PERSISTENT VIRUSES

J. Stevens and G. J. Todaro, Organizers

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Mechanisms by Which Viruses Persist

559 THE ADENOVIRUS TUMOR ANTIGENS, Arnold J. Levine, Susan Ross, Jane Flint, Department of Biochemical Sciences, Princeton University, Princeton, N.J. 08540.
Antiserum from hamsters carrying adenovirus induced tumors derived from five different transformed cell lines, has been employed to identify and map the adenovirus tumor antigens. These sera were used to immunoprecipitate ^{35}S-methionine labeled adenovirus proteins synthesized during productive infection of human cells. Each different antiserum immunoprecipitated a unique subset of the adenovirus early proteins as follows: Ad2 serum, 72K, 58K, 20-21K, 10-15K; Ad1-SV40 serum, 72K, 58K, 18-19K, 10-15K; ND-1 serum, 58K, 49K, 18-19K, 10-15K; ND4 serum, 72K, 58K, 49K, 25K, 20K, 18-19K, 10-15K; 14b serum, 58K and possibly 10-15K. This analysis identified seven immunologically distinct adenovirus proteins. By determining which portions of the adenovirus genome (and viral RNA sequences) are present or absent in these transformed cell lines, it has been possible to map the structural genes of these tumor antigens on the adenovirus genome.

560 DEFECTIVE INTERFERING PARTICLES: THEIR GENERATION AND ROLE IN VIRUS PERSISTENCE,** Alice S. Huang, Sheila P. Little, M.B.A. Oldstone* and Donald Rao, Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, MA 02115.
Defective interfering (DI) particles are efficient and specific inhibitors of virus growth. For vesicular stomatitis virus (VSV), the inhibition occurs during the replication of genome RNA. This is demonstrated by the absence of genome RNA synthesis and subsequent secondary transcription in cells co-infected with standard and DI VSV. Despite the drastic reduction in total viral RNA synthesis and in the formation of progeny virions, primary transcription by input standard virions and primary translation takes place normally. Also, shedding of virus-specific soluble antigens from the co-infected cells still occurs. Examination of the co-infected cell surface by immunofluorescence indicates that the cell-associated viral antigens are less accessible to antibody on these cells than on cells infected with only standard virus. The alteration of the infected cell surface and the shedding of viral antigens by cells co-infected with standard and DI virus may aid in the maintenance of viral persistence in the infected host animal.

Although replication of the standard virus RNA is not detected in these co-infected cells, replication of DI RNA readily occurs. The ability of DI RNA to compete successfully against standard genome RNA resides in sequence alterations found in DI RNA. These alterations result in inverted complementary nucleotide sequences at the termini of the DI genome. The termini are characterized by oligonucleotide mapping after T1 ribonuclease digestion and by self-annealing. The physiologic role performed by these RNA sequences, as well as the generation of these unusual deletions will be discussed.

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561 THE WAYS OF EBV PERSISTENCE IN HUMAN LYMPHOID AND CARCINOMA CELLS. Klein, G Dept. of Tumor Biology, Karolinska Inst.

Available information will be summarized concerning the interaction of EBV with human B-lymphocytes, starting from early events after infection (synthesis of EBNA, immunoglobulin and cellular DNA) and followed by the characterization of established EBV-carrying lines. Cellular and viral controls of spontaneous and induced EBV production will be reviewed and discussed. The review will be concluded with a consideration of the phenotypic changes induced by EBV in its host cells, human and non-human primate B-lymphocytes and human nasopharyngeal carcinoma cells.

562 REQUIREMENTS FOR PERPETUATION AND ERADICATION OF VIRUSES FROM ANIMAL POPULATIONS, N. Nathanson, and J. Martin, Johns Hopkins University, Baltimore, MD 21205.

Persistence of a virus in a population (perpetuation) is a distinct phenomenon from persistence in an individual animal or cell culture. All presently known viruses are necessarily capable of perpetuating in animal populations, although smallpox will soon become extinct. Eradication is of great importance as the ultimate method of control of viral disease, and a rational approach to eradication is best achieved through understanding the requirements for perpetuation.

Among the ecological patterns which viruses exhibit, the majority are confined to a single species and are transmitted directly from individual to individual. This common pattern is also the one most vulnerable to eradication. An analysis of the factors which determine perpetuation, under these ecological conditions, indicates that parameters of importance include: (i) population size; (ii) rate of population turnover; (iii) population density or frequency of contact between individuals; (iv) level of immunity; (v) duration of infectiousness following primary infection; (vi) ease of transmission during period of infectiousness; (vii) prevalence of acute infections and introduction of new infections. A stochastic model may be constructed for computer simulation, and this can be used to study the quantitative impact of these parameters.

A body of empiric observations, based mainly on serological studies in isolated populations, indicates important differences in the ability of different viruses to perpetuate under natural conditions. The history of efforts to eradicate specific viruses from animal or human populations provides another source of data establishing the requirements for eradication. Based upon these considerations, an attempt is made to predict which human viral infections it might be feasible to eradicate in the future.

Genetic Transmission of Tumor Virus Genes

563 ORGANIZATION OF THE GENOMES OF ENDOGENOUS AND EXOGENOUS AVIAN LEUKOSIS VIRUSES. Paul E. Neiman, Mark Groudine, Maxine Linial, Robert Eisenman. Division of Medical Oncology, Fred Hutchinson Cancer Research Center & University of Washington, Seattle, Washington 98104.

The genetically transmitted endogenous virus of chickens, is called RAV-0. All or nearly all of the genome nucleotide sequences of this virus can bedetected in the DNA of normal uninfected cells by molecular hybridization techniques at a level of about 1 to 2 copies/haploid genome. These endogenous viral genes are organized into chromatin subunits, or nu bodies similar (or identical) to those observed for cellular genes. The expression of endogenous viral genes are regulated by a partially characterized set of host cell genes. A number of transcriptionally active cellular genes appear to be selectively digested when chromatin or whole nuclei are treated with DNase 1. About ½ of the endogenous viral genes are susceptible to DNase 1 digestion when such studies are carried out with nuclei from chicken embryo cells regardless of the level of viral expression in these cells. Thus none of the known regulatory cell functions appear to alter viral nu body conformation. Exogenous leukosis viruses are not regulated by cells in the same manner as RAV-0. These agents replicate efficiently on chicken embryo cells while the replication of RAV-0 is restricted by the activity of host cell genes even when the virus is introduced as an exogenous agent. Finally RAV-0 appears to lack the oncogenic properties of the exogenous viruses. Despite these differences the genome of RAV-0 has extensive homology (about 80%) with those of exogenous leukosis viruses. A large portion of the sequence divergence between these agents is concentrated in a segment of the genome located within 1000 nucleotides of the 3' terminus. We are studying the relationship of this genome segment to the gene *env* which maps near this region and codes for the viral envelope glycoproteins or to a possible additional function in this divergent region which might specify some of the biological differences between endogenous and exogenous viruses.

564 THE ENDOGENOUS AND ACQUIRED PROVIRUSES OF MOUSE MAMMARY TUMOR VIRUS, Harold E. Varmus, J. Craig Cohen, Gordon M. Ringold[+], Peter R. Shank, Vincent L. Morris[+++], Robert Cardiff[++], and Keith R. Yamamoto[+], Depts. of Microbiology and [+]Biochemistry and Biophysics, UCSF, San Francisco, Calif., 94143, [++]Dept. of Pathology, UCD, Davis, Calif., 95616, and [+++]Dept. of Bacteriology, Univ. of Western Ontario, London, Onterio.

The mouse mammary tumor virus (MMTV) is an RNA tumor virus with several unusual features: its capacity to produce mammary carcinomas in susceptible mice, regulation of viral RNA synthesis by glucocorticoid hormones, the resistance of most cultured cells to infection, and the absence of a biological assay for infection *in vitro*. Virus strains can be transmitted through the milk of females from mouse strains with a high incidence of tumors or inherited as proviruses found endogenous to all tested mouse strains. These endogenous proviruses are present in 3-5 copies per haploid genome in most strains of laboratory mice, and they are distributed over at least two or three chromosomes. Most virus-induced mammary tumors contain a increment of copies of MMTV DNA sufficient for detection by measurement of hybridization kinetics with radiolabeled viral cDNA (*J. Mol. Biol.* 114:73, 1977).
We have used restriction endonucleases which cleave the DNA of MMTV at one site (*Eco* RI) and at several sites (*Pst* I) to study infection and tumorigenesis in BALB/c mice foster nursed by virus-producing C3H females. Proviruses newly-acquired during infection can be distinguished from endogenous proviruses by the nature of fragments generated with these enzymes. The new proviruses are linked to the DNA of mammary tumors and infected lactating mammary glands within a limited region of the viral DNA; the location of this region, based upon mapping studies with unintegrated MMTV DNA, suggests that the orientation of these proviruses is probably co-linear with the viral RNA genome. Comparisons of many mammary tumors and studies of lactating mammary glands with a large proportion of infected cells indicate that a large number of sites in the cellular genome can accommodate a new provirus; the acquired proviruses are rarely, if ever, found in tandem with each other or with endogenous proviruses. We cannot, however, distinguish between random integration and integration into a large number of preferred sites in the host genome. Similar conclusions about sites of integration have been reached in parallel studies of rat hepatoma cells, heterologous host cells which lack endogenous MMTV-specific sequences and have been infected in culture with MMTV (*Cell* 10:11-18, 1977).
Since cleavage of DNA from each mammary tumor with *Eco* RI generates a unique set of virus-specific fragments, we propose that the tumors constitute clones derived from a single cell among the many infected cells in a mammary gland; this proposal is supported by our finding that *Eco* RI digestion of DNA from several transplants of a primary tumor yields the pattern characteristic of the primary tumor.

565 VIRUS-SPECIFIC EXPRESSION AND GENE AMPLIFICATION IN MICE CARRYING MOLONEY LEUKEMIA VIRUS IN THEIR GERM LINE, Rudolf Jaenisch
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The exogenous Moloney leukemia virus (M-MuLV) has been established in mice as an endogenous virus by infecting preimplantation mouse embryos. The subline of mice derived from infected embryos was shown to transmit the virus as a single Mendelian gene and molecular hybridization experiments demonstrated that a single virus copy was integrated at this locus (1,2). All animals become viremic after birth and develop a virus-specific leukemia during the following months. It was shown that development of leukemia is accompanied by a somatic amplification of M-MuLV specific sequences in the tumor tissues.

In the present experiments, the relation of virus gene amplification to virus expression was examined. Molecular hybridization methods were employed to quantitate virus-specific DNA and RNA sequences in target and non-target organs of mice at different ages. The results demonstrate that low levels of M-MuLV specific RNA are present in target organs (spleen and thymus) of newborn animals, followed by a rapid increase during the next weeks of life. Between three and four weeks of age, maximal levels of M-MuLV specific RNA are synthesized in spleen and thymus and no further increase in virus expression occurs in tumor tissues. Non-target organs do not express virus-specific sequences.

The number of M-MuLV specific DNA copies was determined in the same tissues as used for RNA quantitation. Spleens of young mice (7-14 days of age) carry 1.2-1.4 M-MuLV copies per haploid mouse genome equivalent, whereas spleens of 30-day old mice carry about 2 virus copies. In tumor tissues, a further virus gene amplification to 3-4 copies per haploid mouse genome equivalent is observed. These results suggest a close correlation between virus expression and virus gene amplification. The extent of virus gene amplification appears to occur in several steps, one correlated to M-MuLV expression and a second correlated to leukemic transformation.

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566 FIVE DISTINCT GENETICALLY TRANSMITTED RETROVIRUS GROUPS IN PRIMATES. George J. Todaro, Laboratory of Viral Carcinogenesis, National Institutes of Health, Bethesda Maryland 20014

In the last year our laboratory has isolated and partially characterized three new genetically transmitted viruses of primates.

(1) A type C virus (MAC-1) from an Old World monkey, stumptail monkey (Macaca arctoides).
(2) A type C virus (OMC-1) from a New World monkey, owl monkey (Aotus trivirgatus).
(3) A type D virus (LAD-1) from an Old World monkey, langur monkey (Presbytis obscurus).

We have studied the frequency of retrovirus isolations from primate cell cultures. Primary tissues or established cell lines were cocultivated with a variety (8-10) of indicator cells that had previously been shown to be permissive for mammalian type C and type D virus replication. Of 22 cocultivations with various baboon tissues (Papio spp.), 17 yielded new baboon type C virus isolates; of 17 cocultivations with squirrel monkey tissue (various Saimiri subspecies), 14 yielded type D virus isolates. Cells from these species, regardless of tissue of origin, are "high probability yielders" of endogenous viruses. In contrast, each of the three new isolates has come from a single rare cocultivation. For example, of 65 cocultivations with cells from various Macaca species, only one isolate (MAC-1) has so far been obtained. The stumptail monkey, owl monkey and langur monkey appear, then, to be "low probability yielders" of complete infectious retroviruses.

All three of the new isolates are represented in multiple copies in normal cellular DNA of the species of origin; and related virogene sequences are found in genetically related species. The type D virus has proteins and nucleic acid sequences that are partially related to the previously described Mason-Pfizer monkey virus (MPMV) of rhesus monkey. Of all the primate DNAs tested, DNA transcripts to the genomes of either LAD-1 or MPMV hybridize with the highest thermal stability to langur cellular DNA (Benveniste and Todaro, Proc. Natl. Acad. Sci. U.S.A. 74: 4557, 1977). This would suggest that MPMV, now transmitted under natural conditions as an infectious virus among rhesus monkeys, may have been derived from an "escaped" endogenous virus of langur monkeys.

Neither of the new type C viruses are closely related by hybridization or by immunologic parameters to the previously described baboon type C viruses or to the squirrel monkey endogenous type D viruses. A single Old World monkey species, for example, the African green monkey, can be shown to transmit multiple copies of at least three distinctly different retroviral classes (two type C's and one type D) in its normal cellular DNA. Cell lines in culture that are not releasing virus can be shown, nevertheless, to be transcribing viral specific RNA.

567 QUANTIFICATION AND TOPOLOGY OF ENDOGENOUS AND EXOGENOUS PROVIRUSES IN THE CHICKEN GENOME, Marcel A. Baluda, Chang-Yan Chen and Lawrence M. Souza, University of California, School of Medicine & Molecular Biology Institute, Los Angeles, Ca. 90024

Endogenous proviral sequences are present in the nuclear DNA of all chicken cells. Endogemouse virus (RAV-0) production occurs spontaneously, or can be induced, only from some chicken strains. After infection with exogenous retroviruses, only a few of the newly synthesized proviruses become integrated in cellular DNA. The restricted number of proviruses which can integrate results either from site specificity and/or provirus induced restriction. We investigated the possibility of site specificity by studying the arrangement of proviral DNA in cellular DNA. First, we determined the number of integrated endogenous proviruses in non-producer $gs^{-}chf^{-}$ chick embryonic fibroblasts (CEF) and in CEF transformed by the Schmidt-Ruppin-Rous sarcoma virus strain A (SR-RSV-A). Two types of experimental approach were used to characterize the integration site: i) reassociation kinetics of proviral DNA present on randomly sheared cellular DNA fragments of defined size, and ii) autoradiography of proviral DNA in cellular DNA fragments generated by restriction endonucleases and separated by gel electrophoresis according to size. Saturation experiments with cellular DNA hybridized to an excess of 35S H^3-labeled RAV-0 RNA indicate that there are 2 copy equivalents of the endogenous provirus per haploid cell genome after correction for DNA replication in unsynchronized CEF. Comparison of proviral DNA bands in restriction enzyme generated DNA from RAV-0 producing cells and from non-producing cells as well as hybridization of 35S RAV-0 RNA with an excess of normal chicken DNA indicate that all the endogenous proviral DNA represents two complete, or nearly complete, RAV-0 genomes. The two endogenous proviruses appear to be integrated at two different sites, i.e., not in tandem in cellular DNA between cellular sequences reitereated 1000-2000 times. The hybridization of poly(A) selected 3' proximal viral RNA to DNA fragments which contain both viral and cellular sequences indicates that one end of the integrated provirus corresponds to the 3' end of the viral RNA; this implies that there is no permutation of the endogenous viral genes in the integrated state. In CEF transformed by SR-RSV-A, only one exogenous provirus becomes integrated per haploid cell genome. The SR-RSV-A provirus, like the endogenous provirus, is integrated between cellular DNA sequences reiterated 1000-2000 times but at a different location. Current analysis of restriction enzyme generated DNA fragments from several SR-RSV-A transformed clones should elucidate whether the SR-RSV-A provirus integrates at a unique site.

568 ANALYSIS OF RECOMBINANTS BETWEEN MURINE LEUKEMIA VIRUSES. Nancy Hopkins, Douglas V. Faller, Jean Rommelaere, and John Schindler, Department of Biology, Center for Cancer Research, Massachusetts Institute of Technology, Cambridge, MA 02139.

We have used genetic and molecular biological approaches to analyze endogenous murine leukemia viruses and recombinants between them. The goal of these studies is to identify and map viral genes that determine particular viral phenotypes and to characterize and compare endogenous murine leukemia viruses from different strains of mice. Two systems have been studied in detail. 1) "Exogenously derived" recombinants between leukemia viruses of the BALB/c mouse. 2) Viruses, designated MCF, that appear to arise by recombination *in vivo* in AKR mice. These studies have allowed us to determine approximate positions on the viral genome for a genetic determinant(s) of N- or B-tropism and for a determinant of properties of the viral glycoprotein, gp70. The studies have implications for the mechanisms by which biologically new, potentially highly leukemogenic viruses may arise in mice.

Activation, Restriction and Functions of 'Latent' Viral Genetic Information

569 TRANSFORMATION OF MAMMALIAN CELLS BY HUMAN CYTOMEGALOVIRUSES, Fred Rapp and Laszlo Geder, Department of Microbiology, The Pennsylvania State University College of Medicine, Hershey, PA 17033.

The transforming potential of different isolates of human cytomegalovirus (CMV) has been established. Initially, normal hamster embryo fibroblasts were transformed to malignancy (1). More recently, a CMV isolated from a normal prostatic cell culture established a long-term latent infection of normal prostate cells and a long-term persistent infection of human embryo lung cells in vitro (2). The virus-carrier state eventually resulted in the development of transformed cell lines (3). DEAE-treated primary hamster embryo fibroblast cells inoculated with the same non-inactivated CMV also resulted in transformation. The transformed cells ceased to demonstrate contact-inhibition, grew in soft agarose, possessed easily detectable CMV-antigenic markers at early in vitro passages, and induced poorly differentiated tumors when transplanted into athymic nude mice and hamsters (4). Diverse alterations in oncogenicity, CMV-related antigenicity, karyotypic markers, resistance to superinfection with herpesviruses, and induction of immune response in nude mice were observed in transformed cell lines during prolonged in vitro cultivation. The oncogenicity of the transformed cells was inversely proportional to the rate of expression of virus-related antigens in the cell population (5). One of the transformed cell lines spontaneously yielded a new herpesvirus with biological and biochemical properties resembling CMV and herpes simplex virus (HSV). Base sequence homology between the new virus DNA and HSV-2 DNA has been detected, although the density and the restriction enzyme patterns of the new virus DNA resemble neither CMV nor HSV DNA. The potential of the "hybrid" virus to transform human epithelioid cells in vitro contributes to its significance.

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570 ABELSON MURINE LEUKEMIA VIRUS AND HELPER VIRUS INTERACTION WITH DIFFERENTIATED CELLS, David Baltimore, Naomi Rosenberg*, Owen Witte, Anthony Shields and Edward Siden, Department of Biology and Center for Cancer Research, Massachusetts Institute of Technology, Cambridge, MA 02319; *Present address: Cancer Research Center and Department of Pathology, Tufts University School of Medicine, Boston, MA 02111.

Inoculation of mice with different murine leukemia viruses (MuLVs) results in a variety of types of leukemic cells. To attempt to understand this phenomenon, we have concentrated on Abelson MuLV (A-MuLV) because of the availability of a direct in vitro transformation system for this virus. A-MuLV is defective and must be studied in conjunction with a helper virus. For most experiments, A-MuLV has been rescued from a non-producer fibroblast culture using Moloney MuLV and such stocks are able to transform bone marrow cells or fibroblasts. The transformed marrow cells have the characteristics of immature lymphoid cells and many of them make immunoglobulin heavy chains.

By rescuing A-MuLV from non-producer cells with a variety of helpers it has been possible to show that the helper virus plays a more critical role in lymphoid cell transformation than in fibroblast transformation. Stocks made with helpers that are oncogenic on their own (like Friend and Moloney MuLV) produced Abelson disease in mice and transformed bone marrow cells. Stocks made with non-oncogenic helpers could neither cause Abelson disease in mice nor could efficiently transform bone marrow cells. Both types of A-MuLV stocks, however, could transform fibroblasts.

Apparently the interaction of helper A-MuLV with bone marrow target cells is different from its interaction with fibroblasts. Non-producer transformed fibroblasts are easily derived: true non-producer lymphoid cells are rare but a unique class of cells producing a defective virus is a common result of lymphoid cell transformation and has not been found with fibroblasts. These defective producers make particles that have p28 instead of p30, have no 70S RNA and have a normal or excess amount of reverse transcriptase.

