interleukin-2 production all decline with the infection. This model has been used to evaluate the antiviral efficacy of a variety of immunomodulating substances. Those considered markedly inhibitory to the PTV infection include ampligen, bropirimine, MVE-1, MVE-2, AM-3 and mannozym. Combinations of ribavirin, bropirimine, ampligen, AM-3, MVE-1 or MVE-2 have been studied in this model. (Supported by contract DAMD 17-86-C-6028 from the U.S. Army Medical Research Development Command).

CM 328 **ISOLATION OF cDNA ENCODING THE tat AND PRESUMPTIVE rev GENE PRODUCTS OF EQUINE INFECTIOUS ANEMIA VIRUS.** Robert M. Stephens and Nancy R. Rice, Laboratory of Molecular Virology and Carcinogenesis, BRI-Basic Research Program, NCI-Frederick Cancer Research Facility, Frederick, MD 21701-1013
In order to determine the structure of equine infectious anemia virus (EIAV) regulatory gene products, a cDNA library was constructed from a single-cell clone of EIAV-infected canine cells. Fourteen cDNAs were isolated that were at least 1.3 kb in size and that hybridized with probes from both the 5′ and 3′ ends of the genome. Splice junctions have been determined in five of these, and three have been completely sequenced. All five had the same splicing pattern and contained four exons. Exon 1 was drawn from the 5′ end of the genome, as is typical of retroviruses. Exon 2 contained the tat-related region located between *pol* and *env*. Exon 3 contained bases 126-226 of the *env* gene, and exon 4 contained the rev open reading frame at the 3′ end of the genome. The structure of the cDNAs predicts a bicistronic message. The tat protein is apparently encoded by exons 1 and 2 and is initiated without an AUG codon. The rev open reading frame of exon 4 is fused to a segment of envelope in exon 3, such that the presumptive rev protein contains 30 envelope and 135 rev residues. Using peptide antisera this protein has been detected in EIAV-producing equine cells. (This research was sponsored in part by the NCI, DHHS, under contract NO. NO1-CO-74101 with BRI.)

CM 329 SHORT OPEN READING FRAME STUDIES IN VISNA AND CAEV-ANIMAL MODELS OF HIV. Glenda Watson and Gordon Harkiss. Department of Veterinary Pathology, University of Edinburgh, Edinburgh, Scotland.
Visna virus and caprine arthritis and encephalitis virus (CAEV) are lentiviruses which cause lesions of the brain, joints and lungs in sheep and goats respectively. In common with HIV, visna virus has several short open reading frames (sorfs) which code for regulatory proteins. One of these sorfs, tat, encodes a protein which transactivates viral gene expression. Previous reports have suggested that this activity is lacking in CAEV.
We report here the results of our studies with the sorfs of visna and preliminary evidence for a tat homologue in CAEV.

CM 330 ANALYSIS OF SIV GENE EXPRESSION IN VIVO IN ACTIVATED T CELLS AND RESTING, MEMORY CELLS. Dennis M. Willerford*, Beverly Dere*, Edward A. Clark† and W. Michael Gallatin*. *Fred Hutchinson Cancer Research Center, Basic Sciences Division, 1124 Columbia Street, Seattle, WA 98104; †Department of Microbiology and the Washington Regional Primate Research Center, University of Washington, Seattle, WA 98195.

We have shown that a subset of $CD4^+$ lymphocytes expressing high levels of CD44 are selectively infected with SIV *in vivo* (Gallatin *et al.* Proc. Natl. Acad. Sci. USA 86:3301, 1989). Within this subset both activated $CD45R^+$ cells and non-cycling, $CD45R^-$ cells, which have been linked to immunologic memory, harbor viral DNA by PCR analysis. We hypothesize that these actively cycling and non-cycling populations represent productive and latent infections, respectively. To investigate the nature of viral infection in each of these subsets, we have used PCR to amplify cDNAs corresponding to specific spliced mRNAs. Sequence analysis of these products indicate that we can reliably identify individual transcripts for tat, rev, env, vpr, vpx and nef, as well as several other virus-specific mRNAs. In addition to using this approach to study viral activity in functionally distinct subpopulations among $CD4^+$ lymphocytes, an assessment of specific viral gene expression in SIV-infected macaques at different clinical stages may shed light on factors responsible for progression of SIV and HIV disease.