All A-MuLV transformed lymphoid cells make a discrete polypeptide not made by Moloney MuLV-infected cells. It is also recovered from A-MuLV-transformed non-producer rat fibroblasts that lack any detectable Moloney MuLV and is therefore presumably a product of the A-MuLV genome. It could be the transforming protein of A-MuLV.

At present it is difficult to interpret these various aspects of A-MuLV transformation but interaction of an A-MuLV-specific product with a helper virus-specific product may be required for lymphoid cell transformation while the A-MuLV product alone can transform fibroblasts.

571 MURINE MODELS OF CYTOMEGALOVIRUS LATENCY, M. C. Jordan, Department of Medicine and Reed Neurological Research Center, UCLA, Los Angeles, CA 90024.

Although direct experimental evidence is not yet available, cytomegalovirus (CMV), a member of the herpesvirus group, appears to establish latent infections in man, and many of the clinical manifestations of infection are probably the result of reactivation of latent virus. This is particularly true of life-threatening disseminated CMV infection in patients whose defenses are compromised either by disease or by immunosuppressive treatment regimens. Recently, model systems have been developed in the mouse in an effort to study various aspects of latency. Using a murine CMV (MCMV) strain attenuated by passage in tissue culture, Olding, et al.,[1] described latent MCMV infection of splenic B lymphocytes which could be activated in vitro by co-cultivation with allogeneic but not syngeneic mouse embryo cells. Subsequent studies[2] indicated that MCMV-DNA could be detected in amounts equivalent to 3 to 4 virus genomes per 100 latently infected spleen cells. Mayo, et al.,[3] reported a model of MCMV latency in which latent virus could be reactivated in the salivary gland by immunosuppression with cyclophosphamide. In addition, transfer of MCMV infection by inoculation of spleen cells from latently infected mice into both syngeneic and allogeneic recipient mice was demonstrated and could be enhanced by pre-treatment of recipient animals with cyclophosphamide. In our laboratory,[4] a model of latent MCMV infection in which virulent virus can be reactivated with anti-lymphocyte serum and cortisone has been developed. Mice undergoing reactivation of latent MCMV developed widespread disease involving predominantly the liver and lung, which are the major organs involved in CMV infections of immunosuppressed human patients. In this model, it has not yet been possible to activate latent MCMV from spleen cells, and the site(s) of virus latency remains undefined at present. Further investigation of these murine models will undoubtedly provide important information on the pathogenesis of latent CMV infection in man.

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572 CHARACTERIZATION OF A DEFECTIVE ENDOGENOUS VIRUS OF MURINE CELLS, Edward M. Scolnick, Richard Howk, and William Vass, Tumor Virus Genetics Laboratory, National Cancer Institute, Bethesda, Maryland 20014

A 30S ribonucleic acid has been identified in rat and mouse cells with properties of a defective endogenous virus. The rat 30S RNA was originally recognized because a portion of it had been incorporated into Kirsten sarcoma virus by recombination. This endogenous 30S RNA has been previously classified as type-C viral in nature based on many properties in common with other helper-independent endogenous type-C viruses: (1) The genetic information is present in multiple copies in the DNA of their species of origin. (2) The genetic information is inducible to high levels of expression with halogenated pyrimidines. (3) The 30S RNA is specifically pseudotyped by helper-independent type-C viruses. (4) The 30S RNA forms multimeric structures with itself, presumably dimers, like retravirus RNA. Cells expressing this type-C like RNA,however,do not make viral particles or antigens cross-reactive with known helper independent type-C viruses. Up to now, evidence has been lacking in the definition of this RNA as viral with regards to the transmissibility of this RNA. The current studies will show that both the 30S rat RNA and the 30S mouse RNA can be transmitted in pseudotype form to heterologous cells. A woolly leukemia virus pseudotype of the rat RNA has been transmitted to bat lung cells. A Moloney leukemia virus pseudotype of the mouse RNA has been transmitted to certain rat cells. The data provide proof that the 30S RNA can be transmitted to heterologous cells and further strengthen the thesis that this RNA is a replication-defective endogenous virus. Furthermore, the experiments emphasize the necessity of detailed pedigrees for retravirus clones since, if at any time a virus has been grown in a cell expressing another defective endogenous virus, viral stocks may be contaminated by species of RNA that are not readily appreciated.

Hepatitis Viruses

573 ANTIVIRAL THERAPY IN HEPATITIS B INFECTION. Thomas C. Merigan, Joseph L. Smith, Richard B. Pollard, E. Andrew Neal, Vinod Sawhney, Peter B. Gregory, and William S. Robinson, Divisions of Infectious Diseases and Gastroenterology, Stanford University Medical Center, Stanford, CA 94305.

Markers of hepatitis B virus tend to be stable in patients chronically infected for 1 year or longer with this virus. Assays for HBsAg and Dane particle markers in serum were used to assess the effect of two antiviral substances. Thirteen patients with established chronic active or persistent hepatitis and detectable Dane particles in serum were treated with interferon (IF) and 6 with adenine arabinoside (VIRA-A).

Dosages of interferon $\geq 6 \times 10^3$ u/kg produced a rapid fall in the level of Dane particle associated polymerase (DNAP) in all 13 patients. Two were treated for only short periods, but the remainder are being given long term therapy in an attempt to eradicate the infection. In one man and both of the women treated daily for more than 1 month, DNAP fell to undetectable levels and failed to return over several months of observation after IF was discontinued. Additionally these 3 patients had significant reductions of HBsAg titer. A permanent 4 fold reduction of HBsAg titer by CF was observed in the male patient and both female patients became HBsAg negative by RIA. Parallel changes in HBsAg were noted by FA in liver biopsies. Serum from one female patient was tested after interferon therapy for infectivity in chimpanzees. It was not infectious when undiluted, but prior to treatment it was infectious at 10^{-8} dilution.

Similar effects on the DNAP levels were observed with VIRA-A administration at $\geq$ 2.5 mg/kg. Dane particles failed to return in the only woman so treated and she had a 256 fold fall in HBsAg titer. Her DNAP was totally suppressed during treatment. However in 5 men, the effect was not complete but in all, DNAP was suppressed during treatment in a dosage dependent fashion up to >90% with no significant changes in HBsAg titer detected.

Decreased white blood cell or platelet counts were observed with both substances at the highest dosage level employed. Initial fever, fatigue, and malaise were common with higher dosage IF therapy. Transient cramping abdominal pain and weight loss were observed in some VIRA-A treated patients at 15 mg/kg, the highest dosage level employed.

These studies are being extended to confirm the initial findings and to determine the optimal dosage regimen which will produce permanent eradication of viral markers. Correlations between changes in virologic markers, infectivity, and liver function are being carefully studied. Studies of combinations of interferon and VIRA-A have begun and additional women patients are being enrolled to establish whether or not they are more responsive to the treatment regimen.

574 IMMUNOREGULATORY EVENTS IN HEPATITIS B VIRUS INFECTIONS. Thomas S. Edgington, Department of Molecular Immunology, The Research Institute of Scripps Clinic, La Jolla, California 92037.

A variable evolution of disease is observed for infection with the human Hepatitis viruses. Hepatitis B virus (HBV) appears to be non-cytopathic, and evidence has implicated immunologic mechanisms in the pathogenesis of both hepatic and extrahepatic disease. Immune responses to HBV coded antigens as well as to hepatocyte autoantigens have been observed. A pattern of these responses has been observed to correlate with the biological evolution of infection as acute self-limited infection or development of chronic disease. An immunologically hyper-responsive state in respect to selected antigens is variably observed. This is manifest by the presence of anti-hepatocyte surface autoantibody, excessive responses to selected viral antigens, increased NK lymphocytes cytotoxic to Chang cells, aberrant function of T lymphocyte E receptor function, a humoral immunopotentiating factor, and reduced cell mediated suppression of lymphocyte responses to allogeneic cells. T lymphocyte E receptor function in HBV infections has been characterized and divided into two types, one intrinsic to the lymphocyte and the other secondary to the presence of a humoral factor. The intrinsic defect in E receptor function is induced in most individuals with acute HBV infection, it is frequently persistent, it does not correlate with biological evolution or recovery, and it is not reversible upon in vitro short term culture. The extrinsic defect is reversible in short term cultures of lymphocytes and has been attributed to the presence of an inducible serum factor (RIF). This factor has been purified, is active at 10^{-13}M, and is a lipoprotein with a density of 1.050 gm/ml. It is composed of apolipoprotein chains B, A_{II}, and C_{III}; and it binds to a specific lymphocyte receptor with a density of approximately 2,900 receptors per cell and a binding affinity of 9.3×10^{10}L/M. RIF is induced by hepatitis viruses A, B, and "non-A, non-B". It is present transiently during acute disease but when persistent is found in 93% of slowly resolving active hepatitis or chronic active hepatitis. The biological effect of RIF has been explored in mice where it appears to potentiate the immune response to heterologous antigen. Studies of suppressor cell function of acute viral hepatitis indicates a selective and transient loss of capacity of mononuclear cells to suppress the MLC reactivity of a standard responder cell. These data suggest that RIF might function through the suppression of T suppressor cell differentiation or other selected T cell functions that facilitate potentiation of immune responsiveness to selected antigens. These observations suggest that the pattern of regulation of the immune response influences the pathology and evolution of disease.

575 EVIDENCE FOR THE ASSOCIATION OF A CHRONIC DISEASE (LIVER CANCER) WITH A PERSISTENT VIRUS (HBV), Joseph L. Melnick, Department of Virology, Baylor College of Medicine, Houston, TX 77030.

Patients with primary carcinoma of the liver (hepatoma) have a high prevalence of persistent infection with hepatitis B virus (HBV). Three interpretations have been offered for this association of HBV infection and liver cancer.

(1) HBV infects patients with liver cancer or with cirrhosis (a precursor condition), who have a high susceptibility to infection and the development of the chronic carrier state. However, in areas of high hepatoma prevalence, HBV carrier infections occur most frequently in childhood, and it seems that the virus carrier state occurs before the tumor and not after.

(2) The virus carrier state is a cause of cirrhosis, and the hepatoma arises from regenerative nodules by mechanisms in which HBV is not involved. This view is supported by finding an increase of hepatoma among alcoholic cirrhosis patients, but many cases (perhaps the majority) of hepatoma associated with HBV develop in persons without cirrhosis.

(3) HBV is an oncogenic virus transforming the liver cells it invades. Other cocarcinogenic influences might be necessary for the induction of the cancer; these might be genetic, hormonal, immunologic or environmental.

If HBV is an essential oncogenic factor, then prevention of hepatitis B infection by vaccination should also reduce the occurrence of hepatoma. Such vaccines are now under study.

Viroids, Insertion Sequences, Naked Genomes: Do They Have Counterparts in Animals and in Man?

576 TRANSPOSABLE GENETIC ELEMENTS, RESTRICTION ENDONUCLEASES, AND GENOME EVOLUTION, S. N. Cohen, S. Chang, J. Chou, P. Kretchmer and C. A. Miller, Stanford University School of Medicine, Stanford, CA 94305.

Recent evidence indicates that recombination between structurally discrete and ancestorally unrelated segments of DNA may play a major role in genome evolution. Insertion sequence (IS) regions and transposable genetic elements (Tn elements) are implicated in such "illegitimate" recombinational events, which in Escherichia coli are independent of the bacterial recA gene product. Inverted repeats or palindromic nucleotide sequences present at the termini of at least some transposable genetic elements appear to serve as recognition sites for the interaction of DNA with the proteins involved in illegitimate recombination. Insertion of some elements seems to involve regional specificity; the site of insertion may be in the general vicinity of, but not exactly at the same site as, the recognition sequence. In other instances, recombination in vivo can be accomplished by the combined intracellular action of the DNA ligase and previously characterized restriction endonucleases such as the EcoRI enzyme. In these cases, recombination occurs in vivo at precisely the same sites shown to be cleaved and joined by the same enzymes using in vitro recombinant DNA techniques. Such findings suggest that an important biological function of the so-called "restriction" endonucleases may be site-specific, illegitimate genetic recombination.

577 POSSIBLE ROLES FOR NONCODING POLYNUCLEOTIDE SEQUENCES IN ADAPTIVE GENETICS, D.C. Reanney, Department of Biochemistry, Lincoln College, Canterbury, New Zealand.

Bacterial and extrachromosomal DNAs contain a variety of noncoding sequences: these include regulatory sequences (promotors, operators, initiators), untranslated "leader" sequences and specific *recombination sequences* (RS units) (5). Many of these RS units appear to be transposible, either per se, or as subsections of larger units. Available evidence suggests that RS units and perhaps "fixed" noncoding sequences do not tolerate the same degree of nucleotide divergence as structural genes; thus homologous non-coding units may occur in the DNAs of diverse taxa. Such units play a hinge role in adaptive genetics by providing favourably positioned targets for recombination events. (2,3). It follows that the transposibility of genes within and between replicons inside *one* cell must be coupled to effective mechanisms for polynucleotide exchange *between* cells to realise its full evolutionary potential. The resulting composite process is dubbed *coupled evolution* (3,4). Data presented in this paper illustrate the principles of coupled evolution. It is shown that plasmids drawn from a common ecological source and carrying a variety of adventitious genes can promiscuously infect multiple bacterial genera. It is also suggested that phages, like plasmids, often carry "somatic" genes but that these are less likely to be detected in experimental situations because of the lytic effect of phages on their hosts. "Conversion" phages then, are believed to be the rule, not the exception.

Among higher organisms, noncoding sequences, of similar modal length to RS units appear crucially involved in genome evolution (1). Some ideas concerning possible roles for these sequences are presented in the context of supporting data. Nucleotide rearrangements may also occur at the RNA level as exemplified by the apparent coalescence of non-contiguous sequences during the genesis of mRNA in adenovirus. Collectively, these data suggest that polynucleotides are much more dynamic and plastic structures than had been supposed.

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578 INCREASED REPRODUCTIVE FITNESS OF E. COLI LYSOGENS GROWN IN CHEMOSTATS CORRELATE WITH CHANGES IN OUTER MEMBRANE PROTEINS, Gordon Edlin, Department of Genetics, University of California at Davis, Davis, California 95616.

Lambda lysogens of E. coli reproduce more rapidly than isogenic nonlysogens when mixed populations of bacteria are grown in glucose limited chemostats. Lysogens of phage P1, P2 and Mu also show a reproductive growth advantage in glucose limited chemostats. In batch cultures, in which growth is not limited, lysogens and nonlysogens are equally fit. The outer membrane protein composition of chemostat grown bacteria is different from that observed in batch grown bacteria even though the carbon source is the same. Moreover, the presence of the lambda prophage results in an alteration of the amount of the bacterial major outer membrane proteins. A speculative hypothesis will be presented suggesting that the presence of prophage and plasmid DNA in bacteria confers a reproductive growth advantage to such bacteria in addition to infection immunity and resistance to antibiotics and colicins. This increased reproductive fitness of lysogens may account for the co-evolution of plasmid and prophage DNA in bacteria and might explain the ubiquity of viral DNA in higher organisms also.

579 VIROIDS: AUTOINDUCING REGULATORY RNAs? T. O. Diener, Plant Virology Laboratory, Agricultural Research Service, U. S. Department of Agriculture, Beltsville, MD 20705. Viroids are low molecular weight RNAs that are present in certain plants afflicted with specific maladies. Viroids are not detectable in healthy individuals of the same species but, when introduced into such individuals, they replicate and cause the appearance of the characteristic disease syndrome (1). Viroids are the smallest known agents of infectious disease; they are responsible for a number of destructive diseases of crop plants (2). Electron microscopy of native viroids discloses a uniform population of seemingly double-stranded rods of about 500 Å length (3). After denaturation, two types of molecules are evident: a majority of linear, single-stranded molecules, about 900 Å long, and a minority of covalently-closed circular molecules, about 1100 Å long (4). Both types of molecules are infectious (5). Although formation of hairpin structures and collapsed circles indicate the presence of large regions of intramolecular complementarity, thermal denaturation and other properties of viroids demonstrate that they are not regularly base-paired structures (6, 7). Viroids are not translated in several cell-free protein-synthesizing systems (8) and no new proteins are detectable in infected plants (9). Replication of viroids occurs in the cell nucleus (11) and is actinomycin D-sensitive (10). Sequences complementary to one viroid are present in the DNAs of uninfected and infected host plants (12). Based on these properties, a speculative model of viroid RNA replication and pathogenesis will be presented. Viroids are considered as abnormal regulatory RNAs that are able to interfere directly with gene regulation in their hosts.

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580 MONKEY DNA SEQUENCES IN DEFECTIVE SV40 VARIANTS, M. Singer, M. Rosenberg, H. Rosenberg, T. McCutchan, T. Wakamiya and S. Segal, Laboratories of Biochemistry and Molecular Biology, National Cancer Institute, Bethesda, MD 20014.

Between 10 and 20 percent of the genome of the African Green monkey is a single highly repetitive class of DNA termed α-component. The bulk of the α-component consists of long tandem repeats of a segment that is 172 bp in length. A single endo R·HindIII site occurs within most of the 172 bp repeats permitting isolation of the monomeric unit. An unambiguous sequence defining the most abundant nucleotide at each of the 172 positions in the monomer has been determined by both direct DNA and cRNA-fingerprint methods. However the data indicate that the many repeats of the 172 bp monomer unit are not identical. Thus, the highly repeated DNA component is a set of closely related sequences differing from one another at one or a few residues: the sequence divergence appears to be nonrandom. Neither the number of members in the set nor their relative frequency is known. The data do show that the most abundant residue at each position occurs in at least 90 percent of the members of the set. Nevertheless, it is important to note that the determined sequence may not represent the structure of any member of the set.

Some of the defective variants of SV40 that arise upon high multiplicity serial passage of virus in African Green monkey cells contain covalently linked monkey and wild type viral DNA sequences. In a large proportion of these recombinant (substituted) variants a portion of the monkey DNA is homologous to α-component. We have compared the nucleotide sequence of the monomer of α-component with that of the homologous sequences in two independently derived defective variants. In each of the two variants the homologous sequences differ from one another in length and are less than a complete copy of the 172 bp fragment but represent overlapping portions of the monomer. Within the region of the monomer common to both defectives there are variations in sequence in a few residues, suggesting that different members of the set of sequences comprising the monomer may have been incorporated in each case. In several instances the sequence data define joints between α-component monomer sequence and other sequences. In some cases the joints are with wild type SV40 DNA and in others the joined component may represent low reiteration frequency monkey DNA.

The mechanism(s) by which the SV40-monkey recombinants arise is not understood. Both hybridization experiments and sequence data show that the monomer has no extensive homology to wild type SV40 sequences: there are however short regions of homology near the joints as well as in other regions of the monomer. These homologies as well as certain other features of the sequences surounding the joints may be involved in the recombinational events leading to formation of defective viruses.

Tissue and Cell Specificity of Persistent Virus Infection

581 THE BIOLOGIC ROLE OF HOST-DEPENDENT CLEAVAGE OF VIRAL GLYCOPROTEINS. P.W. Choppin, A. Scheid, M. C. Graves, S. M. Silver, The Rockefeller Univ., New York, N.Y. 10021

Myxoviruses and paramyxoviruses possess two surface glycoproteins which are involved in similar reactions with cells, however in the two viruses the biological activities are distributed differently among the proteins. In myxoviruses, receptor binding is associated with one protein (HA) and neuraminidase activity with another (NA), whereas in paramyxovirus these activities reside in one protein (HN) and the other glycoprotein (F) is involved in cell fusion, hemolysis, and initiation of infection. In both viruses host-dependent proteolytic cleavage of a precursor glycoprotein, yielding two disulfide-bonded polypeptides, activates or enhances infectivity; in myxoviruses, the HA protein is cleaved and in paramyxoviruses, the F protein. Activation of infectivity appears to be at the penetration step, and in paramyxoviruses is correlated with activation of hemolysis and cell fusion, including fusion between viral and cell membranes. Thus with regard to receptor binding and neuraminidase activities, the paramyxovirus HN protein is functionally analogous to both HA and NA of myxoviruses, whereas the paramyxovirus F protein is analogous to HA of myxoviruses with regard to initiation of infection activated by cleavage. These functional interrelationships suggest possible evolutionary relatedness of the proteins on the same virus as well as across virus groups. Studies with wild type (wt) and protease activation (pa) mutants of paramyxoviruses and different cells have shown that host proteases cause the cleavage and that host range, tissue tropism, and spread of infection through multiple cycles are determined by availability of appropriate proteases in the tissue. Possibilities raised by these results include the natural occurrence of mutants which could infect tissue not infected by wt virus, and failure of cleavage being a factor in persistent infection in the absence of production of infectious virus. To investigate further this biologically important cleavage, the precursor (F_o) and polypeptide cleavage product (F_1 and F_2) are being characterized physically and chemically. Analysis of N-terminal sequences of F_1 polypeptides exposed by cleavage of SV5, NDV, and Sendai virus has revealed stretches of hydrophobic amino acids which are strikingly similar in the different viruses, supporting other evidence with different proteases and pa mutants for a strict requirement for cleavage at a specific site. Studies with oligopeptide inhibitors of cell fusion, hemolysis, and penetration by measles virus have revealed a similarity between the structure of the inhibitors and the N-terminal sequence of the F_1 polypeptide of paramyxoviruses, suggesting that they may act competitively, and again emphasizing the importance of this region of the protein in biological activity. The evidence suggests that virus-induced membrane changes involved in cell fusion, hemolysis, and virus penetration result from a conformational change in the glycoprotein following cleavage to yield a biologically active complex of two disulfide-linked polypeptide chains.

582 HERPES SIMPLEX - NEURONAL INTERACTION DURING ACUTE AND LATENT INFECTION, Stevens, J. Dept. of Microbiology and Immunology, Univ. of CA, Los Angeles, CA 90024.

Evidence from several experiments of differing designs, when taken together, indicate that latent Herpes simplex virus is selectively harbored in neurons (1,2). As is detailed elsewhere (3), studies employing temperature sensitive mutants of the virus, both in vivo (nervous system of the mouse) and in vitro ("differentiated" murine neuroblastoma cells) have been initiated. Here, the ultimate goal is a definition of the biochemical events necessary for the establishment and maintenance of latent infections. In the mouse, both "latency positive" and "latency negative" mutants have been indentified.

Through additional biochemical and morphological studies both in vivo and in vitro, several characteristics relating to latency have been defined. As examplse, 1) both viral DNA positive and DNA negative mutants have been found to establish latent infections 2) those mutants which synthesize the fewest morphologically indentifiable viral products during acute infection at the restrictive temperature are the most likely to establish latent infections 3) the synthesis of Immediate Early (2) proteins appears not to be sufficient for establishment of latent infections. Appropriate mutants and revertants of these agents are now being studied in greater detail, particularly in "differentiated" neuroblastomas maintained in vitro.

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583 EPSTEIN-BARR VIRUS INFECTION OF EPITHELIAL CELLS AND LYMPHOCYTES, Joseph S. Pagano, M.D., Cancer Research Center, The University of North Carolina, Chapel Hill, North Carolina 27514.

The association of the Epstein-Barr Virus with three such disparate conditions as nasopharyngeal carcinoma (NPC), Burkitt's lymphoma (BL), and infectious mononucleosis (IM) has seemed paradoxical inasmuch as NPC is an epithelial malignancy, BL is a malignant lymphoma, and IM represents benign lymphoproliferation. The recent identification of a missing link in the pathogenesis of EBV infection, namely the primary target cell of infection, makes it possible to construct a unified pathogenetic scheme for all three conditions. In this scheme primary infection with virus replication in respiratory epithelium mediated by virus-induced DNA polymerase occurs in all three conditions. Secondary infection with limited viral DNA replication in lymphocytes is a key feature of both BL and IM. The plasmid form of the EBV genome has a central role at the molecular level. It is presumed to be the form in which latent EBV genome persists and can be reactivated in cells; the plasmid may mediate covalent insertion of viral genes into host chromosomal DNA; and, possibly benign B-cell lymphoproliferation in IM may be a consequence of expression of genes in the EBV plasmid. The persistence of virus genome both in lymphocytic and epithelial cells is accounted for by this scheme and would be a necessary element of the mechanism of the three diseases.

584 PERSISTENT MuLV INFECTION IN WILD MICE, Murray B. Gardner[1], Vaclav Klement[2], Suraiya Rasheed[1], Robert W. Rongey[1], John D. Estes[1] and P. Roy-Burman[1,3], Departments of Pathology[1], Pediatrics and Microbiology[2] and Biochemistry[3], University of Southern California School of Medicine, Los Angeles, Calif. 90033

Persistent lifelong infection with endogenous murine leukemia virus (MuLV) characterizes a certain population of wild mice (Mus musculus) located at a squab farm near Lake Casitas (LC) in southern California. The great majority (≥85%) of LC mice are congenitally infected with MuLV, primarily by maternal milk. These mice are prone to lymphoma, paralysis, and certain other tumors, all occurring after 8 months of age. The lymphomas are of B cell origin, arise in spleen and bone marrow, and usually terminate with leukemia. Lower limb paralysis is caused by direct non-immunogenic injury to lower motor neurons in the lumbar spinal cord. An autogenous humoral immune response to the congenitally acquired virus is not detectable. Passive immunization of newborns with heterologous anti-virus antiserum reduces infectious virus expression at weanling age.

The endogenous MuLV consists of a mixture of amphotropic, ecotropic, and xenotropic virus classes. The amphotropic virus is most prevalent and is a new class of MuLV; it is not a recombinant of known viruses. Experimentally the amphotropic virus induces lymphoma while the ecotropic virus induces paralysis as well as lymphoma. Except for a shorter latent period the induced diseases in both mice and rats closely resemble the natural diseases in LC mice.

A small minority (≤15%) of LC mice escape congenital infection, even though their littermates may be infected; these uninfected mice are also apparently resistant to natural contact or venereal infection but are susceptible to inoculated virus. They generally remain persistently non-viremic and are free of lymphoma and paralysis. By selective breeding in the laboratory of non-viremic LC mothers, both lymphoma and paralysis, but not the other tumor types, are eliminated in the progeny. The total evidence thus conclusively shows that indigenous MuLV is the essential etiologic determinant of lymphoma and paralysis in LC wild mice. The natural history and characterization of MuLV in LC wild mice indicate that, in several major aspects, this is a different system than that in lymphoma-prone laboratory mice.

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585 ROLE OF D.I., VIRUS MUTATION, AND HOST RESPONSE IN RNA VIRUS PERSISTENCE.
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We reported previously that long-term, persistent noncytocidal infection of BHK_{21} cells in culture by Vesicular Stomatitis virus requires the initial presence of defective interfering virus particles (DI), and found that DI remain present in the carrier cultures over years of persistence at 37°C (1,2). We report here the chemical and biological characterization of infectious virus and DI released from these carrier cells after more than four years of persistence. The infectious virus is a very slow-growing, small plaque mutant with slight temperature sensitivity. Temperature sensitivity does not explain the lack of cytopathology in carrier cells since these cells survive incubation for 7 days at 25°C or 32°C. Virus mutations alone do not explain the lack of cytopathology since cloned virus free of DI destroys 100% of BHK_{21} cells exposed at any multiplicity, whereas this infectious virus mixed with DI from the carrier cells does not destroy all exposed BHK_{21} cells, and a new carrier state is readily reestablished.

We will present data on the chemical characterization of infectious virus and DI isolated after 4 years of persistence and show evidence from oligonucleotide mapping that the infectious virus is a multiply mutant compared to the original input virus, and that many DI from carrier cells are unique in their oligonucleotide map patterns as compared to a variety of VSV DI from other sources (including the DI originally used to establish the persistent infection).

We will present data showing that tumorigenic BHK_{21} cells or Hela cells lose their tumorigenicity (as assayed in nude mice) when they are persistently infected with VSV and other enveloped RNA viruses. This appears to be due to recognition of viral envelope proteins at the cell surface, and some radiation-sensitive cell(s) of the nude mice are involved. Although carrier cells do not usually form tumors in nude mice, they do form a benign module from which carrier cells, carrier virus and DI can be reisolated after months or years in an in vivo environment. Preliminary characterization of these "in vivo" carrier cells, their virus and their DI will be presented.

Finally, we will present recent information regarding DI prophylaxis of virus infection in mice and evidence regarding the uniqueness of the 3' terminus of VSV DI RNA as compared to the 3' OH end of the infectious virus from which they are derived (3).

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Viral Involvement in Chronic Neurological Diseases

586 ESCAPE FROM IMMUNOLOGIC SURVEILLANCE DURING PERSISTENT VIRUS INFECTION, Michael B. A. Oldstone, Michael Buchmeier, Raymond Welsh, Department of Immunopathology, Scripps Clinic and Research Foundation, La Jolla, CA 92037.

The biology of lymphocytic choriomeningitis virus (LCMV) infection and the pathogenesis of associated tissue injury during persistent infection are becoming increasingly well understood. Recent investigations in our unit have defined the structural proteins of LCMV and their role in the immune complex disease of persistently infected mice. Purified LCMV contains three major polypeptides, a single nonglycosylated nucleoprotein with an estimated molecular weight of 63,000, and two surface glycoproteins of 54,000 and 35,000. Immunoglobulin eluted from the kidneys of three strains of LCMV persistently infected mice reacts against all of the major viral polypeptides of LCMV by immunoprecipitation tests. In contrast, antibody eluted from kidneys of mice with immune complex disease unrelated to LCMV did not show deposition of LCMV antigen in glomeruli and these mice did not react against LCMV antigens. Thus, it is clear that mice infected at birth with LCMV and persistently infected throughout their adult life make antibodies to all the known structural polypeptides of a virus and that these antibodies interact with LCMV antigens to form the immune complexes which deposit in their glomeruli, choroid plexus and arteries.

In other experiments the expression of viral antigens on the surfaces of LCMV infected cells and the ability of such cells to escape immunologic attack by specifically sensitized cytotoxic T lymphocytes or antibody and complement were examined. Expression of viral antigens on the surfaces of infected cells peaked 2-4 days postinfection and thereby precipitously declined. Little or no viral antigen was expressed on the plasma membrane surfaces of persistently infected cells but LCMV antigens were clearly present in the cytoplasms of most of these cells. Cells early after acute infection (days 2-4) were lysed by both virus specific antibodies and complement and immune T lymphocytes. To the contrary, antibody and complement did not kill persistently infected cells, but T lymphocytes did kill such cells although at significantly lower efficiencies than acutely infected cells. The expression of viral antigens on the surfaces of infected cells was regulated by the virus-cell interaction in the absence of immune reagents and was closely associated with the presence of defective interfering (DI) LCMV. DI LCMV blocked the synthesis and cell surface expression of LCMV antigens, and DI LCMV generation immediately preceded a precipitous reduction in cell surface antigenicity during acute infection. It is proposed that DI virus mediated interference with viral protein synthesis may allow cells to escape immunologic surveillance during persistent infection while still producing sufficient viral antigens to elicit immune responses.

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587 NATURAL HISTORY OF HUMAN POLYOMAVIRUS INFECTIONS, Duard L. Walker and Billie L. Padgett, Department of Medical Microbiology, University of Wisconsin Medical School, Madison, WI 53706.

During the last few years information has developed (1,2,3) indicating that two newly recognized papovaviruses are circulating in the human population. BK virus (BKV) and JC virus (JCV) both belong in the polyomavirus genus of the papovaviruses. They have a weak antigenic relationship to each other and to SV40 through capsid antigens and a stronger relationship through T antigens. Serologic surveys have revealed antibodies against BKV and JCV in normal human populations in many parts of the world. Prevalence of antibodies in various age groups indicates that infections occur most commonly in childhood with BKV infections occurring earlier than those of JCV. In Western Europe and the U.S.A., 65-90% of adults have antibody evidence of past infection. Seroepidemiologic evidence also indicates that BKV and JCV circulate completely independently. This fits with biologic and biochemical evidence that they are quite distinct viruses. All data suggest that only humans are involved in the natural cycle. Although BKV and JCV infect large numbers of people, their full disease-producing potential is not yet clear. Most primary infections occur in children but little is known of the nature of such infections or of routes of transmission. From a few clues, however, it seems likely that such infections are subclinical or mild and that excretion in urine is important in spread of virus. BKV and JCV are opportunistic viruses that tend to become active in persons with reduced immune competence. BKV has been isolated repeatedly from the urine of persons with compromised immunity due either to disease or therapy, but there is little evidence that BKV itself causes disease in these persons. JCV, on the other hand, is clearly associated with progressive multifocal leukoencephalopathy (PML), which is a progressive, lethal brain infection in persons who have defective immunity due either to disease or therapy. JCV has been identified in the lesions of 36 cases of PML (4,5). Whether or not JCV causes other disease is not yet clear. JCV has been found in the urine of immunosuppressed persons and in the urine of a pregnant woman (6), so there is evidence that the virus is active in extraneural tissues. Both JCV and BKV are oncogenic in hamsters. JCV is particularly notable in that it induces a wide variety of malignant tumors in the nervous system of hamsters.

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588 VIROLOGICAL STUDIES ON CENTRAL NERVOUS SYSTEM DISEASES OF UNKNOWN ETIOLOGY, Richard I Carp and Richard Kascsak, Department of Virology, N. Y. S. Institute for Basic Research in Mental Retardation, 1050 Forest Hill Road, Staten Island, New York 10314

Serological studies were performed on the sera of patients with amyotrophic lateral sclerosis (ALS), their contacts, neurological controls and age-matched normal controls. Antibody responses to polio types 1, 2, and 3, herpes simplex, adeno and measles viruses were similar for the various groups. The spectrum of viral antigens was expanded to include agents never before examined serologically in this disease. Complement fixation (CF) and hemagglutination inhibition antibody responses to a wide range of arboviruses and a number of unclassified viruses were studied. Again no difference in response was noted among the different groups. CF antibodies to adeno-associated viruses (AAV) were also analyzed. The proportion of positive individuals for the ALS group was significantly higher than the values obtained for each of the other groups. Also, the proportion of positives in the ALS contact group was significantly higher than that seen for the control groups. Explant cultures derived at autopsy from the CNS tissues of 12 ALS cases were established and examined for: CPE, changes after fusion with various indicator cells and standard and intrinsic interference. These tests were negative. With the serologic data serving as the basis, explant cultures were examined for the presence of AAV. Adeno-associated virus was isolated, after the addition of helper adeno virus, from the cervical cord and muscle explant cultures of one ALS case and from the lumbar cord culture of another case. All other ALS cases (4) and all control cases (4), tested similarly, have been negative. A series of experiments were initiated to study molecular alterations occurring either in explant cultures or cultures treated with CNS material from ALS or multiple sclerosis (MS) patients. A radioactive peak (TCA insoluble) was observed in the cytoplasmic extracts of a high proportion of MS-treated cultures but only rarely seen in cultures infected with control material. The peak was seen employing DNA and protein but not RNA precursors, in rate zonal but not isopycnic density gradients, in cytoplasmic but not nuclear extracts. In one experiment, brain explant cultures were established from suckling mice inoculated IC with MS and normal human brain homogenates. Two of 3 MS explant cultures demonstrated a rate zonal peak of similar sedimentation properties to that seen in *in vitro* treated cultures. None of 3 control cultures exhibited a similar peak. Current work is aimed at investigating the presence of this peak or other molecular changes in cell cultures established from various tissues of MS, ALS and control individuals.

589 POSSIBLE ROLE OF HERPES VIRUS IN THE CHRONIC CNS DISEASES.

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Herpes simplex virus (HSV) infection is acquired during childhood, as indicated by the presence of anti-herpes antibodies in more than half of the population by the age of 5 years, and the virus persists in latent form in nervous tissue of most (60 - 80%) humans during their lifetime. Isolation of HSV from trigeminal, vagus and superior cervical ganglia of humans points to the possibility that HSV, in addition to being the cause of encephalitis, may be involved in the etiology of chronic diseases of the central nervous system of man, such as multiple sclerosis (MS). It is possible that one of the not uncommon features of MS, a plaque of demyelination localized at the nerve root entry zone, may be attributable to centripetal migration of the virus from ganglia to the CNS.

One of the most interesting aspects of HSV infection of nervous system is the mechanism involved in maintaining latency of the virus. Temperature, immune response and other factors are probably responsible for the latency of the virus in nervous tissue. What "activates" and facilitates the spread of the virus with the result of clinical manifestations such as herpes labialas or possible involvement in chronic CNS disease is still unknown.

590 PATHOGENETIC ASPECTS OF SUBACUTE SCLEROSING PANENCEPHALITIS (SSPE).

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The pathogenicity of SSPE, a slowly evolving disorder of the central nervous system (CNS), is still not understood, despite the fact that a measles like-virus (referred to as SSPE virus) has been demonstrated in SSPE brain material (1). The epidemiology of a rural prevalence of this disease and the rarity of its occurrence cannot be directly linked to the ubiquitous measles virus infection. Therefore, both, genetic predisposition and an immunological defect have been postulated to explain this disease. However, no laboratory evidence is available to support either possibility (2). The failure to demonstrate a host factor, responsible for this disease, has stimulated studies of the infectious agent associated with this chronic CNS infection.

Recently biochemical investigations have revealed genomic RNA differences between SSPE and measles viruses (3). The results indicated that the SSPE strains may have information in addition to that contained in the measles virus. Moreover, immunological analysis of isolated M protein of measles and SSPE viruses showed distinct antigenic differences, suggesting a mutation or modification in the gene of SSPE virus coding for the M protein. It is conceivable that such changes could lead to a membrane protein which is incapable of maintaining its normal function in virus assembly. It is possible that such a breakdown of membrane protein function could result in blocking the assembly of virus particles causing a non-productive or persistent infection.

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591 SCRAPIE: VIRUS, VIROID OR VOODOO?, Richard F. Marsh, Terry G. Malone, Wayne D. Lancaster, Robert P. Hanson and Joseph S. Semancik, Department of Veterinary Science, University of Wisconsin, Madison, WI 53706 and the Department of Plant Pathology, University of California, Riverside, CA 92502.

Scrapie, a natural disease of sheep and goats, is the prototype member of a group of diseases (others include kuru, Creutzfeldt-Jakob disease and transmissible mink encephalopathy) caused by microorganisms having unusual biological and biochemical properties. These unconventional viruses stimulate no detectable antibody responses, have never been positively visualized, and produce no recognizable cytopathologic effect on any in vitro culture system.
Studies on the scrapie mouse model have shown that the agent is highly membrane associated and it has been suggested that scrapie is produced by replication of an aberrant membrane fragment (1). An alternative hypothesis, based on the small radiation target size of the scrapie agent, speculated that scrapie may be caused by small, naked nucleic acids similar to those producing disease in plants (2). However, subsequent experiments failed to demonstrate scrapie infectivity using free nucleic acids (3). Recently, a new hamster scrapie model has been developed having incubation periods only one-half the length of those in the mouse and concentrations of scrapie agent in brain which are 10 to 100 fold greater than any other animal model. Examination of subcellular fractions of scrapie-infected hamster brain has confirmed the membrane association of the scrapie agent, but has been unsuccessful in isolating a homogeneous membrane fraction having high scrapie activity (4). The ubiquitous distribution of scrapie infectivity in plasma membrane, and in smooth and rough endoplasmic reticulum, suggests a non-specific association with membrane which we have now confirmed by subcellular fractionation of hamster brain at intervals during the asymptomatic and clinical course of disease.
We have found that 8-9% of the total scrapie infectivity in hamster brain remains in the high speed supernatant (HSS) after centrifugation at 100,000 x g for 1 hour. Electron microscopic examination of material pelleted from the HSS reveal no visable virus-like particles or membrane structures. Thus, for the first time it is now possible to dismiss the concept of intact membranes being necessary for scrapie infectivity. Characterization of the minimal scrapie infectious unit in the HSS is proceeding along several lines including experiments designed to identify an essential nucleic acid component.

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592 EVIDENCE FOR MULTIPLE MOLECULAR FORMS OF THE SCRAPIE AGENT, Stanley B. Prusiner, William J. Hadlow*, Carl M. Eklund*, S. Patricia Cochran, Carol J. Hooper, J. Richard Baringer, Richard E. Race*, and David E. Garfin, University of California, Departments of Neurology and Biochemistry and Biophysics, San Francisco, CA 94143, and *Rocky Mountain Laboratory, NIAID, NIH, Hamilton, MT 59840.

Scrapie infection is attended by a slow, progressive degeneration of the central nervous system. A variety of studies suggest that the scrapie agent is a novel infectious entity differing from conventional viruses in many respects. To date, the scrapie agent has eluded isolation and identification in large part as a consequence of the titration assay for scrapie in mice which requires nearly one year. Preparatory to developing a purification protocol, the agent in extracts of murine spleen was studied by analytical differential centrifugation (1). The sedimentation profiles of the scrapie agent in spleen extracts suggested that it is an infectious particle with a $S_{20,w}$ of ~400S (2). These profiles were unchanged by sonication, by detergent treatment with 0.5% sodium deoxycholate, or by heating the extracts to 80° for 30 min. Similar sedimentation profiles for the agent in extracts of murine brain were observed, indicating that the size of the agent is independent of the tissue in which it replicates. From these profiles a partial purification scheme was developed using a series of differential centrifugations in the absence and presence of deoxycholate. A fraction (P_5) enriched ~20-fold for the scrapie agent, with respect to cellular proteins, has been obtained from murine spleen homogenates. This fraction was observed to be free of membranous structures by electron microscopy but contained numerous ribosomes. After storage of the P_5 fraction at -70° under conditions where infectivity is completely preserved, rate-zonal sucrose gradient centrifugation of P_5 showed that the agent can exist in a continuum of particle sizes ranging from 40S to greater than 500S. Destruction of ribosomes in P_5 by treatment with 0.5 M KCl and 5 mM EDTA at 37° for 30 min, by treatment with ribonuclease at 37° for 30 min, or by heat treatment at 80° for 30 min was associated with distinct changes in the patterns of infectivity on rate-zonal centrifugation in sucrose gradients. After any of these treatments, the agent sedimented with a $S_{20,w}$>500S. These apparent changes in the size of the scrapie agent suggest that the agent is a multimeric particle which can undergo aggregation and dissociation. The putative monomeric form of the scrapie agent appears to be 40S or smaller, assuming a density of ~1.2 gm/cm^3 in sucrose. The unusual sedimentation behavior of the agent would seem consistent with the hypothesis that the agent contains a hydrophobic protein which can aggregate and dissociate with itself as well as with lipid molecules.