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CM 331 COMBINATION THERAPY UTILIZING ZIDOVUDINE, ALPHA INTERFERON, AND IL-2 IN THE TREATMENT OF FELV-INDUCED IMMUNODEFICIENCY SYNDROME (FELV-FAIDS), Nordin S. Zeidner*, Matthew H. Myles* Candace K. Mathiason-Dubard*, Lucy M. Rose**, Donald L. Hill**, James I. Mullins*** and Edward A. Hoover*, *Department of Pathology, Colorado State University, Ft. Collins, CO 80523, **Biochemistry Research, Southern Research Institute, Birmingham, AL 35255, ***Department of Microbiology and Immunology, Stanford University, Palo Alto, CA 94305.

Zidovudine inhibited replication of an immunodeficiency inducing strain of feline leukemia virus *in vitro* at concentrations of 0.5-0.005 μg/ml. A 30% additive antiviral effect was achieved *in vitro* when AZT was combined with human recombinant alpha interferon 2a (IFNα) at concentrations of 500-1000 units/ml. When activated, immune lymphocytes were transferred onto infected fibroblast targets, antiviral activity was demonstrated at effector-target ratios as low as 5:1. This antiviral activity was enhanced another 20% when cells were transferred onto infected targets in combination with AZT plus IFNα, at a dose of IFNα which only minimally inhibited virus replication.

Oral administration of AZT (20mg/kg) alone or in combination with IFNα or interleukin-2 (IL-2) throughout a 6 week treatment period enabled cats to resist challenge with FeLV-FAIDS. In contrast, those cats treated with IFNα or IL-2 alone became persistently antigenemic in parallel with placebo treated controls. Although antigenemia remained undetectable in AZT treated cats throughout an 80 day period post inoculation, latent FeLV-FAIDS in bone marrow was detectable by *in vitro* culture of progenitor cells. Serial analysis of circulating p27 antigen, neutralizing antibody, and quantification of latent, reactivatable virus indicated that only those cats receiving AZT in combination with IFNα were able to completely resist FeLV-FAIDS challenge. We are presently investigating the use of these protocols in combination with adoptive transfer of activated immune lymphocytes to treat established persistent retrovirus infection in the FeLV-FAIDS model.

CM 332 SIMIAN FOAMY VIRUS (SFV) IS A SPUMARETROVIRUS ENCODING A TRANSACTIVATOR THAT FUNCTIONS ON SEQUENCES IN THE LONG TERMINAL REPEAT (LTR)
Ayalew Mergia, Karen E. Shaw, Elissa Pratt-Lowe, and Paul A. Luciw, Department of Pathology, University of California, Davis, CA 95616.

We have molecularly cloned the genome of simian foamy virus-type 1 (SFV-1), a spumaretrovirus isolated from rhesus monkeys (J. Virol. 64:406-410, 1990). Sequence analysis reveals that SFV-1 is 60% to 80% related to the human foamy virus (HFV) reported by Flugel and coworkers (J. Virol. 62:1590-1597, 1988). To investigate mechanisms regulating viral gene expression directed by the promoter in the long terminal repeat (LTR), we are using transient expression assays in several types of tissue culture cells. In these transfection experiments, levels of LTR-directed gene expression are more than 50-fold higher in cultures of SFV-1 infected cells than in uninfected cells. We have found that SFV-1 contains several open reading frames (ORFs) extending from the end of the envelope gene into the 3' LTR. Spliced RNA transcripts from the ORF region have been detected and these have been used to make cDNA libraries for detailed characterization. Current efforts are directed at (i) identifying the ORF(s) that has transactivator function, (ii) localizing the cis-acting responsive element in the LTR, and (iii) elucidating the mechanism of transactivation.