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Proven Viral Induced Diseases of Animals: Do They have Human Counterparts?

593 EQUINE INFECTIOUS ANEMIA: A PERSISTENT RETRAVIRUS OF HORSES, Timothy B. Crawford, Travis C. McGuire, W.P. Cheevers and Paula Klevjer Anderson, Department of Veterinary Microbiology and Pathology, Washington State University, Pullman, WA 99164.

Equine infectious anemia is a recurrent disease of horses caused by a persistent, non-endogenous, horizontally transmitted retravirus. Typically, short (2-5 day) "cycles" of clinical disease occur at unpredictable intervals, separated by asymptomatic periods of increasing length, until in most cases cycling eventually ceases. However, the disease is occasionally fatal within a short period following infection. Clinical disease (fever depression, anorexia, declining PCV) occurs in association with bursts of viral replication in macrophages. During these periods, destruction of RBC's is immunologically mediated through antibody against viral antigens which apparently attach to the RBC surface through a viral hemagglutinin. Though the macrophage is the predominant site of replication during clinical cycles, the site of viral persistence between cycles has not been identified. In vitro, replication of the virus in macrophages results in cell death, whereas the infection is persistent and non-cytolytic in replicating fibroblasts. In persistently infected fibroblast cultures, the number of cells containing antigen detectable by immunofluorescence varies in concert with the phase of the growth cycle, whereas release of virions from the cells remains relatively constant. The virion possesses typical C-type morphology and contains a magnesium-dependent RNA-directed DNA polymerase. The sequence composition of the DNA product of the endogenous polymerase reaction is complementary to the viral genome, which consists of two subunits of single-stranded RNA of molecular weight 2.8×10^6 held in a heat-dissociable high-molecular-weight complex with equine tRNA. The structural proteins of EIA virions resolve by SDS-polyacrylamide gel electrophoresis (p30, p15), at least four glycosylated polypeptides (gp77/79, gp64, gp45, gp10), and 5 to 7 minor components. Horses make antibody against most of the virion proteins as well as a viral-induced cell membrane antigen. The presence of antibody against p30 is used to identify infected animals. Lymphocytes sensitive to virion antigens are found following infection. No antigenic relatedness to any other retraviruses has been shown. The envelope protein(s) involved in neutralization undergo antigenic shifts, a new serotype appearing at each successive disease cycle. This tendency to undergo antigenic shifts probably is the basis for the cyclic nature of the clinical disease. Whether or not it is instrumental in viral persistence is uncertain.

594 THE LUCKE TUMOR: A MODEL FOR HUMAN HERPESVIRUS LATENCY AND ONCOGENESIS. Allan Granoff and Robert F. Naegele, Division of Virology, St. Jude Children's Research Hospital, Memphis, Tennessee 38101.

The frog renal adenocarcinoma (Lucké tumor) and its etiologic agent, the Lucké herpesvirus (LHV), offer a unique model for the study of the relationship of viral latency to cancer etiology. Tumor cells of frogs maintained at 4°-9°, whether in nature or in the laboratory, contain intranuclear inclusion bodies (Cowdry type A) and herpesvirus (LHV). In contrast, neither inclusions nor LHV is found in tumor cells of frogs captured in the warm months of the year (usually summer) or held in the laboratory at 20°-25°. However, transcripts of LHV DNA and virus-specific membrane antigens are present in virion-free tumors. Exposure to low temperature (4°-9°) of (i) virus-free, tumor-bearing frogs, (ii) normal frogs bearing anterior eye-chamber transplants of virus-free tumor tissue, or (iii) fragments of virus-free tumor cultured *in vitro* induces complete expression of the viral genome and virus replication. Thus, virus replication is dependent on low temperature.

LHV extracted from naturally occurring Lucké tumors induces virus-free Lucke tumors in developing frogs. When virus-free tumor fragments from an induced tumor are incubated at low temperature *in vitro*, herpesvirus can be recovered. The virus is oncogenic when injected into developing frog embryos.

In toto, these results, in many ways, reflect a striking similarity in the behavior of the Lucké herpesvirus to certain conditional lethal mutants of Herpes simplex virus, which can successfully establish latent infections at restrictive temperatures.

The genome of LHV is a linear duplex DNA molecule. Preliminary experiments indicate that its molecular weight is about 70×10^6 suggesting that LHV may carry only the fundamental genetic sequences which are believed required for specifying a herpesvirus. The molecular association between host and LHV genome is unknown as is the significance of a minimal genome size and its relationship to latency and malignant transformation.

595 ALEUTIAN DISEASE OF MINK, David D. Porter, Department of Pathology, UCLA School of Medicine, Los Angeles, California 90024

Aleutian disease (AD) is a common and economically important persistent virus infection of mink. In vivo, the initial replication of Aleutian disease virus is rapid, and peak titers of 10^8 to 10^9 ID50 per gram of spleen and liver are observed 10 days after experimental intraperitoneal infection with 10^5 ID50 (1). Virus titers then fall slowly to 10^5 ID50/gm of tissue and 10^4 ID50/ml of serum by 60 days after infection. ADV antigens are demonstrable principally in macrophages in various organs. Mink first develop antibody to ADV antigens 7 to 10 days after infection, and the persistently infected mink eventually develop extraordinarily high levels of ADV antibody together with a marked elevation of serum gamma globulin. Levels of 4 to 6 gm of gamma globulin per 100 ml of serum are common in advanced AD, and the gamma globulin often shows restricted heterogeneity. Immune complexes of various sizes are demonstrable in the serum of mink with AD, and ADV is present in the serum in the form of infectious virus-antibody complexes. Although antibody coats and agglutinates the virions, no neutralization of infectivity can be shown in vivo or in vitro (1,2). The deposition of immune complexes in the glomeruli and blood vessels causes glomerulonephritis and arteritis, which may lead to death of the mink from renal failure or arterial rupture (1,3). Host factors such as genotype and age play a major role in determining the outcome of infection, and mink of some genotypes may have an inapparent or non-persistent infection by ADV (4). Although mink of any age may develop persistent ADV infections, those infected transplacentally have fewer lesions, less ADV antibody and higher ADV titers than mink infected as adults (5). ADV is a partially defective parvovirus which will initially replicate in cell culture only at temperatures markedly lower than the body temperature of mink (2). Continued passage of several ADV strains eventually results in virus which will replicate at higher temperatures. Persistence of ADV in vivo may be linked to the defective nature of the virus, or to ineffectiveness of the host immune response in neutralizing the virus.

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596 THE VIRUS OF ALEUTIAN DISEASE OF MINK, H. J. Cho, Animal Diseases Research Institute (Western), Agriculture Canada, Lethbridge, Alberta, Canada T1J 3Z4

Aleutian disease virus (ADV) causes a persistent infection in mink. Studies were undertaken to develop a reliable and practical serological test to detect ADV infection and to purify and characterize ADV. In recent years, a procedure for extracting ADV antigen from early infected mink tissues has been established (1). This procedure involves fluorocarbon extraction of infected mink tissue and subsequent concentration and activation of the antigen by dissociating the virus-antibody complexes and removing the antibody. Utilizing this ADV antigen, extremely high-titered ADV antibody has been detected in persistently infected mink serum by counterimmunoelectrophoresis (CIEP). The test has been successfully utilized for the eradication of AD from infected mink populations (2). AD virions were first visualized in 1972 (3) in preparations of purified ADV antigen from columns of Sepharose 6B and in immune precipitates formed in a liquid phase after CIEP. Both the immune precipitates and the purified ADV antigen were infective. ADV has an icosahedral structure and is 23-25nm in diameter. Infected mink tissues (10-13 DPI) were examined by EM and numerous virus-like particles about 20-22nm in diameter were observed in vacuoles within the cytoplasm of: macrophages in spleen and mesenteric lymph node; and Kupffer cells. ADV antigen was detected in similar locations by an immunoferritin technique. In addition, ADV antigen was observed in the nuclei of some cells (4). Equilibrium centrifugation of a highly concentrated ADV preparation (20 ml of antigen from 20 kg of early infected mink tissue) yielded three bands at buoyant densities of 1.29, 1.33, and 1.41 in CsCl (5). All three populations were antigenically indistinguishable. Light ADV (ρ = 1.33) had a particle to CIEP antigen ratio comparable to that of dense ADV (ρ = 1.41) but possessed much lower infectivity, thus it is a defective particle. Acridine orange staining of purified ADV and immune precipitate gave a brilliant flame-red color (6). The reaction of 1.8% formaldehyde with the intact ADV causes an increase in absorption and a shift of the absorption maximum to a longer wavelength, from 264nm to 268nm. ADV nucleic acid has been visualized and characterized (7). It is a single strained DNA, has a buoyant density in CsCl of 1.733 g/cm^3 and MW 1.2 x 10^6 dalton. Polypeptide analysis of ADV by polyacrylamide gel electrophoresis revealed the presence of four polypeptides with MW of 30K, 27K, 20.5K, and 14K. These biochemical characteristics and the behavior of ADV in cell culture (8) suggest that ADV is a member of the parvovirus group.

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597 CURRENT UNDERSTANDING OF THE SLOW AND PERSISTENT INFECTION CAUSED BY VISNA VIRUS, A. Haase, M. Brahic, J. Scott, L. Stowring, B. Traynor, School of Medicine, Department of Medicine, University of California, San Francisco, University of California Service, Veterans Administration Hospital, 4150 Clement Street, San Francisco, CA. 94121.

Visna virus is a retrovirus responsible for a persistent and slowly evolving infection of sheep in which pathological damage accumulates in the lungs and central nervous system over a period of many months to years. Virus is able to survive the humoral and cellular immune response mounted by its host because of host mediated restriction in virus gene expression: The genetic information in the RNA genome of the virus is transferred to a stable DNA intermediate in the infected cell, but later steps in the life cycle are blocked; consequently a cohort of latently infected cells survives to perpetuate infection (1). Evidence will be presented to show that visna virus is remarkably resistant to the effects of interferon, thus establishing a further adaptation to intracellular survival. The nature of virus gene regulation in vivo is the focus of current experiments. Methodological approaches to delineating the level at which host restriction is imposed will be discussed.

In the course of infection, limited replication does occur; antigenic variants arise (2); these variants temporarily escape the immunological interdiction to virus spread, and cause tissue destruction until immunological control over extracellular virus is reimposed. The cumulative effects of recurrent episodes of this kind are dysfunction and death. Both the limited production and spread of virus account for the slow tempo of disease production. Data will be presented that bears on the potential genetic mechanisms responsible for antigenic variation, as well as information on the unusual nature of visna virus DNA synthesis in vitro that might generate virus mutants at an unusually high frequency.

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598 CHRONIC CENTRAL NERVOUS SYSTEM INFECTION PRODUCED BY THEILER'S MOUSE ENCEPHALOMYELITIS VIRUSES, Howard L. Lipton, Department of Neurology, Northwestern University, Chicago, Illinois 60611.

The Theiler's mouse encephalomyelitis viruses (TMEV) are picornaviruses which cause widespread enteric infection and, occasionally, spontaneous paralysis of colony-bred mice. Since their discovery in the 1930's, numerous TMEV isolates have been recovered from the intestinal contents of normal mice and from the central nervous systems (CNS) of paralyzed or, more rarely, encephalitic mice. The literature tends to delineate two groups of TMEV on the basis of their biological behavior in vivo. All isolates from the CNSs of spontaneously paralyzed mice and stools of asymptomatic animals resemble Theiler's original virus isolates, hence they are referred to as TO strains. Experimentally, the TO viruses cause flaccid paralysis after a long incubation period (7 to 30 days), but such isolates from animals do not grow directly in tissue culture cells. In contrast, two more neurovirulent strains of TMEV, GDVII and FA viruses form another group. Both of these viruses cause encephalitis following a short incubation period (2 to 8 days), and they have been readily propagated in cell culture. Only the TO strains have been associated with persistent CNS infections.

Our recent success in adapting a number of TO strains of TMEV to cell culture has provided a basis for evaluation of the properties of these two groups of TMEV. Further, it has provided a way to approach in vivo the mechanism(s) of establishing and maintaining this persistent infection. We have found that GDVII and FA viruses are large plaque variants. Both of these viruses are highly virulent. When as little as 50 plaque-forming units (pfu) of virus is inoculated intracerebrally (ic), adult outbred mice develop lethal encephalitis. However, the survivors of infection with one LD50 do not carry virus in their CNSs. In contrast, all TO isolates that we have adapted to cell culture have formed small plaques in L929 and BHK21 cells. These tissue culture adapted TO strains are relatively avirulent since more than 10^5-10^6 pfu do not produce a lethal or generally even an early (30 days) symptomatic infection following ic inoculation. But these TO viruses regularly initiate a chronic CNS infection. It is of interest that this persistent infection results in intense mononuclear cell inflammatory infiltration and concomitant primary demyelination of the host's spinal cord leptomeninges and white matter, a pathologic reaction similar to that previously reported for DA virus (mouse brain stocks used). In addition, chronically infected mice develop clinical symptoms from demyelination several months after inoculation.

Further contrasts of the properties of these two groups of TMEV have been exploited and will be discussed as well as the possible mechanism(s) of virus persistence.

Possible Role of Viruses in Other Chronic Diseases

599 MOLECULAR BASIS OF REOVIRUS VIRULENCE, Bernard N. Fields[1,2], Howard L. Weiner[1,2], Robert F. Ramig[1], and Thomas A. Mustoe[1], Department of Microbiology and Molecular Genetics, Harvard Medical School(1) and Department of Medicine, Peter Bent Brigham Hospital (2), Boston, MA. 02115.

The individual dsRNA genome segments of reovirus types 1, 2 and 3 can be distinguished by differences in their migration on SDS-PAGE. Ts^+ recombinants have been isolated following mixed infections of ts mutants of reovirus type 3 and clones of type 1 or 2. Utilizing these recombinants we have determined that the S1 dsRNA segment (that codes for the σ1 outer capsid polypeptide) is the hemagglutinin as well as the gene coding for the type specific polypeptide (as determined by neutralization tests). Furthermore, using such clones to infect newborn mice, we have found that the σ1 polypeptide is the determinant of cell tropism, determining whether the virus will infect ependyma to produce a "non-lethal" disease (type 1 pattern) or infect neurons to produce a highly lethal necrotizing encephalitis (type 3 pattern). The highly specific interaction of the reoviral hemagglutinin (σ1) with cell surface receptors, is thus the major determinant of differential neurovirulence associated with the different reovirus serotypes.

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600 VARIED ROLE OF VIRUSES IN CHRONIC NEUROLOGIC DISEASES, Richard T. Johnson, Opendra Narayan and Janice Clements, The Johns Hopkins University School of Medicine, Balto., Md. 21205

The central nervous system (CNS) appears uniquely predisposed to chronic disease related to viral infections. Its structural organization, lack of lymphatic system, intense cellular specialization, high metabolic requirements and paucity of regenerative capacity may, in part, explain why viruses tend to persist in CNS or why systemic infection may be manifest solely as neurological disease (e.g., spongiform encephalopathies). However, chronic disease does not necessitate persistent infection; acute, self-limited infections may be followed not only by static chronic disease (e.g., paralytic sequelae of poliomyelitis) but by progressive disease (e.g., hydrocephalus following mumps virus infection) or apparently progressive disease resulting from pathologic sequelae which become manifested during host maturation (e.g., deficits after infantile Western equine encephalitis).

During persistent infections chronic disease may result from either defective replication of the virus in immunologically intact hosts (e.g., subacute sclerosing panencephalitis) or from normal replication of virus in immunologically compromised hosts (progressive multifocal leukoencephalopathy). However, chronic disease may also result from complex host-virus interactions in which neither the virus is defective nor the host compromised by immunological or age-dependent factors. For example, in visna of sheep the virus is sequestered as proviral DNA, virus replication is restricted, antigenic mutants evolve under pressure of host antibodies and ultimate progressive disease may result from compounded immunopathologic responses.

601 EVOLUTION OF VIRUS POPULATIONS IN PERSISTENT INFECTIONS OF CELL CULTURES BY CYTOLYTIC RNA VIRUSES, Julius S. Youngner and Olivia T. Preble, Department of Microbiology, University of Pittsburgh, School of Medicine, Pittsburgh, PA 15261.

We have reported previously that non-cytocidal persistent infections at 37C of mouse L cells (L_{VSV}) with infective B particles of vesicular stomatitis virus (VSV) could be established only in the presence of large numbers of defective interfering (DI) particles. Under these conditions there was a rapid spontaneous selection of temperature-sensitive (ts) virus. As early as 10 days after infection under the conditions described there was a significant rise in the frequency of ts clones in the virus population and by 63 days 100% of the clones isolated were ts at 39.5C, the non-permissive temperature used. All of the clones in the persistent infection had an RNA^- phenotype and belonged to VSV complementation group I. By using low multiplicities of a clonal isolate of an RNA^- group I mutant from L_{VSV} cells, persistent infections of L cells at 37C could be initiated under conditions in which few, if any, DI particles were present. In light of these results, persistent infections of L cells at 37C were established with low multiplicities of RNA^+ mutants representing VSV complementation groups III and V in order to study the evolution of virus populations under these conditions. Six-times cloned populations of ts-O-23, a group III mutant, and of ts-O-45, a group V mutant, were employed in order to minimize the role of DI particles. In the case of ts-O-23 (III), by 8 days after establishment of persistence clonal analysis of the virus recovered from the cultures revealed the population contained ts virus with a reduced ability to synthesize RNA at 39.5C, the non-permissive temperature. By 43 days, a majority of the clones isolated had a clearly RNA^- phenotype, i.e., at 39.5C they synthesized less than 20% of the RNA synthesized by the parental group III mutant or by wild-type VSV. By 211 days the virus population present in the cultures was ts and even more RNA^-; a majority of virus clones synthesized less than 10% of the RNA levels of the parental group III mutant. Analysis was carried out to determine the nature of the RNA defect of the virus recovered under these conditions. Evolution of the persisting virus population also occurred when infections were initiated with ts-O-45 (V), an RNA^+ mutant with a heat-labile G protein in the virion. At 7 days after infection, all clones were characterized as ts, hs (heat-sensitive), and RNA^+, i.e., identical to ts-O-45. Within a few days, most clones isolated were ts^+ hr (heat-resistant) RNA^+, and probably represented wild-type revertants. The remaining clones were ts hs RNA^+, ts hr RNA^-, or ts hr RNA^+. The hr clones were more heat-resistant than authentic wild-type VSV, while the hs clones were as sensitive as parental ts-O-45. At much later times after initiation, all clones isolated were ts and more hr than wild-type VSV but both RNA^+ and RNA^- phenotypes were found. When a large number of parental ts-O-45 clones were isolated, all were uniformly ts hs RNA^+. Attempts to characterize the biochemical defects and genetic relations of the evolved populations will be described.

Mechanisms of Persistence, RNA Containing Animal Viruses

602 PERSISTENCE OF AVIAN TUMOR VIRUSES IN CHICKEN MACROPHAGES. Carlo Moscovici, Louis Gazzolo, and M. Giovannella Moscovici, Tumor Virus Lab., V.A.H., Gainesville, FL 32602

Mononuclear phagocytes are known to play a key role in monitoring viral infections in the organism (Mims, Bacter. Rev. 28:30, 1964). They also participate in cooperation with lymphocytes in the induction of cellular and humoral immune responses. Moreover macrophages are able to support the replication of a wide range of viruses implicated in persistent infections (Allison, Progr. Med. Virol. 18:15, 1974). We have observed that replication of avian tumor viruses (ATV) in chicken macrophages cultivated in vitro is subgroup dependent, i.e., virus of subgroups B and C are able to replicate in these cells, while those of subgroups A and D were excluded (Gazzolo, et al., Virology 58:514, 1974). Since fibroblasts from the same phenotype do not show the same selectivity to infection, the question was raised whether replication of ATV in macrophages in vivo is also subgroup dependent. This question was answered by detecting the presence of ATV in macrophages from birds immunized with avian leukosis and sarcoma viruses. It was found that a) the subgroup specificity did not strictly follow the in vitro findings, b) that avian leukosis viruses can persist in macrophages for at least 3 years after inoculation, and c) that sarcoma viruses were never detected in macrophages from immunized chickens. These findings indicate that antibody response against avian leukosis might be dependent on their persistence into macrophages. It remains to be seen whether the antibody response to avian sarcoma virus reflects the fact that they reside in another immunocompetent cell.

602A SITES OF INTEGRATION OF RETICULOENDOTHELIOSIS VIRUS DNA IN AVIAN CELLULAR DNA. Eli Keshet and Howard M. Temin, McArdle Laboratory for Cancer Research, Univ. of WI, Madison, WI 53706

Infectious reticuloendotheliosis virus DNA was found integrated at multiple sites in acutely infected avian cells (soon after infection) and at a unique site in chronically infected avian cells (late after infection) [1]. These conclusions were based on the results of infectivity analysis of DNA fragments generated by restriction endonuclease EcoR1 digestion of DNA from infected cells. It was hypothesized that the cell death that occurs after infection of avian cells by reticuloendotheliosis viruses is a consequence of the integration of viral DNA at multiple sites in the cell DNA and that only the cells that have the provirus integrated at a unique site in the cell DNA survive [1]. These hypotheses were further tested by nucleic acid hybridization with iodinated viral RNA using the blotting technique of Southern [2]. The results are consistent with the hypotheses. Integration of viral DNA at multiple sites was found in acutely infected cells, and loss of the majority of integrated viral DNA was found in chronically infected cells.

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603 LCMV REPLICATION IN LYMPHOCYTES OF VIRUS CARRIER MICE, Michael V. Doyle and Michael B. A. Oldstone, Scripps Clinic and Research Foundation, La Jolla, CA 92037

Mice infected at birth with lymphocytic choriomeningitis virus (LCMV) harbor virus in their circulating peripheral blood lymphocytes (PBL) during their adult life. A plaque assay using Ficoll-Hypaque purified PBL from SWR/J or BALB/WEHI mice and Vero target cells demonstrated between 10 and 5,000 infectious centers/10^6 cells. Similar results were found with lymphocytes harvested from thymus, spleen and bone marrow. Although numbers of infectious centers varied extensively between different animals, serial determinations with individual mice remained constant. Experiments with anti-theta sera and complement as well as nude (thymusless) mice showed that both B and T lymphocytes scored as infectious centers. Disruption of lymphocytes by freeze-thawing abrogated infectious center formation while treatment with anti LCMV antibodies did not inhibit them. This indicates that virus was not present in an infectious state in the lymphocyte and was not carried adsorbed to the cell's surface. Treatment with either B (LPS) or T (Con A) cell mitogens did not enhance the number of infectious centers. LCMV also replicated in PBL of adult mice undergoing acute infection with a peak on day 3-4, and less than detectable levels by day 8 post infection. These results indicate that LCMV is carried in peripheral and central lymphoid tissue in adult mice following infection at birth. This adds to the growing awareness that viruses may persist in lymphocytes and these cells may serve as a vehicle for transmitting infection to other parts of the body. In addition viruses may alter immune responses by their replication in specific cells of the immune system. Supported by AI 09484, NS 12428 & Arthritis Foundation Fellowship (M.V.D.).

603A MODALITIES OF LCM VIRUS TO PERSIST - Mircea Popescu, Heinrich-Pette-Institut für exp. Virologie u.Immunologie, Martinistr. 52, 2 Hamburg 20, West-Germany.

LCM virus persistent infections were studied in L cell cultures and in mice (random bread NMRI) using a cytolytic clear plaque variant of WE_3 strain (C-virus). In both systems the infection starts with a pick production of C-virus and of its defective interfering particles (DIP). During persistence no accumulation of DIP was observed. Instead, a noncytolytic turbid plaque variant (T-virus) accumulated progressively both in cell cultures and in lympoid organs of carrier mice. T-virus apparently is not a temperature sensitive mutant, generates relatively less DIP and interferes with C-virus.

Carrier mice, in which the cellular arm of immunity is impaired, produce excessive amounts of neutralizing antibodies. The virus persists in the blood associated with lymphocytes (~ 90%)+ with antibody (~ 10 %). The infected lymphocytes (~ 0.5 - 2 %) seem to be thymus dependent. They generate spontaneously T-virus. Possibly these lymphocytes are LCM-virus reactive clones, which, being infected, are unable to express their antiviral function.

604 EVOLUTIONARY RELATEDNESS OF TYPE-C AND TYPE-D PRIMATE ONCORNAVIRUSES STRUCTURAL PROTEINS: Sushilkumar G. Devare, Donald L. Fine, Larry O. Arthur, Howard P. Charman and John R. Stephenson, Viral Oncology Program, National Cancer Institute, Frederick, Md. 21701.

The major internal antigens and envelope glycoproteins of the squirrel monkey retravirus (SMRV) and Mason-Pfizer monkey virus (MPMV) were isolated and partially characterized. Immunologic analysis of SMRV p35 led to the demonstration of antigenic determinants common to SMRV and the Mason-Pfizer monkey virus (MPMV). While the major internal antigens of type-C oncornaviruses failed to cross-react with analogous type-D virus structural proteins, immunologic cross-reactivity was demonstrated between envelope glycoproteins of several type-C and type-D oncornaviruses of primate origin. These findings indicate that type-C and type-D oncornaviruses represent two evolutionarily distinct groups of viruses and raise the possibility that their shared envelope glycoprotein antigenic determinants may be a result of genetic recombination. Naturally-occurring antibodies reactive with purified type-D oncornavirus structural proteins were demonstrated in sera of several normal rhesus monkeys with known prior exposure to MPMV-infected animals. The specificity of this reactivity was established by absorption with purified viral protein. This observation, in combination with the previous inability to detect full complements of MPMV genetic sequences in the cellular DNA of normal rhesus monkeys, provide evidence for horizontal transmission of MPMV.

605 THE ROLE OF INTERFERON IN PERSISTENT INFECTION WITH T1026, Ann-Michèle Francoeur, Teresa Lam and Clifford P. Stanners, The Ontario Cancer Institute, Toronto, Canada.

T1026, a double mutant of VSV ($L^{ts}P^-$), though not isolated from persistent infection, shares characteristics common to the viruses that emerge from persistent infections. It has a ts virion RNA polymerase (L^{ts}), has reduced cytopathogenicity in vivo and in vitro, forms smaller plaques than the wild type parental strain, HR, and can establish persistence at multiplicities as high as 20 PFU/cell. T1026 has a second, non-ts lesion in the VSV function, P, for VSV-induced shut off of total protein synthesis in infected cells.

In an assay based on increase in plaque size with time, HR plaques grow linearly with time (PSI^+) while those of T1026 grow linearly for a short time only, then remain constant in size (PSI^-). We have shown that the PSI^- behavior is associated with P^- in that viruses like T1026 and its ts revertant, R1 (L^+P^-), are PSI^-. Induction of interferon by T1026 and R1 appears to be responsible for the PSI^- behavior. Thus T1026 or R1 plaques can inhibit the linear increase in size of adjacent HR plaques making them PSI^-; plaque size increase of unrelated viruses such as VSV (New Jersey), Mengovirus, and Bovine enterovirus can also be inhibited by T1026 plaques; in Vero cells which are genetically unable to respond to interferon induction, T1026 and R1 show PSI^+ behavior. Like the VSV P function, the interferon inducing property is not ts, nor is it required for replication.

Since T1026, but not R1, can establish persistent infection, we suggest that all three viral properties, L^{ts}, P^-, and interferon induction are involved in persistence with VSV.

606 HIGHLY INDUCIBLE CELL LINES DERIVED FROM MICE GENETICALLY TRANSMITTING M-MuLV, Lee Bacheler, Rudolf Jaenisch and Hung Fan, Tumor Virology Laboratory, The Salk Institute, San Diego, California 92112

Permanent, non-virus producing cell lines have been established from a mouse embryo carrying an endogenous, genetically transmitted Moloney MuLV gene. These cells carry the M-MuLV gene as demonstrated by hybridization of cellular DNA to M-MuLV cDNA, but do not express it at the levels of virus production, accumulation of intracellular viral p30, or M-MuLV-specific RNA. Treatment with BrdU (50 μg/ml for 24 hr) results in induction of XC positive NB tropic virus, although only a small fraction of the cells release virus (less than 0.1% after 48 hr). Immunofluorescent staining and flow microfluorimetry indicate that a wave of p30 accumulation occurs in the induced cells, with a maximum at 24-48 hr after the addition of BrdU. Furthermore, most, if not all, cells are induced to produce p30 protein. Similar kinetics are found for the accumulation of M-MuLV-specific RNA in the cytoplasm of induced cells. At 24 hr after induction, the level of virus-specific RNA in induced cells is similar to that found in cells productively infected with exogenous M-MuLV. This rapid induction of virus expression in a majority of cells is dependent on the presence of the M-MuLV gene and probably represents primarily expression of this endogenous virus since induction is not observed in cells similarly derived from a sibling embryo lacking the M-MuLV gene.

607 CHARACTERIZATION OF TRANSMISSIBLE GASTROENTERITIS VIRUS IN ESTABLISHMENT OF PERSISTENT INFECTION IN SWINE LYMPHOID CELLS. Roger D. Woods, NADC, Ames, IA 50010

Macrophages (monocyte derived) and lymphocytes have been successfully cultured from swine peripheral blood so that long term replicating cultures were established. These cultures are maintained both in media and in the frozen state. The swine lymphoidal cells were infected with the virulent Miller #3 strain of transmissible gastroenteritis (TGE) virus at an input multiplicity of 0.01. This strain set up a persistent infection with virus particles observed in the cytoplasm and produced infectious virus titers of 10^4 to 10^6 p.f.u./ml over 2½ years after inoculation of the virus. No other viruses were detected in the TGE-infected cells by electron microscopy and/or tissue culture methods. Immunofluorescence showed over 90% of the cells contained TGE viral antigen without any detectable c.p.e. It was not possible to superinfect this line or to cure the infected cells by culturing them in the presence of high titered hyperimmune TGE antisera. The virus produced minute plaques on McClurkin's swine testes indicator cell line. The virus was completely avirulent when inoculated orally into susceptible 3-day-old swine. Attempts to recover the virus from inoculated swine were not successful although the animals produced TGE VN antibodies. The attenuated virus retained its immunogenic properties for susceptible swine for the duration of the test period.

608 PERSISTENT INFECTION BY VESICULAR STOMATITIS VIRUS: INTERFERON INDUCTION BY ts-MUTANTS, AND CELL SPARING. Margaret J. Sekellick and Philip I. Marcus. University of Connecticut, Storrs, Connecticut 06268.

A *sine qua non* of persistent infection(PI) is survival of host cells in the presence of lethal virus. DI particles, ts-mutants and the interferon(IF) system have been implicated in PI. Conceivably, they could interact to effect a balance between cell killing and sparing, and virus replication and inhibition. Since IF-mediated interference permits survival of susceptible cells in the presence of lethal virus (Virology 69:378, 1976), we sought to link cell sparing by the IF system with DI particles and ts-mutants.We have established the bifunctionality of a new class of [±]RNA defective particles of VSV that can act as homotypic DI particles to inhibit VSV replication but not cell killing (Virology 57:321, 1974), and also as IF inducing particles (Nature 266:815, 1977) to activate the IF system, reduce virus yield and prevent cell killing. In contrast to wildtype virus and ts-mutants that kill cells at 40°C, many mutants of VSV induce IF at that temperature and also at one used to establish PI (37°C). Complementation groups can be subdivided: only mutants which show primary transcription, but do not inhibit cell protein synthesis or kill cells (Virology 79:267, 1977) are capable of inducing high levels of IF. Clonally purified and gradient-banded tsG11(1) induces a maximal yield of IF at $m_{pfp} \approx 1$. IF induction is as sensitive to heat (50°C) inactivation as is plaque-forming particle and virion-associated transcriptase activity. IF induction by [±]RNA DI particles is unaffected by heat. Our studies now link all 3 elements previously implicated in PI, and add a fourth, cell sparing -- for, regardless of the multiple elements that may come into balance in PI, it is clear that survival of the host cell must be considered paramount among them.

609 ANALYSIS OF *IN VITRO* PRODUCT RNA'S AND TERMINAL SEQUENCES OF DI RNA IN ACUTE AND PERSISTENT VSV INFECTIONS, B.L.Semler, J. Perrault*, and J. J. Holland, Dept. Bio., Univ. of CA San Diego, La Jolla, CA 92093

The *in vitro* RNA polymerase activity of defective interfering particles (DI) of vesicular stomatitis virus (VSV) produces a small product RNA. The product RNA's from DI derived from a number of different B virion isolates of VSV have been characterized, and the major species found is 45 nucleotides long. The RNA sequence has been determined for DI derived from two different infectious B virion strains (MS and ts G31) of VSV. Both of these sequences are characterized by several adenosine-rich stretches of 4-6 nucleotides each, separated by other nucleotides. These purified RNA's have been shown to anneal back to the DI virion template but not to B virion genome RNA. We have shown further that the product RNA is synthesized from the 3' end of the DI template. These and other data indicate that the 3' end of the VSV B virion RNA is not conserved in DI RNA, but is replaced by a complementary copy of the B virion 5' terminus. This suggests that these sequence differences at the termini of B virion RNA and DI RNA may play a significant role in DI interference in acute infections. We have recently begun to analyze the terminal sequences of a number of different DI appearing at various times in a BHK_{21} cell culture (CAR-4) persistently infected with VSV for over four years. The genome RNA from the first of these to be analyzed annealed to significant fraction (>50%) of a ^{32}P-end-labeled, single stranded RNA probe derived from the terminal ~60 nucleotides at the 3' ends of ts G3] DI. We have also begun a comparative sequence analysis of the RNA products synthesized *in vitro* by several purified DI derived from the CAR-4 carrier culture. *Present address: Dept Microbio & Immun., Washington U., Sch. Med., St Louis, MO 63110

610 CHRONIC INFECTION WITH VESICULAR STOMATITIS VIRUS OF INTERFERON-TREATED L-CELLS, Janet M. Ramseur and Robert M. Friedman, N.I.H., NIAMDD, Bethesda, Maryland 20014

When Ly cells were treated with 100 units per ml of mouse interferon and then infected with vesicular stomatitis virus (VSV) at a multiplicity of 10-60 PFU per cell, a prolonged infection of cultures ensued, lasting as long as 60 days. After 4 passages in Ly cells at high multiplicities of VSV from a prolonged infection there was no inhibition of virus growth and viral intracellular and extracellular RNA forms were no different than viral RNA found in wild-type virus passed four times in Ly cells. Infection of BHK cells with a mixture of VSV and defective interfering forms (DIF) of VSV produced in BHK cells resulted in marked inhibition of virus production; however, the same mixture in Ly cells did not result in any decrease in virus production. A morphological examination of VSV from a prolonged infection did not show a significant number of small defective viral forms after four passages in Ly cells at a high multiplicity of infection. Therefore, DIF of VSV do not seem to play a role in this form of chronic infection. Temperature-sensitive mutants of VSV did emerge during the course of the prolonged infection. There was an increase in virus production and virus-induced cytopathology after temperature downshift to 32°. The virus produced during prolonged infections grew to higher titers at 32° than at 37° and its plaque size at 39° progressively decreased with the length of time that the infection persisted. Furthermore, interferon production also played a role in the persistence of such infections as low titers of interferon were present and treatment with rabbit anti-mouse interferon globulin resulted in a significant rise in virus production and viral cytopathology.

611 PERSISTENT INFECTION OF MOUSE PERITONEAL EXUDATE CELLS BY LACTATE DEHYDROGENASE-ELEVATING VIRUS (LDV) IN VITRO, J. Stueckemann and P. G. W. Plagemann, University of Minnesota, Minneapolis, MN 55455.

Primary cultures of starch-stimulated mouse peritoneal macrophages maintained in L cell-conditioned medium remained susceptible to LDV replication for 90 days. When infected with LDV after 1-4 weeks in culture, productively-infected cells detected by autoradiography for LDV-specific RNA and by fluorescent antibody staining were found in colonies of replicating cells. LDV production decreased rapidly 12-30 hr after the initial infection of 24-hr or older cultures, even though no cytopathic effects were observed. The macrophages continued to replicate normally, and significant amounts of interferon were not detected. LDV production, however, continued in these cultures at a low level (2-3 logs lower than the initial viral yield) for 75 days. LDV production was too low to be detectable by biochemical methods, and no increased virus production could be induced by superinfection. This transient high level production of LDV and subsequent persistent infection of macrophage cultures mimics the replication of LDV in mice, which is also associated with a life-long chronic stage with low level viremia, but the mechanism by which this persistent infection is maintained is uncertain.

612 MuLV-ENVELOPE GLYCOPROTEIN, gp70 IN ONCORNAVIRUS-INDUCED AUTOAGGRESSION
B.C. Del Villano, G. Butler, A.R. Vessey, and M.R. Proffitt, Dept. of Immunology Research Division, The Cleveland Clinic Foundation, Cleveland, Ohio 44106.

Thymocytes from C3H mice neonatally infected with MuLV-Moloney (MuLV-M) are autoaggressive for normal fibroblasts, but are not so for cells infected with MuLVs. C3H/He target cells which are resistant to kill at low passage (<P25) by autoaggressive MuLV-M-carrier thymocytes become resistant to kill at higher passage (P30-P40). This resistance coincides with 1) a detectable change in the morphology and growth characteristics of the cells, 2) the spontaneous expression of MuLV-envelope glycoprotein, gp70, and 3) the spontaneous production of infectious ecotropic MuLV. By analysis of BrCN peptide maps of gp70s, we have shown that different molecular species of gp70 were present on cells which were exogenously infected with MuLV-M than were present on those spontaneously expressing endogenous MuLV.

Autoaggressive cytotoxicity by thymocytes from MuLV-M-carrier mice is a complex phenomenon apparently involving changes both in effector thymocytes and in target cells. This could be the obverse of the reactivity seen in studies involving haptenated or virally infected target cells, where reaction by normal lymphocytes against altered self recognition units is an integral part of the immune response to the hapten or the virus. It is possible that viral antigens are recognized as self in MuLV-M-carrier mice, and that the recognition unit is a complex involving both viral and cell surface molecules. In such a case, a non-infected cell would be recognized as "non-self" by MuLV-altered thymocytes because the target cell lacks an essential part (viral antigen?) of the recognition unit.

613 EXPRESSION OF SIMIAN RETRAVIRUSES ANTIGENS IN PATIENTS TREATED FOR RENAL DISEASES AND IN PREGNANT WOMEN, Lise Thiry, Suzy Sprecher-Goldberger, Michel Bossens and Pierre Vereerstraeten, Institut Pasteur, Brussels, 1040 Belgium

Serum antibodies to Mason-Pfizer virus (M-P V), Baboon C particle virus (BeV) and Simian Sarcoma Virus (SSV) were assayed by 3 methods : neutralization of syncytium plaque forming units on KC and XC cells; neutralization of vesicular stomatitis pseudotypes with retravirus envelope antigens;complement dependent cytotoxic antibodies to retravirus infected cells. There was a positive correlation between the 3 methods. Cell-mediated response was studied by monitoring (^{3}H) thymidine incorporation into human lymphocytes treated in vitro with the retraviruses antigens. A high frequency of antibodies to M-P V was observed in patients treated for chronic glomerulonephritis and in those with a kidney graft. Seroconversions were observed during the treatments, which were accompanied by multiple transfusions. On the other hand pregnant women with a history of many pregnancies frequently showed transient specific cell-mediated response to either M-P V or BeV antigens. Immune responses to SSV were observed in about 10 % of the adults, with no specific incidence among the groups studied. It is suggested that grafts and/or transfusions may induce the expressions of antigens related to Simian retraviruses.

614 PERSISTENT INFECTIONS OF TWO CELL LINES WITH THE FLAVIVIRUS JAPANESE ENCEPHALITIS VIRUS. Connie Schmaljohn and Carol D. Blair, Dept Microbiol, Colorado State University, Fort Collins, CO, 80523.

Persistent infections by Japanese encephalitis virus were established in a line of rabbit kidney cells (MA-111) by serial undiluted passage of the virus. A small percentage of cells released low levels of infectious virions and a larger and more variable percentage was shown to possess viral antigen by fluorescent antibody staining. Virus released from persistent infections interfered with replication of wild-type Japanese encephalitis virus. Persistently infected cultures could not be superinfected with wild-type virus but could be superinfected with two heterologous viruses. Transfer of cell culture medium from persistently infected MA-111 cells resulted in new persistent infections in a line of African green monkey kidney cells (Vero). Host cell interferon production and temperature sensitive viruses were not responsible for establishment or maintenance of persistence. Ratios of physical particles to infectious virions demonstrated that many defective, non-infectious viruses were present. Cell clones were isolated from persistent infections and examined for the presence of viral antigen, release of infectious virions and ability to be superinfected with wild-type Japanese encephalitis virus.

615 MULTIPLICITY OF PROVIRAL SEQUENCES IN VIRUS-SHEDDING AND NON-PRODUCING TRANSFORMED MOUSE CELLS, Hiromi Okabe and Masakazu Hatanaka, Viral Oncology Program, Frederick Cancer Research Center, Frederick, MD 21701

The presence of endogenous viral genomes and the integration of infecting viral genomes into the host chromosomal DNA are well documented with oncornaviruses. However, little is known about differences in the proviral state between virus-producing and non-producing cells. We have isolated several cell lines with diverse biological characteristics from a cloned Ki-SV transformed Balb/3T3 cell line (K-Balb). All of these sub-clones, including persistently virus-producing and non-producing lines, contained at least two distinct sets of proviral DNA, one representing the endogenous ecotropic virus, and the other Ki-SV. Hybridization kinetics of cellular DNA with total ^{3}H-cDNA, prepared from a virus produced in one of the variants (58-2T), indicated that the virus-producing clones contained 1.5 to 2 times more viral genome equivalents compared to the non-producing clones; the latter clones had the same provirus copy number as uninfected Balb/3T3 cells (about 20 per haploid genome). A Ki-SV specific ^{3}H-cDNA, prepared by removal of MuLV specific sequences by prior hybridization with MuLV 70S RNA, detected 2-4 times more proviral DNA in producing clones than in non-producing clones; the latter contained only 1 copy per haploid genome. The distribution of free and chromosomally localized proviral DNA in these cells, as determined by the Hirt extraction procedure, indicated that the increase was not from free proviral DNA. Thus, in these sub-clones of K-Balb cells, DNA of virus-producing variants contain more proviral sequences of both endogenous and exogenous viruses in coordinate fashion.