Animal Models for Papillomavirus and Respiratory Virus Infections

CM 400 TRANSGENIC MICE WITH RESISTANCE TO A VIRAL DISEASE, Heinz Arnheiter, Susan Skuntz, Mathieu Noteborn, Ellen Meier, Stephen Chang, and Charles Weissmann, Lab. of Viral and Molecular Pathogenesis, NINDS, NIH, Bethesda, MD 20892, and Institute for Molecular Biology I, University of Zürich, CH-8093 Zürich, Switzerland.

The Mx1 protein of mice is a nuclear antiviral protein known to protect tissue culture cells specifically and efficiently against infection with influenza virus. We have placed an Mx1 cDNA behind an interferon-responsive regulatory element, and we have introduced this construct into the genome of *Mx*-negative, influenza-susceptible mice. According to the levels to which transgene mRNA and protein are induced by application of double-stranded RNA, transgenic lines can be separated into high-, low- and non-responders. When challenged intracerebrally with a neurotropic influenza virus, high responders produce high amounts of Mx protein - locally at the sites of initial viral replication - and little virus, and they survive infection over a large range of viral doses. Non-responders show no detectable Mx production, rapid viral spread, and the animals die of infection as do non-transgenic littermates. Surprisingly, animals of a low-responder line survive infection with high virus doses but they die, though somewhat later than control animals, after infection with low virus doses presumably because too little Mx protein is produced in the early phases of infection. These results illustrate how animals can be rendered virus-resistant by gene-therapy, and how the outcome of a viral disease is determined by a subtle balance between the dose of the infecting virus, and the sites and levels of accumulation of antiviral host factors.

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CM 401 THE IMMUNOPATHOLOGY OF INFLUENZA A VIRUS INFECTION IN THE MOUSE, Jeffery A. Engelhardt, Cheryl A. Brodhecker, Joseph Tang, Joseph M. Colacino and Carlos Lopez, Departments of Morphologic Pathology and Virology Research, Lilly Research Laboratories, Indianapolis, IN 46285

Adult CD-1 mice were infected intranasally with approximately 10^3 PFU of influenza A/Ann Arbor. Lungs from infected and control mice were examined histologically at 1, 3, and 5 days post-infection (pi). Acute bronchiolitis was evident at 1 day pi. By 3 days pi, the bronchiolitis had increased in severity and extended into the adjacent interstitium. Alveolar degeneration was accompanied by the influx of alveolar macrophages and lymphocytes. At 5 days pi, there was a severe, diffuse necrotizing alveolitis accompanied by pulmonary congestion and edema, fibrin exudation, and an intense inflammatory cell inflitrate composed of alveolar macrophages and lymphocytes admixed with low numbers of neutrophils. The lymphocyte population was subtyped in frozen sections of lung using antibodies directed against T4 and T8 surface markers. The identity of alveolar macrophages was confirmed by antibodies directed against MCA antigen. The most extensive alveolar damage was related to the influx of lymphocytes and alveolar macrophages, indicating the potential of a host-mediated pathogenesis for the necrotizing alveolitis.

CM 402 THE DEVELOPMENT AND COMPARISON OF THREE MODEL SYSTEMS IN MICE INFECTED WITH INFLUENZA A, IN WHICH TO EVALUATE THE ANTIVIRAL POTENTIAL OF NOVEL COMPOUNDS.

D Michael Ryan, Fiona C. Cook and Elizabeth Macdougall, Glaxo Group Research Limited, Greenford, UK