616 THE RNA OF REPLICATION-DEFECTIVE MAMMALIAN TYPE C TRANSFORMING VIRUSES CONTAINS NUCLEOTIDE SEQUENCES HOMOLOGOUS TO THE 5'-TERMINUS OF THE HELPER VIRUS GENOME, Steven R. Tronick, William A. Haseltine*, Cirilo D. Cabradilla, Keith C. Robbins, and Stuart A. Aaronson, Laboratory of RNA Tumor Viruses, National Cancer Institute, Bethesda, Md. 20014, and Sidney Farber Cancer Center, *Harvard Medical School, Boston, Mass. 02115

Replication-defective mammalian type C retroviruses that induce solid and hematopoietic tumors in vivo and transform fibroblasts in vitro, have been isolated from several mammalian species. A helper type C virus provides functions necessary for the replication of the defective transforming virus. The genomes of transforming viruses isolated from murine and primate species have been shown to contain, in a genetically stable form, nucleotide sequences homologous to the original helper virus in addition to unique sequences. The extent of representation and expression of helper viral sequences varies with each transforming virus isolate. The availability of cDNA probes specific for the 5'-terminal region of the helper virus genome made it possible to study the composition and arrangement of helper virus sequences in the genomes of defective transforming viruses. The RNAs of a large number of these viruses were shown to contain sequences homologous to the helper viral 5' cDNA. These 5'-specific sequences were conserved relative to sequences representative of the entire helper virus genome. The conservation of helper viral 5'-terminal sequences suggests they play an essential role in the life cycle of mammalian transforming viruses.

617 ORIGIN AND SPECIFICITY OF A 32,000 DALTON ENDONUCLEASE FROM AVIAN RETROVIRUS CORES Miriam Golomb, Duane P. Grandgenett, Ajaykumar C. Vora, and Ron D. Schiff, Institute for Molecular Virol, St. Louis Univ. Med. Ctr., St. Louis, Mo. 63110

A 32,000 dalton nucleic-acid binding protein (p32), possessing endonuclease activity, has been identified in cores isolated from avian myeloblastosis virus (AMV) and Rous sarcoma virus (RSV) (Prague B). p32 elutes from phosphocellulose at high salt and can thereby readily be resolved from other nucleic acid-binding core proteins, including AMV α and $\alpha\beta$ DNA polymerase. p32, which represents 1-2% of the total virion protein, has been purified to near homogeneity from whole AMV virions. Purified AMV p32, labeled with ^{125}I, is specifically immunoprecipitated by hamster serum prepared from an animal inoculated with passage-cloned cells derived from an RSV-induced hamster tumor, indicating that p32 is virus-specified. An endonuclease activity is found associated with p32 protein throughout purification. This nuclease converts a variety of supercoiled DNAs to relaxed circles by means of single-stranded interruptions, but lacks DNA exonuclease or RNAse H activity and is relatively inactive in nicking single- or double-stranded linear DNAs. Analysis of the products of p32 digestion of supercoiled Col E_1 DNA, followed by Eco Rl cleavage , shows that p32 cuts at a limited number of preferred sites on this DNA. p32 endonuclease is tenfold more active in the presence of Mn^{++} than of Mg^{++} but loses site specificity. We will present structural and enzymological evidence that p32 is the polypeptide cleaved from the β subunit of AMV DNA polymerase (92 K daltons) to yield the α subunit (62 K daltons).

618 COMPLETE MULTIPLE COPIES OF RAUSCHER MURINE LEUKEMIA VIRUS-RELATED SEQUENCES ARE PRESENT IN INBRED MOUSE STRAINS AND ABSENT IN A WILD MOUSE STRAIN, Gurmit S. Aulakh, E. Prem-Kumar Reddy, Hari S. Aulakh, and W. John Martin, The Bureau of Biologics, N.I.H., Bethesda, Md., 20014

A ^{3H}-cDNA probe of Rauscher Murine Leukemia Virus (RLV) was synthesized, recycled with 70 S viral RNA, and shown to represent the complete viral genome. By using this probe in DNA-DNA hybridization with excess unlabelled DNA from various inbred mouse strains, strains of wild mice, other rodents, and mammals, it was demonstrated that: 1) complete viral genomes are present in all the inbred strains of mice tested, 2) these sequences are absent in at least one strain of wild mice, 3) there are multiple copies (60-70) present in every cell of the inbred mice tested, 4) RLV-related sequences are widely distributed among rodents. We suggest that Rauscher Murine Leukemia Virus should be classified as an endogenous mouse virus. Other implications of these results will be discussed.

619 PERSISTENT EMC VIRUS INFECTION IN MURINE CELL LINES, A. J. Hackett, J. Weaver, and F. Schaffer, University of California, Berkeley, CA 94720

Fifteen murine cell lines were challenged with encephalomyocarditis virus (EMC); nine lines not producing oncornavirus were completely lysed by EMC. Of oncornavirus-producers, two cell lines were totally lysed, but four others (all high-level oncornavirus producers) yielded survivors from which new sublines were developed.

Sublines continued to produce high levels of oncornavirus. They produced low levels of EMC with about 1 cell per 1000 showing viral antigen; they were resistant to superinfection with EMC. Passage in the presence of EMC antibody "cured" EMC production but not resistance to superinfection. However, further multiple passage in the absence of antibody resulted in loss of resistance to EMC. The possibility that a DNA copy of the EMC genome has been integrated is suggested by preliminary transfection experiments.

620 DECREASED PRODUCTION OF TRANSFORMING VIRUS BY CULTURED TUMOR CELLS, Mark A. Wainberg, Lady Davis Institute for Medical Research, Montreal, Quebec, Canada

Tumor cells, which are grown in vitro and induced in chickens as the result of inoculation with avian sarcoma viruses, are persistently infected with these viral agents. In cases in which tumors are derived from birds with progressing neoplasms, much de novo virus is released, while tumor cells cultured from regressing neoplasms produce few or no transforming particles. The latter cell type is frequently capable, however, of producing transformation-defective, non-oncogenic particles which contain normal levels of the enzyme RNA-dependent-DNA polymerase (RDDP). In similar fashion, tumor cell cultures (derived from progressing neoplasms) which initially produce complete de novo virus cease after several months and 8-10 passages either to produce virus particles altogether or manufacture virus which is RDDP-containing but transformation-defective. Furthermore, tumor cells, which are unable to produce complete de novo virus, lose the ability to interact with the cellular immune system of sensitized tumor-bearing hosts, as judged by the technique of peripheral lymphocyte stimulation in response to culture supernatant fluids. This is accompanied by senescence-like changes in the appearance and behavior of these cells such as increased granularity and decreased plating efficiency. These results suggest that tumor cells, growing either in vivo or in vitro, become less efficient in the production of de novo virus as a consequence of senescence, and that such changes in virus production status are accompanied by alterations in immunological appearance as well. Supported by the National Cancer Institute of Canada.

621 PROTEIN-PROTEIN INTERACTIONS WITHIN THE SENDAI VIRION, Mary Ann K. Markwell and C. Fred Fox, University of California, Los Angeles, CA 90024

The components of protein complexes formed by native disulfide bonds or produced by chemical crosslinking within the Sendai virion were elucidated using two-dimensional gel electrophoresis. Egg-grown virus was purified from either early or late harvests of chorioallantoic fluid using centrifugation through sucrose and Renografin gradients. Chemical crosslinking of the purified virus was performed at 0° and 37° using dimethyl-3,3'-dithiobispropionimidate and methyl 3-[(p-azidophenyl)dithio]propionimidate. Results indicate that HN is present in the virion as a dimer and tetramer and that P is present as a trimer in native disulfide complexes. Chemical crosslinking additionally produces a dimer of $F_{1,2}$ and oligomers of NP and of M.

622 CHARACTERIZATION OF $GaLV_H$, A NEW AND DISTINCT ISOLATE OF GIBBON APE LEUKEMIA VIRUS, K. Green, F. Wong-Staal, M. Reitz, C. D. Trainor, R. C. Gallo, and R. E. Gallagher, National Institutes of Health, Litton Bionetics, Inc., Bethesda, MD 20014, and Hem Research, Inc., Rockville, MD 20852.

A gibbon ape with lymphoblastic leukemia, obtained from a colony in Bermuda with no previous history of leukemia or exposure to GaLV, contained high levels of type-C virus and viral components in various tissues (Gallo et al., Virology, in press). This isolate, called $GaLV_H$, is related to other isolates of GaLV and also to simian sarcoma virus (SiSV) by reverse transcriptase and p30 serologic homology. We have shown that these viruses also share nucleotide sequence homology with $GaLV_H$. However, by both extent of hybridization and thermal stability of the hybrids formed with 1) viral RNA and infected cell DNA; 2) viral cDNA and viral RNA; and 3) viral cDNA and infected cell DNA, this virus can be readily distinguished from GaLV-SEATO, GaLV-Br, and GaLV-SF, the three previously described strains of GaLV, and more readily distinguished from SiSV. An analysis of the "strong stop" cDNA (by W. Haseltine), representing the 5' 1% of the viral RNA, also shows that $GaLV_H$ can be distinguished from these previously described isolates. $GaLV_H$ thus represents yet another strain of GaLV.

623 GENETICALLY TRANSMITTED RETROVIRUSES ISOLATED FROM ASIAN RODENT SPECIES: RELATIONSHIP TO PREVIOUS ISOLATES FROM RODENTS AND PRIMATES. Robert Callahan and George J. Todaro, National Cancer Institute, Bethesda, MD 20014

We have isolated two distinct classes of endogenous type C viruses (C I and C II) and one new class of endogenous type B retroviruses (designated M432) from the Asian murine species Mus cervicolor. The class C I viruses are antigenically related to the leukemia- and sarcoma-inducing infectious primate type C viruses isolated from a woolly monkey and gibbon apes. Since DNA transcripts of the woolly monkey sarcoma viruses and the gibbon leukemia viruses hybridize to rodent, and especially Mus, cellular DNA and not to primate DNA, it would appear that this group of infectious primate RNA tumor viruses has been derived under natural conditions from endogenous viruses of certain Mus species. Xenotropic retroviruses, capable of replicating in cells of heterologous species, including human, have been isolated from M. caroli, M. pahari, M. shortridgei (Thailand), M. dunni (India) and a distant relative, Vendeluria oleracea. The ability to release infectious type C and type B viruses is clearly not limited to inbred laboratory mouse species, but, rather, is also found with high probability in many different outbred rodent species. Hybridization of unique sequence cellular DNA shows that some of these species are as genetically distant from Mus musculus and Mus cervicolor as are the rat species. The retroviruses they release are related to, but distinct from, laboratory mouse and rat viruses. Breeding colonies of some species have been established and spontaneous leukemias and breast cancers have been observed; the role of activated endogenous viruses and/or natural recombinant viruses in disease production is under study.

624 INTERFERON EFFECT ON MuLV INFECTION: FORMATION OF UNINFECTIOUS VIRUS IN CHRONICALLY INFECTED CELLS, Paula M. Pitha, Nelson A. Wivel and Bruce F. Fernie, Johns Hopkins Oncology Center, Baltimore, MD 21205 and National Cancer Institute, Bethesda, MD 20014
Treatment of SC-1 cells chronically infected with MuLV with interferon (150 u/ml) leads to a 100-fold decrease in the amount of infectious virus in the medium, but only to a 10-fold decrease in the number of released virus particles. In the cells the amount of infectious virus was not greatly affected, while the number of cell-associated virions quantitated by electron microscopy was 2-5 fold higher in interferon-treated cells than in the controls. The interferon block of MuLV replication occurred at the post-budding stage, and the virus particles produced in the presence and absence of interferon were morphologically indistinguishable. Interferon had no effect on the transcription of viral RNA, and interferon-treated particles contained 70S RNA and all the major structural proteins. The particles synthesized in the presence of interferon contained 3 novel polypeptides. Two of these are glycosylated (m.w. 85,000 and 68,000), and one (m.w. 70,000) is glucosamine deficient. The ratio of the relative amounts of p30/gp71 in the virions made in the presence of interferon is 3 fold higher than in the control virus. The pulse-chase experiments demonstrated that the precursor synthesized in the presence of interferon could be cleaved and assembled into the virions after interferon removal. These results show that the interferon-induced inhibition of MuLV replication is due to a deficiency in the virion assembly which leads both to the formation of uninfectious particles and to the decrease in virion production.

625 INTEGRATION OF MOLONEY AND AKR PROVIRUSES IN THE MOUSE AND RAT GENOME. Robert A. Weinberg and David Steffen, Biology Dept. and Center for Cancer Research, MIT, Cambridge, MA 02139.
DNA from cells infected with Moloney or AKR murine leukemia viruses can be cleaved with EcoRI endonuclease which has no cleavage sites within the proviral DNA sequences. The resulting DNA fragments can then be resolved by gel electrophoresis and detected by Southern gel-filter blotting. Examination of a series of independently infected rat cell clones reveals several integrated proviruses, each represented by a differently-sized fragment. Since the sizes of the fragments varies from clone to clone, there are apparently a large number of integration sites of the provirus in rat cell DNA.

Examination of normal and leukemia mouse cells reveals 15-20 endogenous viral bands, which are incremented by additional bands in the leukemic tissues. Specific probes are being used to enhance the detection of the exogenously-introduced proviruses.

626 DI PARTICLES OF SENDAI VIRUS: ROLE IN ACUTE AS WELL AS IN PERSISTENT INFECTIONS, Laurent Roux, John J.Holland, Department of Biology, University of California San Diego, La Jolla, CA 92093
In an attempt to understand the role that the defective interfering(DI)particles of Sendai virus may play in acute as well as in persistent infections, stocks of standard(ST) and DI virus were characterized and examined. Cytopathic effect of these different stocks was then studied during acute infections of BHK cells. It was found that, although after an infection with ST stock more than 99% of the cells died within 3 days, coinfection with ST plus DI virus could protect more than 99% of the cells from dying. During these primary mixed infections, the replication of the full length 50 S viral RNA is inhibited by more than 95-99%. Long term persistently infected cell cultures were established either by waiting many weeks for the few surviving cells of an infection with ST virus to reestablish growth, or by repeatedly passaging those cultures which were originally infected with both ST and DI viruses. Their characterization is in progress. It is already known that these cultures are partly or totally resistant to a challenge by ST Sendai virus, although they are totally sensitive to Vesicular Stomatitis virus, that smaller RNAs are present in equal or greater amount along with 50 S RNA in intracellular NC structures and that one of these cultures shed virus during a crisis episode.

627 MEASLES VIRUS PERSISTENT INFECTIONS - BIOLOGICAL AND BIOCHEMICAL STUDIES, Hans J. Zweerink and Edward C. Hayes, Department of Microbiology and Immunology, Duke University Medical Center, Durham, N.C. 27710.

Biochemical and biological analyses of HEp-2 cells lytically and persistently infected with measles virus have been carried out to elucidate parameters that are responsible for the establishment and maintenance of persistent infections. All cells in cultures of persistently infected HEp-2 cells (HEp-2-PI) produced infectious virus although the yield was a thousand-fold reduced compared to a lytic infection. The plaque purified infectious virus produced by HEp-2-PI cells differed in two ways from the prototype Edmonston vaccine strain of measles virus that was used to establish the persistently infected culture. It was temperature-sensitive and it generated rapidly in HEp-2 but not in Vero cells a persistent infection with the concomittant release of interfering particles.

Both lytically and persistently infected HEp-2 cells contained 55 S genomic RNA although significantly more accumulated in HEp-2-PI cells. Another RNA species sedimenting at 30S, with an as yet undetermined polarity, also accumulated in HEp-2-PI cells. The major viral polypeptides in particles released by both cell types did not differ in their electrophoretic mobility in SDS-polyacrylamide gels and the extent of glysosylation. Differences were observed in virus-induced polypeptides in both cell types. HEp-2-PI cells expressed considerably more of the two surface polypeptides (a glycoprotein and the fusion protein) than lytically infected HEp-2 cells. No differences were observed in the molecular weights of the virus induced polypeptides.

628 PROGRESSIVE ANTIGENIC DRIFT OF VISNA VIRUS IN PERSISTENTLY INFECTED SHEEP. Opendra Narayan, Diane Griffin and Janice Clements. Johns Hopkins Medical School.

Visna virus is a retrovirus of sheep and replicates both in sheep cell cultures and in the animal by means of intermediate proviral DNA. The virus replicates exponentially in cell cultures causing cytopathic effects. However in infected sheep exponential viral replication does not occur and virus is recovered mainly by explantation of tissue or co-cultivation of cells with indicator cell cultures. Virus can be repeatedly recovered from the peripheral blood leukocytes (PBL) of an infected sheep despite the development of virus neutralizing antibody. At all times, virus recovery from PBL is inefficient, requiring 10^5-10^6 cells. Visna viruses isolated from sheep 2 to 3 years after inoculation is antigenically distinct from the virus inoculated. This provides one explanation for virus persistence in an immunologically competent host. The effect of serum containing virus neutralizing antibody on virus-infected cell cultures was studied. Mutant virus was consistently isolated within three weeks during the first passage. Detectable mutant viruses emerge more slowly in vivo. Viruses were isolated from PBL of infected sheep at monthly intervals for 1 ½ years after inoculation. Although animals developed virus neutralizing antibody 8 weeks post inoculation all the viruses isolated during the first 12 months were of parental antigenicity. Subsequently both parental and mutant viruses were identified. Thereafter, antigenic drift of the virus was progressive with continuous evolution of new virus strains and gradual sequential development of neutralizing antibody to the preexisting mutant viruses.

629 IMMUNE RESPONSES IN VISNA VIRUS INFECTION OF SHEEP. Diane Griffin, Opendra Narayan, and Robert Adams. Johns Hopkins University School of Medicine.

Visna is a slow progressive neurological disease of Icelandic sheep which has a prolonged incubation and is caused by a persistent virus infection. Visna virus is a retrovirus and replicates by means of a proviral DNA intermediate which probably integrates into host cell DNA. In addition, antigenically distinct mutant viruses can be recovered from the peripheral blood leukocytes (PBL) of infected sheep. Both mechanisms are probably important for persistence. The host immune response appears unable to clear the virus infection. For this reason a detailed study of the initial immune response to visna virus infection was done. Virus specific cell mediated immunity, measured by stimulation of PBL and cerebrospinal fluid (CSF) cells appeared within 1 week, peaked by 2 weeks, and then fell to control values by 4 weeks post inoculation. Cell free virus was recoverable from CSF only during the first 2 weeks. Histopathology at 10 days post inoculation showed an acute meningoencephalitis. Thereafter the inflammatory changes became more chronic with the development of dense perivascular infiltration and germinal centers. Virus neutralizing antibody is detectable within 2 months of virus inoculation. Thus, the initial immune responses of sheep to infection with visna virus are vigorous and temporally similar to those seen in acute viral infections except that virus is not cleared. Late in the infection antigenically distinct mutants arise possibly leading to repeat cycles of virus replication, immune responses and the accumulation of acute and chronic lesions ultimately resulting in clinical disease.

630 THE PROVIRAL DNA OF VISNA VIRUS: SYNTHESIS AND PHYSICAL MAPS OF PARENTAL AND ANTIGENIC MUTANT DNA. Janice E. Clements, Opendra Narayan and Diane Griffin. Johns Hopkins Medical School.

The synthesis of proviral DNA of visna virus was measured at various intervals after inoculation of sheep cell cultures at multiplicities of 0.3, 1 and 10 pfu/cell. The DNA from the infected cells was fractionated by the Hirt procedure (Hirt, B., J. Mol. Biol. 26:365) into low and high molecular weight DNA and each fraction was quantitated for infectivity by plaque assay using the calcium phosphate transfection technique (Graham, F.L. and Van Der Eb, A.,J. Virology 52: 456). The appearance of infectious DNA in the Hirt supernatant was biphasic at all multiplicities, apparently reflecting two rounds of low molecular weight DNA synthesis. The amount of infectious DNA in the Hirt precipitate increased with time, reaching maximum levels in all cultures at the time of peak virus production.

Using the DNA from the Hirt supernatant and the blotting technique of Southern (Southern, E., J. Mol. Biol. 98: 503), restriction maps of visna viral DNA (strain 1514) have been constructed. Since visna virus changes antigenically in the presence of neutralizing antibody, both in sheep and in cell culture, a comparison was made of the restriction maps of the DNA from visna virus strain 1514 and its natural antigenic varian LV1A, to determine whether these antigenic changes were reflected in their DNA.

Genetic Expression, RNA Containing Animal Viruses

631 GENE EXPRESSION BY A DEFECTIVE INTERFERING PARTICLE OF VESICULAR STOMATITIS VIRUS IN THE ABSENCE OF HELPER. Leslye D. Johnson and Robert A. Lazzarini, NIH, Bethesda, Maryland 20014

Vesicular stomatitis virus (VSV) defective interfering (DI) particles whose RNA is the same as that from the 5' half of the standard viral RNA are generally genetically inert. Results have been different with DI-LT whose RNA is derived from the 3' half of viral RNA and contains four genes and the proposed transcription initiation sequence (5'-G-M-NS-N-TI-3'). The genome of the DI-LT particle is transcribed and replicated in the absence of infectious VSV (PNAS, 74, 4387, 1977). Continued studies of the biological capabilities of DI-LT indicate an impaired ability to shut off host protein synthesis. However, M protein is readily detected by isotopic labeling. The new full length + and - strand DI-LT replicative RNAs sediment as ribonucleoprotein particles which contain N protein made during the infection. G protein is detected in cells using a fluorescent antibody technique. Mixed infections with DI-LT and a temperature sensitive VSV mutant also show that competent G protein is produced. These and earlier results demonstrate that not all DI particles are genetically silent. Indeed, DI-LT is able to express independently and extensively its genome. This class of DI particles may enhance the immune response as well as interfere with production of the standard virus.

632 GENETIC ORGANIZATION OF THE SRC AND THE ENV GENES IN AVIAN ONCOVIRUSES. Michael M.C Lai, Sylvia S.H.Hu and Peter K. Vogt, Dept. of Microbiology, University of Southern California, School of Medicine, Los Angeles, California 90033

The organization of src gene was studied by electrophoresis and heteroduplex mapping of transformation-defective (td) viruses. It was found that many td viruses still retained about 25% of the src gene. The residual src sequences were all derived from the 5'-end of the gene. It appears that this is the favored site for deletion within the src gene. Furthermore many td viruses incorporated a stretch (0.6 kilobases) of cellular sequences at the site of the src deletion.

The organization of the env gene was studied by heteroduplex mapping between DNA of ring-necked pheasant virus and RNA of chicken oncoviruses. It was found that the env gene was located at 0.6 kb from the 3'-end of the genome in td viruses and 2.5 kb from the 3'-end in nondefective avian sarcoma viruses. It spans 1.5 to 1.7 kb in length. Furthermore, the 3'-half of the env gene is conserved while the 5'-half is more diverged. A small area at the very 3'-end of env also shows divergence. There is no evidence for the presence of any gene between env and src. The distribution of variable and conserved regions within the env gene suggests interesting structure for the viral glycoproteins.

633 CELL-FREE TRANSLATION OF ROUS SARCOMA VIRUS RNA, Karen Beemon and Tony Hunter, The Salk Institute, San Diego, CA 92112

Rous sarcoma virus 70S virion RNA has been translated in the messenger-dependent reticulocyte lysate system. Three major products, which were synthesized from RNA of nondefective RSV but not from RNA of transformation-defective deletion mutants, have been characterized. These proteins were found to have apparent molecular weights of approximately 55,000, 25,000, and 17,000 daltons by electrophoresis in a SDS-polyacrylamide slab gel. Tryptic peptide analysis of these proteins showed all three to be overlapping proteins with shared amino acid sequences. However, both similar and distinct peptides were observed when the 55 K protein synthesized from RNA of the PR-B strain was compared to the 55 K protein from SR-D RSV RNA, suggesting that these RNAs code for related but different 55 K proteins. These proteins were found to be unrelated to any of the RSV virion proteins by tryptic peptide analysis and by immunoprecipitation. The RNAs coding for the 55 K, 25 K, and 17 K proteins were found to be polyadenylated and to sediment in sucrose gradients with peaks at approximately 24S, 20S, and 18S, respectively. It appears likely that these proteins are synthesized from viral RNA because of strain-specific differences in them. The size of the messenger RNA activity and the absence of the 55 K, 25 K and 17 K proteins in the translation products of td RSV RNA, suggest that they may be coded for by the RSV src gene.

634 ANTIBODIES TO BABOON RETROVIRUS REVERSE TRANSCRIPTASE IN SERA FROM INFERTILE HUMANS, Steven S. Witkin, Paul J. Higgins and Aaron Bendich, Memorial Sloan-Kettering Cancer Center, New York, New York 10021

Human sperm nuclei contain particulate DNA-synthesizing complexes that resemble retroviruses in biochemical properties. In addition, the immunoglobulin G (IgG) fraction of rabbit antisera to human sperm nuclei specifically inactivates the reverse transcriptase (RTase) activity in disrupted endogenous type C viruses of baboon (BEV) and cat (RD114); the RTase in oncogenic primate, feline, murine and avian retroviruses remain unaffected. Since one cause of infertility is the presence of antibodies to sperm, we examined the IgG fraction of sera from infertile men and women for the presence of antibodies to both sperm DNA polymerase and BEV RTase. IgG inhibitory to sperm DNA polymerase and BEV RTase, but not to the RTase of simian sarcoma virus, was present in sera from infertile persons of both sexes. A maximum of 80% of dT_{12-18}·poly rA-directed BEV reverse transcriptase activity could be inhibited by human IgG. This inhibition was completely abolished by prior incubation of the IgG with partially purified human sperm DNA polymerase. We propose that the sperm DNA-synthesizing complex may represent the product of the expression, during spermatogenesis, of human genes that code for a BEV-like endogenous virus.

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635 GENETIC AND SIALYLATION HETEROGENEITY OF MuLV gp70, Mark J. Murray and David Kabat, Department of Biochemistry, School of Medicine, University of Oregon Health Sciences Center, Portland, OR 97201

Using a combined biochemical and genetic approach, we have analyzed the gene products of Eveline virus (Friend MuLV). The envelope glycoprotein of MuLVs (gp69/71) exhibits both size and charge microheterogeneity. We have found that cell lines producing gp69/71 are doubly infected; cloned viruses from these cell lines produce either gp69 or gp71. The charge heterogeneity is caused by heterogeneous sialylation of carbohydrate side chains. However, there are two carbohydrate side chains, only one of which is sialylated. Related studies of a temperature sensitive mutant of Rauscher MuLV indicate that at 39° a 90,000 dalton glycosylated precursor of gp70 is formed but not cleaved and the plasma membrane lacks gp70 antigens. Processing is therefore required for insertion of the glycoprotein into the plasma membrane. Additionally, particles appear to be released from cells in the absence of mature gp70. The Eveline virus is widely assumed to be Friend-MuLV. Analysis of Eveline virus proteins and the *gag* and *env* precursors indicate that Eveline is substantially different from authentic Friend-MuLV.

636 BIOCHEMICAL AND IMMUNOLOGICAL PROPERTIES OF gag GENE-CODED STRUCTURAL PROTEINS OF TYPE-C VIRUS ISOLATES OF DIVERSE MAMMALIAN SPECIES: Roberta K. Reynolds, Sushilkumar G. Devare, Fred H. Reynolds and John R. Stephenson, Viral Oncology Program, National Cancer Institute, Frederick, Md. 21701.

The major nonglycosylated structural proteins of murine leukemia virus, coded for by a gene designated gag, are synthesized in the form of a 65,000 molecular weight precursor polypeptide which becomes subsequently cleaved, giving rise to proteins with molecular weights of 30,000 (p30), 15,000 (p15), 12,000 (p12) and 10,000 (p10). The availability of conditional lethal mutants of the Rauscher strain of murine leukemia virus with temperature-sensitive defects in cleavage made possible the identification of several pairs of adjacent proteins within the type-C viral gag gene and indicated the internal sequence from the 5' to the 3' end of the gag gene to be: p15-p12-p30-p10. The biochemical properties of immunologically cross-reactive structural proteins of type-C virus isolates of additional mammalian species were analyzed. The results obtained indicate that the isoelectric points and hydrophobic/hydrophylic nature of immunologically cross-reactive proteins have been highly conserved throughout the evolution of this group of viruses. Moreover, by identification of biochemically and immunologically analogous proteins, the gag gene sequences of a broad range of mammalian type-C viruses have been deduced. Extension of these studies to cell lines nonproductively-transformed by diverse mammalian sarcoma virus isolates, including those of mouse, rat and feline origin, has indicated expression of different numbers of gag gene proteins occurring progressively from the 5' to the 3' end of the gag gene.

637 IDENTIFICATION OF A TRANSFORMATION-SPECIFIC FELINE SARCOMA VIRUS-CODED PROTEIN: Arifa S. Khan, Max Essex, Ann H. Sliski, and John R. Stephenson, Viral Oncology Program, National Cancer Institute, Frederick, Md. 21701 and Harvard University School of Public Health, Boston, Mass. 02115.

The feline oncornavirus-associated cell membrane antigen (FOCMA) acts as a target for natural immunosurveillance against tumor development in the cat. In the present study we demonstrate the expression of FOCMA as well as the 5' terminal feline leukemia virus (FeLV) gag gene proteins, p15 and p12, in mink and rat cells nonproductively transformed by feline sarcoma virus (FeSV). In contrast, such cells lack detectable levels of other FeLV gag gene-coded proteins or the env gene product, gp70. FOCMA, p15 and p12 are initially expressed in the form of an 80,000-100,000 molecular weight precursor which upon post-translational cleavage gives rise to a 65,000 molecular weight component which contains FOCMA and a 25,000 molecular weight protein containing p15 and p12. This observation, in combination with immunologic evidence establishing the FeLV gag gene sequence as NH_2-p15-p12-p30-p10-COOH, suggests that cellular sequences coding for FOCMA may have been acquired as a result of recombination within the FeLV gag gene. Feline lymphoma cells, including those from several tumors which lacked detectable levels of FeLV structural protein expression, were shown to be FOCMA-positive. These findings strongly suggest that FOCMA represents an FeSV-coded transformation-specific protein and provide preliminary information regarding the position within the FeSV genome coding for its synthesis.

638 ORGANIZATION OF AVIAN ENDOGENOUS RETROVIRUS IN THE HOST GENOME, Lawrence M. Souza and Marcel A. Baluda, University of Calif., Mol. Biol. Inst., Los Angeles, Ca. 90024

All chicken cells contain endogenous proviral sequences in their nuclear DNA. The virus can be spontaneously released or induced from some, but not all strains of chicken and has been designated Rous associated virus, RAV-0. Two models that could explain a difference in ability to produce virus are: (1) deletion of part of the RAV-0 genome; or (2) differences in neighboring cellular sequences, which might affect control of virus production. We are analyzing the topography of the endogenous provirus in producing and in nonproducing chicken strains. To study the location of the endogenous provirus within cellular DNA, we have used the following approach: (1) digestion of cellular DNA with site specific endonucleases; (2) gel electrophoresis in agarose and transfer of the DNA to nitro cellulose filters as developed by Southern; (3) detection of proviral sequences by hybridization with ^{125}I-labeled viral RNA; and (4) visualization of bands containing viral specific sequences by autoradiography. This technique permits us to detect 10^{-11}g of viral DNA. Using three different restriction enzymes and unfractionated or poly(A) selected 3' proximal ^{125}I viral RNA probes, we made the following observations: (1) in the two chicken strains studied, line 100 (virus producer) and gs^-chf^- (nonproducer), there are molecular weight differences in bands containing both viral and cellular sequences; (2) bands which probably contain only viral DNA are similar in both strains; and (3) the 3' end of the viral RNA hybridizes to a subset of bands which contain both viral and cellular sequences. Thus, observations (2) and (3) suggest that the same gene order exists in both strains.

639 MOLONEY MuSV MUTANTS EXHIBITING TEMPERATURE SENSITIVE EXPRESSION OF TRANSFORMATION FUNCTIONS, D.G. Blair, Cancer Research Laboratory, University of Western Ontario, London, Ontario, Canada N6H 5B7

Genetic studies of mammalian sarcoma viruses have been limited to a small collection of temperature sensitive mutants of the Kirsten strain of MuLV[1], and a single cold sensitive isolate of Moloney MuSV[2]. I wish to describe new mutants of Moloney MuSV isolated from mutagenized stocks by a procedure which selected for transformed cell clones with a reduced efficiency of agar colony formation at 39°C.

Ts MuSV is rescuable from these cell clones, but about 25% of the rescued virus is wild type. Analysis of cell clones and virus populations during serial cycles of rescue and reinfection suggests the ts phenotype is partially expressed in cells doubly transformed by wt and mutant genomes.

Cell morphology, rate of 2-deoxyglucose uptake, and colony-forming ability in agar are all temperature dependent in NRK cells transformed by cloned ts MuSV. Effects on morphology and sugar uptake are rapidly reversible (<24 hrs). Ts transformed cells incubated at 39°C in agar suspension for 24 hrs are inhibited (>90%) in their ability to form colonies in agar when shifted to 34°C. Growth in liquid media is not affected. Colony formation by ts transformed cells at 39°C can be restored if they are superinfected by wt MuSV, indicating no cell blockage of MuSV expression at 39°C. These mutants are potentially useful in studying the mechanism of MuSV transformation.

1) Scolnick et al J Virol 10 653 (1972)
2) Somers and Kit PNAS 70 2206 (1973)

640 AVIAN SARCOMA VIRUS REPLICATION: LOCATION OF RESTRICTION ENZYME SITES ON UNINTEGRATED AND INTEGRATED VIRAL DNA, J. M. Taylor, T. W. Hsu, J. L. Sabran, M. M. C. Lai, R. V. Guntaka and W. S. Mason. The Institute for Cancer Research, Philadelphia, PA 19111; University of Pennsylvania, Philadelphia, PA 19174; University of Southern California Medical School, Los Angeles, CA 90033; Columbia University, New York, NY 10032

Single-stranded DNA complementary to the avian sarcoma virus (ASV) genome was transcribed in the endogenous reaction of detergent-disrupted virions. The potentially full-size DNA was isolated and converted with E. coli DNA polymerase I into ^{32}P-labeled double-stranded DNA. The latter was then used to localize the sites of action of certain restriction enzymes (Sst I, Pvu I, Pvu II, Hpa I, Xba I, Eco RI, Hind III and Xho I). Identical results have been obtained with three strains of ASV: Pr-C, Pr-B and B77.

The restriction fragments obtained with in vitro DNA could not be distinguished by electrophoresis from those obtained with linear double-stranded viral DNA synthesized in vivo in infected quail QT6 cells and detected by the procedure of Southern.

Several clones of chick, duck and quail cells transformed by certain ASV (Pr-C, Pr-A, B77), have been characterized for their content of integrated viral genomes, using the Southern procedure. We have also examined the manner in which viral DNA has been integrated into the host genome. Preliminary results with transformed quail clones indicate that the attachment site on the cell DNA at which integration occurs is not unique. The attachment site on the viral DNA does seem to be unique for those transformed cell clones that we have selected.

641 EXPRESSION OF ENDOGENOUS VIRAL SEQUENCES IN MOUSE LYMPHOCYTES, John F. DeLamarter, R. Parry Monckton, Christoph Moroni, Friedrich Miescher-Institut, P.O.Box 273, CH-4002 Basel, Switzerland

Previous work from this laboratory demonstrated that several B-lymphocyte mitogens (e.g., LPS and PPD) are efficient inducers of endogenous C-type viruses in mouse spleen cultures. Mitogen stimulated Balb/c lymphocytes release endogenous virus of xenotropic host range. Virus-releasing cell lines have been established by cocultivation of non-mouse lines with these lymphocytes. Recently antisera raised against this virus were shown to suppress the humoral immune response of mouse lymphocytes to several antigens both in vitro and in vivo (Nature 269:600-601, 1977). These findings support the hypothesis that endogenous viral genes play a physiological role in the immune system.

We have examined the mechanism of viral induction and the expression of viral genes at the molecular level. Using the endogenous reverse-transcriptase reaction, we have synthesized cDNA to viral RNA of the induced xenotropic virus. The majority of these transcripts are approximately 150 nucleotides in length while some extend to over 600 nucleotides as measured by polyacrylamide gel electrophoresis. In RNA driven cDNA/RNA hybridizations, 70% of the cDNA product hybridizes to nuclear RNA from infected cell lines. To quantitate viral expression in stimulated lymphocytes, we hybridized our probe to RNA extracted from cultured spleen cells of Balb/c mice which had been treated with LPS in the presence of BrdU. Results from these experiments showed that mitogen-treated cell populations contained approximately 3 times the concentration of viral-specific transcripts as unstimulated cells. These results suggest that mitogenic induction of endogenous virus occurs through de novo synthesis of viral RNA. Furthermore, uninduced cultured spleen cells of three different mouse strains (AKR, Balb/c, 129/J) were all found to contain virus-specific transcripts. The expression of viral genes even in lymphocytes of the non-inducible 129/J strain, is consistent with our hypothesis that these genes play a physiological role in the immune system. Currently we are examining other stimulated, control, and virus negative lymphoid cells and their subpopulations for viral gene expression.

642 PERSISTENT INFECTION OF A MOUSE SARCOMA VIRUS ON DIPLOID LINES OF INDIAN MUNTJAC CELL: Masakazu Hatanaka and Richard Klein, Viral Oncology Program, National Cancer Institute, Fort Detrick, Frederick, Md. 21701.

The diploid lines of Indian Muntjac cells were transformed by a mouse sarcoma virus (43-2XV). The transformed cells were isolated and cloned. The transformed clones were analyzed for karyotype, the persistent presence of the src gene and tumorigenic potential on athymic nude mice. The number of chromosomes in each of the transformed clones were found to be 6 in female and 7 in male after many passages, essentially similar to the chromosome number of the progenitor cells.

Several clones shed the sarcomagenic type-C RNA viruses, while most transformants became non-producer. The presence of the src gene in these non-producer transformants were determined by infectious center assay after super-infection of a mouse xenotropic virus (Balb-2 V). The cell clones were further tested for tumoigenicity on athymic nude mice. Many clones formed progressive or regressive tumors, but some transformants produced no tumors. These observations demonstrate that the persistent presence of the src gene in cells is an essential but not sufficient condition of tumorigenesis.

643 HOST RESTRICTION OF FRIEND LEUKEMIA VIRUS. R. Soeiro, N. Burnette and S. Gupta. Departments of Medicine and Cell Biology, Albert Einstein College of Medicine, Bronx, New York.

Host restriction of murine oncornaviruses is multigenic. However, one host gene, the FV-1 locus, is a major determinant of the ability of Friend Leukemia Virus (LLV-F) to replicate in tissue culture cells. Additionally, this gene regulates the ability of Friend virus to cause leukemia in the mouse. Replication appears to be inhibited at an intracellular step, but as yet the target site on the virus for interaction with the putative host repressor molecule has not been determined. Host range, the relative ability of a virus type to grow in NIH or BALB/c cells (N-tropic or B-tropic virus) can be altered by continuous passage in restricting cell types. We have analyzed the T_1-RNAse resistant oligonucleotides from the genomes of N-tropic, B-tropic and 5 N-B tropic host range variants in order to determine whether host range variation can be reflected in the genome. The results indicate (1) that N-tropic Friend virus differs significantly from the B-tropic type, (2) only minor oligonucleotide changes occur in the genome on conversion of B-tropic to N-B tropic Friend virus and (3) the oligonucleotide maps of 5 N-B tropic variants appear to be identical.

644 Molecular Basis for $(p30)^{gag}$ Murine Oncornavirus Interstrain Variation, William M. Mitchell and W. Neal Burnette, Vanderbilt University School of Medicine, Nashville, TN 37232, and Albert Einstein School of Medicine, Bronx, NY 10461.

The major internal structural protein $(p30)^{gag}$ of the Moloney leukemia virus and the endogenous Y-1 murine oncornavirus has been examined for biochemical and biophysical manifestations of interstrain antigenic variation. Although they share murine group-specific antigenic determinants, the Y-1 virus p30 appears to have both a lower relative number of such determinants and a decreased affinity at the crossreactive sites for Moloney virus p30 monospecific antibodies. Further, immunological anlysis indicates the presence of unique antigenic sites on the Moloney virus p30 not shared by the analogous Y-1 virus molecule. The two polypeptides co-purify and have similar isoelectric points (pH6.2-6.3) and sedimentation coefficients (2.47 S). However, equilibrium sedimentation yields a significant mass difference between the two proteins, 28,300 daltons and 31,000 daltons for the Moloney and Y-1 virus molecules, respectively. Amino acid analysis indicates a concomitant increase in total residues for the Y-1 virus p30, although a number of residues appear to have been conserved between the two viral proteins. Conformational studies and hydrodynamic calculations demonstrate marked secondary and tertiary structural differences with the Y-1 virus p30 being an asymmetric prolate ellipsoid containing 27-28% α-helix and Moloney virus p30 being somewhat more spherical and possessing an α-helical content of 50-55%. Two dimensional mapping of ^{125}I tryptic peptides of each p30 suggests that considerable sequence heterogeneity is responsible for many of the biophysical, biochemical, and immunochemical differencesin these two analogous structural proteins.