The mouse is the most commonly used laboratory model of influenza for screening potentially useful antiviral compounds. For the selected virus strains (A/Texas PR/8 and A/Singapore 1/57) we have developed three distinct model infection systems by using three levels of intranasal challenge. The three systems vary in their biology and utilise endpoints which measure, in decreasing order of challenge:- a) mortality, b) macroscopic lung consolidation scores and lung virus titres in the absence of visible lung consolidation. We used Ribavirin (RBV) and Amantadine (AMT) to compare the relative usefulness of the three models as screens for evaluating antiviral compounds. At the highest challenge level, AMT was effective at preventing death at 10mg/kg/dose, whereas RBV was ineffective at 100mg/kg/dose. However, surviving mice still had obvious lung consolidation and high titres of virus in the lung. In the lung consolidation model, while AMT and RBV were effective at reducing consolidation scores at doses down to 2mg/kg and 20mg/kg respectively, their effect on lung virus titres was minimal even at doses of 100mg/kg. At the lowest challenge level, in the absence of macroscopic lung consolidation, AMT and RBV were effective at reducing lung virus titres at doses of 10mg/kg and 50mg/kg respectively. Reduction in lung virus titres, is regarded as a good indicator of in vivo activity, and it is suggested that the model utilising lung virus titres in the absence of macroscopic lung consolidation or mortality, is the most discriminating model in which to screen for potentially useful antiviral agents.

CM 403 ANIMAL MODELS FOR THE COMMON COLD: TREATMENT WITH THE INTERFERON INDUCING AGENT 7-THIA-8-OXOGUANOSINE, Donald F. Smee, Michael L. Bartlett, Hassan A. Alaghamandan, and Roland K. Robins. Nucleic Acid Research Institute, Costa Mesa, CA 92626. Human coronaviruses and rhinoviruses cause 20% and 50% of common cold episodes, respectively. Animal models were developed that may be predictive of drug efficacy against the common cold. Intranasal (i.n.) inoculation of 3-4 day old rats with rat coronavirus (RCV) leads to pneumonia and death by 6 to 9 days. Treatments with 7-thia-8-oxoguanosine (50-200 mg/kg/day) administered either intraperitoneally or i.n. 24 and 18 hours before virus challenge reduced mortality by ≥75% in infected rats. EMC virus is a picornavirus in the same family as human rhinoviruses, but causes encephalitis and death in mice in 4-5 days. Thus, the pathogenesis of EMC virus is much different than rhinovirus-induced colds in man. However, mice infected i.n. with EMC virus developed high virus concentrations in the nose and lungs, in addition to the spleen and brain. In this model, inhibition of nasal virus replication should equate to antiviral activity in the respiratory tract. Indeed, i.n. treatments with a 1% solution of 7-thia-8-oxoguanosine suppressed nasal virus titers, and also inhibited virus titers in spleen and brain. A three day delay in development of brain virus titer corresponded to a similar delay in death. Radioactive 7-thia-8-oxoguanosine administered i.n. distributed primarily to the nose and mouth of mice and rats in 5 minutes, indicating targeted antiviral drug activity in the nasal area. The animal models we describe may be useful for evaluating treatments of the common cold.

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CM 404 EFFICACY OF LY217896, SODIUM SALT IN ANIMAL MODELS OF HUMAN RESPIRATORY VIRUS DISEASES, Joseph Tang, Joseph M. Colacino, James Terry, Ernie Wu, Wayne Spitzer, and Don DeLong, Lilly Research Laboratories, Indianapolis, IN 46285 The sodium salt of LY217896 (1,3,4-thiadiazol-2-ylcyanamide), was shown to be highly effective against a number of strains of influenza A and B viruses in the mouse model system. LY217896 (3 mg/kg/day) administered in the diet, in the drinking water, by oral gavage, by i. p. injection, or by aerosolization, provided protection to CD-1 mice lethally challenged with influenza A or B virus. Effective administration of drug could be delayed for up to 96 hours post infection; a single dose of 200 mg/kg was found to be highly protective as well. LY217896 was found to be more effective than either adamantyl amine or ribavirin, two compounds which have anti-influenza activity. Mice treated with protective levels of LY217896 were resistant to a subsequent lethal challenge of influenza virus indicating that the animals were able to develop immunity to the initial infection in the presence of drug. Administration of LY217896 to uninfected mice did not induce interferon-like activity or interfere with natural killer cell function. In the ferret, LY217896 was effective in preventing the induction of fever by influenza virus infection. In the hamster, LY217896 inhibited respiratory syncitial virus replication as shown by reduced virus titers in nasal washings.