645 RETICULOENDOTHELIOSIS PROVIRUS INTEGRATION SITES IN CHROMOSOMES OF TRANSFORMED CELLS. by Jane Pfeil, Chil-Yong Kang*, and Henry R. Bose,Jr., Dept. of Micro., Univ. of Texas, Austin, Tx. and*Dept. of Micro., Univ. of Texas Health Sci. Center, Dallas, Tx.

Reticuloendotheliosis virus (REV) is the prototype of a group of avian retraviruses. REV transforms cells of the bone marrow and other lymphoreticular organs both in vivo and in vitro. Lines of bone marrow cells (BMC) obtained from Spafas COFAL chicks produce REV but not avian leukosis virus. The DNA of these cells contains REV sequences. In an effort to determine whether REV DNA is integrated into a specific size class, metaphase chromosomes of a cloned line of BMC were fractionated and analyzed for the presence of REV DNA. Colcemid-treated cells were fixed in 50% acetic acid, lysed, and the chromosomes separated by size on zonal sucrose gradients. The chromosomes were divided into four size classes by pooling gradient fractions after microscopic examination. These four size classes contain chromosome numbers 1 and 2; 3,4 and 5; 6 through 12; and the microchromosomes respectively. The specified chromosomes of each class comprised from 63 to 86% of the chromosomes found in the final pooled fractions for these experiments. The DNA from the chromosomes was extracted, hydrolyzed at alkaline pH to remove contaminating RNA, and hybridized with ^{3}H-REV cDNA. REV sequences were found to similar extents in all four size classes. Our study shows that the integration pattern for REV is similar to that reported for viruses of the AL-SV complex. Transfection experiments are in progress to determine the infectivity of the REV proviral DNA associated with the DNA of the various chromosomes.

646 A GENETICALLY TRANSMITTED TYPE C VIRUS ISOLATED FROM SARCOMA VIRUS-TRANSFORMED MINK (MUSTELA VISON) CELLS. Charles J. Sherr, Raoul E. Benveniste and George J. Todaro, National Cancer Institute, Bethesda, MD 20014

The mink epithelioid cell line, Mv-1-Lu (ATCC line CCL 64) has been extensively used in the isolation of type C viruses, in assays for viral-induced transformation, and in studies of cell surface antigens encoded by leukemia and sarcoma viruses. Transformed, "nonproducer" clones containing sarcoma viral genomes have also been employed in constructing sarcoma virus pseudotypes and in quantitative assays of nontransforming leukemia viruses. Following infection of sarcoma virus-transformed Mv-1-Lu subclones, an endogenous, genetically transmitted mink virus (MiLV) has been isolated and cloned to eliminate focus-forming activity. Multiple viral gene copies related to the MiLV genome are detected in the DNA of normal mink tissues and in other related Mustela species, showing that these viral genes have been genetically transmitted in certain carnivores since their evolutionary divergence from a common ancestor. Immunological and nucleic acid hybridization studies suggest that viruses related to MiLV may have originated in rodents and were acquired by carnivores subsequent to an ancestral trans-species viral infection. While Mv-1-Lu cells and their transformed derivatives transcribe only low levels of MiLV RNA and do not produce detectable mink viral particles, infection of transformed mink cells with helper type C viruses leads to the activation of mink viral RNA and the appearance of MiLV proteins in extracellular virions. Since the Mv-1-Lu cell line and its derivatives have become widely used in studies of retroviridae, the activation of endogenous MiLV should be considered by investigators working with these cells.

647 THE BEVI LOCUS, THE PREFERRED HUMAN CHROMOSOMAL INTEGRATION SITE OF BABOON TYPE C VIRUS. R. S. Lemons, W. Nash, S. J. O'Brien, R. Benveniste and C. J. Sherr, Laboratory of Viral Carcinogenesis, National Cancer Institute, Bethesda, MD 20014.

A chromosomal locus, Bevi, on human chromosome six is described which is the preferred chromosomal integration site for baboon type C virus in human cells. Somatic cell hybrids derived from seven independent fusions between rodent X human parental cells were examined for their ability to support the replication of the M7 baboon endogenous virus. These hybrids preferentially segregated human chromosomes while retaining rodent chromosomes. A total of 194 hybrid clones (1^{o} and 2^{o}) were infected with M7 virus and analyzed for viral replication as well as for the presence of enzyme structural gene markers for 19 of 23 human chromosomes. A syntenic association was seen between baboon virus replication and the expression of three enzyme genes assigned to human chromosome six. Human cells were infected with M7 virus, cloned, and then fused to Syrian hamster cells and analyzed for continued M7 virus production. Karyological and isozyme analysis of 34 hybrid clones showed human chromosome six to be syntenic with M7 virus replication as assayed by both viral reverse transcriptase and p30 protein. In addition, molecular hybridization experiments with representative hybrid clones using (^{3}H) labelled cDNA probes to endogenous baboon type C virus demonstrated the presence of integrated proviral DNA sequences was correlated with the presence of human chromosome six. These data suggest that chromosome six is the site of integration of baboon type C virus in human cells.

648 GENERATION OF ONCOGENIC TYPE C VIRUSES: RAPID LEUKEMIA AND SARCOMA VIRUSES FROM C3H MOUSE CELLS DERIVED IN VIVO AND IN VITRO. Ulf R. Rapp and George J. Todaro, National Cancer Institute, Bethesda, MD 20014

An ecotropic type C virus was isolated from C3H/10T1/2 cells in culture after activation with IdU. Variants with increased infectivity for mouse cells have been derived from this virus both in vivo and in vitro by selecting for high titer growth. The highly infectious variants induced mouse fibroblasts to grow in soft agar. When inoculated into newborn NIH Swiss mice 100% of the animals died of leukemia within 4 months and some solid tumors developed at the injection site. Both mousetropic and dual tropic viruses were isolated from the leukemic mice and plaque purified. Isolates from either group were highly leukemogenic on retesting. In addition, new variants were isolated from foci of morphological transformation that developed on mouse cells infected either with tissue extracts from leukemic mice or with virus that was grown in spontaneously transformed NIH/3T3 cells. These variants induced rapid formation of undifferentiated sarcomas in newborn NIH Swiss mice. The results led to the conclusion that the better a mouse type C virus grows in cell culture the more effective it is as a leukemogen. Further, it is possible to start with a weakly infectious, non-leukemogenic endogenous virus and to convert it to highly leukemogenic or to rapidly sarcomagenic virus by passage either in cell culture or in the animal. The availability of a defined series of viruses from a low leukemia mouse strain that differs greatly in their biologic properties should facilitate studies of the molecular basis for the acquisition of type C virus-transforming ability.

649 LEUKEMIA RESISTANCE IN (AKRxNZB)F_1 HYBRID MICE: Syamal K. Datta, Mark Casey and Robert S. Schwartz. Tufts Univ. Med. Sch., Boston, MA 02111

High ecotropic virus producer AKR mice were crossed with the high xenotropic virus producer NZB strain. As compared to the AKR parent the incidence of thymic leukemia was very low and markedly delayed in the F_1: 0% at 8 m. & 8% at 20 m. age. The F_1 expressed high titers of both N-tropic and xenotropic viruses in spleen, lymph node and marrow cells at all ages tested. However 50 F_1 lymphoid tissues (thymus, spleen and marrow) tested between 6-20 m. age lacked the MCF type dual tropic virus that arises by recombination of ecotropic and xenotropic viruses in preleukemic AKR parents. A striking feature, found only in F_1 thymuses at all ages tested, was an almost complete absence of ecotropic virus and markedly reduced expression of xenotropic virus. T lymphocytes in F_1 spleen and lymph nodes also showed this restriction. Thus a cellular mechanism, present in leukemia-susceptible AKR thymus, that allows virus expression and production of recombinant viruses, is absent in the leukemia-resistant F_1 thymus. Since the backcross F_1xAKR thymus is leukemia susceptible, the thymic mechanism seems genetically controlled.

ECOTROPIC/XENOTROPIC VIRUS TITERS: Infectious Centers($\log_{10}$)10^7 cells

		THYMUS	SPLEEN			BONE MARROW
			Unfractioned	T cells	B cells	
AKR	2-5 m	2.4±0.5/0(.05±0.1)	3.4±0.3/0.6±0.4	2.6±0.2/ NT	3.8±0.3/ NT	3.7±0.4/1.1±0.4
NZB	2-5 m	-- /3±0.5	-- /3.7±0.4	-- /2.9±0.5	-- /4.3±0.4	-- /4±0.3
F_1	2-6 m	0(0.2±0.3)/1.2±0.5	2.6±0.5/2.9±0.6	1.4±0.7/1.9±0.7	3.2±0.5/3.4±0.6	2.5±0.7/3.4±0.6
	20 m	0(0.3±0.5)/1.5±0.7	2±1/2.8±0.6	NT	NT	1.5±1.3/3.4±0.7

650 TRANSCRIPTION AND REPLICATION OF THE INFLUENZA GENOME, G.E. Mark, J.M. Taylor, B. Broni and R.M. Krug, The Institute for Cancer Research, Philadelphia, Pa. 19111 and The Memorial Sloan-Kettering Cancer Center, New York, N.Y. 10021.

Determination of the kinetics of annealing of virus-specific ^{32}P-cDNA and ^{125}I-vRNA probes to RNA isolated from nuclear and cytoplasmic fractions of infected cells allowed for the analysis of the transcription and replication in MBCK cells of influenza virus (strain WSN). Transcription of the infecting vRNA into cRNA, first evident at 1 hr p.i., increase significantly after 2 hrs p.i. During the course of transcription greater than 75% of the cRNA was detected in the cytoplasmic fraction. Replication of vRNA molecules commenced between 1.5 and 2 hrs, at which times ~60% of these molecules are found in the nuclear fraction. After 2 hrs a substantial accumulation of replicated vRNA was detected in the infected cell cytoplasm. Primary transcription was investigated by inhibiting vRNA replication by the addition of cycloheximide (100 µg/ml) at the time of infection. This resulted in a 3-6 fold decrease in the total number of cRNA transcripts compared with untreated infected cells, when measured at a time prior to vRNA replication (1.5 hrs). Also, in contrast to the control cultures, the primary transcripts were approximately equally distributed between the nuclear and cytoplasmic fractions. One interpretation of our data is that cRNA is transcribed in the nucleus and cycloheximide interferes with the subsequent migration to the cytoplasm. Further analysis of these primary transcripts shows the majority of them to be polyadenylated.
By employing probes specific for individual virus RNA segments the primary transcripts were shown to contain sequences representative of each segment, however, the number of copies of each segment is not equal. We conclude that primary transcription is a regulated process.

651 A GROUP III TEMPERATURE SENSITIVE MUTANT OF VESICULAR STOMATITIS VIRUS AMPLIFIES SPECIFIC TRANSCRIPTS AT THE NONPERMISSIVE TEMPERATURE,

Gail M. Clinton, Sheila P. Little, Frederick S. Hagen and Alice S. Huang, Harvard Medical School, Boston, Mass. 02115

Of the mutants of vesicular stomatitis virus (VSV) tested, only a group III temperature sensitive mutant, tsG31, along with its homologous defective interfering particle regularly established a persistantly infected culture in vitro (Holland and Villarreal, 1974). The VSV group III mutants are thought to have a lesion in the membrane-associated M protein (Lafay, 1974). This lesion has been reported to affect the transport of uradine which leads to an apparent difference in the amounts of RNA synthesized at the permissive and nonpermissive temperatures (Genty, 1975).

We have investigated the molecular biology of the tsG31 mutant and found an over production of RNA at the nonpermissive temperature which was unrelated to the transport of uridine. Analysis of the RNA synthesized at the non-permissive temperature compared to that synthesized under permissive conditions revealed that the messages coding for the glycoprotein, G, and the polymerase protein, L, were specifically over produced at the nonpermissive temperature. On the basis of these observations we propose that the M protein regulates the synthesis of specific VSV transcripts.

652 SELECTIVE ALTERATION OF MURINE LEUKEMIA VIRUS CELL SURFACE ANTIGENS BY EXOGENOUS VIRUS INFECTION OF T-LYMPHOBLASTOID CELLS, Kim S. Wise and Ronald T. Acton, University of Alabama in Birmingham, Birmingham, Alabama 35294.

Changes in the expression of several antigens residing on the surface of murine T-lymphoblastoid cell lines were assessed following infection of these lines with vesicular stomatitis virus (VSV), an agent known to augment the immunogenicity of murine tumors. Quantitative absorption of cytotoxic antisera recognizing 1) Thy-1 T-cell differentiation alloantigens 2) H-2 histocompatibility antigens and 3) MuLV-associated antigens p30, gp70 and Gross Cell Surface Antigen (GCSA) were employed to compare the amount of each antigen on uninfected or VSV-infected cells. Infection of the AKR thymus-derived BW5147 line (phenotype: Thy-1.1/H-2^k/GCSA/gp70/p30) had no effect on the quantity of Thy-1.1, H-$2K^k$, or gp70 expressed, whereas a marked (10-20 fold) increase in GCSA and p30 was observed. Similarly, selective increases in GCSA and p30 were found when the C57BL/6 derived EL4(G+) line (phenotype Thy-1.2/H-2^b/GCSA/gp70/p30) was infected with VSV. The increase in GCSA followed VSV maturation but preceded the cytopathogenic effect of this virus. The increase was not observed with uninfected cells either osmotically lysed or treated with actinomycin D or cycloheximide. Anti-GCSA activity could not be removed by absorption with VSV purified from infected lymphoblastoid cell lines. These results demonstrated that VSV infection caused a highly selective increase in the surface expression of antigens comprising MuLV gag (but not env) gene products. This effect is not due to VSV-induced cell damage, nor to inhibition of RNA or protein synthesis by this virus. These data are consistent with a selective VSV-induced alteration in the processing of endogenous MuLV gag gene products on the cell surface.

653 CHARACTERIZATION OF THE 25-30S RNA COMPONENT OF THE INDUCTION SUPERNATANT OF JLS-V9 CELLS, D. S. Dolberg, T. Hunter, and H. Fan, Tumor Virology Laboratory, The Salk Institute, San Diego, California 92112

A variety of retroviruses with typical 35-40S genomes can be induced from the cell line JLS-V9 with halogenated pyrimidines. In addition, the induction supernatant contains a 25-30S species which is present in four- to five-fold excess of the 35-40S material. By hybridization, the 25-30S species has little sequence homology to ecotropic or xenotropic components. It has previously been demonstrated that although this RNA is present on polyribosomes in uninduced cells, these cells are negative for the production of p30, reverse transcriptase and gp70. These studies have been extended to show that, in the messenger-dependent lysate system of in vitro translation, the 25-30S RNA is an extremely poor message relative to the xenotropic message.

654 GENOMIC STABILITY OF PERSISTENT GIBBON ONCORNAVIRUS, Lily Sun, Thomas G. Kawakami, and Sam I. Matoba, Comparative Oncology Laboratory, University of California, Davis, Calif

Gibbon type-C virus has been isolated independently by several investigators from clinically healthy and leukemic gibbons. The gibbon can have persistent infection over several years *without overt* clinical symptoms, although all leukemic gibbons we have examined were found infected with type-C virus. Based on molecular hybridization studies all viruses we have isolated were found to have some variation in the viral genome. The sequence homology may vary from 30-100%. Since gibbon viruses were found to be exogenous to the species, we attempted to determine whether the sequence variation in the viral genome was the consequence of virus transmission through unrelated gibbon hosts.

The effect of horizontal transmission on the virus genome was evaluated by examining the 70S RNA of viruses from experimentally inoculated gibbons. Virus transmission was achieved by serial passage through three unrelated gibbons. Each animal developed persistent viremia before the onset of leukemia. Infectious virus was isolated from each animal. Using cDNA prepared by endogenous reverse transcriptase reaction, the homology of viral genomes of these viruses were examined by cDNA-70S RNA hybridization. Each virus was found to share more than 93% homology with the others. T_m analysis further indicated the genomes of these viruses were essentially identical. Viral genomes of viruses passaged from parent to progeny were similarly found identical.

The conclusion which can be drawn from this study indicates that the genome variation of each new isolate is not the consequence of horizontal or parent-progeny transmission.

656 PROCESSING AND TURNOVER OF PROTEINS RELATED TO THE P27 OF MOUSE MAMMARY TUMOR VIRUS, Clive Dickson and Maureen Atterwill, Imperial Cancer Research Fund, London.

The mouse mammary tumor virus (MMTV) contains several low molecular weight proteins which together with the RNA genome constitute the core structure of the virion. The most abundant protein of the virion core is the p27 which probably forms the core shell. Using immunoprecipitation procedures and SDS-polyacrylamide gel electrophoresis, several proteins related to the p27 of MMTV have been detected in two mouse mammary tumor cell lines (GR and Mm5MT). After a short pulse with ^{35}S-methionine the major component immunoprecipitated from cell extracts with an anti-p27 serum migrates on SDS-polyacrylamide gels as a doublet of 77,000 and 75,000 daltons (p77/75) respectively. Several lower molecular weight proteins are also detected; these are apparently derived from the precursor p77/75 molecules by proteolytic cleavage. During a 3 hour chase, the amount of p27 in the cells and in the culture fluids increases with time but remains at a comparatively low level. The immunoprecipitated proteins are all related to the virion p27 as shown by an analysis of their methionine or arginine-lysine containing peptides following trypsin digestion. These results indicate that most of the p27 related proteins are rapidly turned over in these cell lines. The small amount of p27 released into the culture fluids in virions probably represents a minor portion of these proteins which are processed via a proteolytic cleavage mechanism.

657 EVIDENCE FOR INVOLVEMENT OF TRANSCRIPTIONAL CONTROL IN THE REGULATION OF AKR VIRUS GENE EXPRESSION, P. J. Laipis, J. L. Stein, G. S. Stein, J. A. Thomson and S. K. Chattopadhyay, Univ. of Florida, Gainesville, Fl. 32610 and National Cancer Institute Bethesda, Md. 20014

The expression of AKR C-type RNA tumor virus genetic information has been studied in cell lines derived from AKR mouse embryos. Although all AKR mouse cell lines examined contain the AKR proviral sequences integrated into their genomes, viral producer and nonproducer cell lines have been identified. Using a ^{3}H-labeled DNA probe complementary to AKR viral RNA sequences, the presence of viral sequences in RNA isolated from the nucleus and cytoplasm of viral producer cells has been detected. Chromatin from these viral producer cells is an effective *in vitro* template for transcription of AKR viral RNA sequences. In contrast, a significantly reduced level of hybridization is observed between AKR viral cDNA and nuclear RNA, cytoplasmic RNA and the *in vitro* chromatin transcripts from nonproducer AKR cell lines. Regulation of viral gene expression mediated at least in part at the transcriptional level is therefore suggested. The low level of hybridization of viral cDNA observed with *in vivo* and *in vitro* transcribed RNAs from nonproducer cells may reflect either partial AKR viral transcripts or transcripts from other endogenous, closely related C-type viral proviruses, which are also present in these mice. Evidence to distinguish these alternatives will be presented.

658 CLONING AND ANTIBODY STUDIES OF A VARIANT OF SENDAI VIRUS WITH TEMPERATURE SENSITIVE HEMOLYTIC ACTIVITY, Arlene R. Collins and Thomas D. Flanagan, State University of New York at Buffalo, Buffalo, NY 14214

Sendai$_{AS}$ is a variant recovered *in ovo* from persistently infected mouse cells maintained in the presence of Sendai antibody. Replication of Sendai$_{AS}$ at 37C *in ovo* was abortive. Less than 50% of the eggs inoculated at dilutions -2 to -13 $\log_{10}$ yielded infectious virus. Virus produced at 37C showed weak hemagglutinating activity ($HA^{\pm}$), no detectable neuraminidase (NA^{-}) or hemolysis (F^{-}). Sendai$_{AS}$ replication at 33C was productive and lethal in eggs ($10^{13}ELD_{50}$). Virus produced at 33C showed $HA^{\pm}$, $NA^{\pm}$ (0.19 NAU_{OD540}) but high F^{+} activity (10^{3} HEU). Hemolysis was temperature sensitive. Sendai$_{AS}$ particles produced at 33C were purified by sucrose gradient centrifugation (15-60% sucrose, 16 hrs, 40,000 xg), polypeptides separated by polyacrylamide gel electrophoresis and antisera were produced in rabbits to polypeptides HN and F. The ability of the antisera to inhibit hemolysis was examined. Anti F inhibited activity of the AS variant and to a lesser extent the wild type virus. Anti HN showed much less antihemolytic activity which was directed against the AS variant exclusively, suggesting HN was the defective polypeptide. Attempts to clone Sendai$_{AS}$ at 33C by terminal dilution *in ovo* yielded fluids with one of two types of activity F^{-} or F^{+}. Passage of either type of fluid again yielded both types of activity at terminal dilution. In conclusion we suggest that Sendai$_{AS}$ has a defective HN peplomer. The HN defect is temperature sensitive in that at 33C the expression of F activity is permitted for at least a part of the population of particles.

659 RNAs OF SENDAI VIRUS DELETION MUTANTS, Carroll Pridgen and David W. Kingsbury, St. Jude Children's Research Hospital, Memphis, Tennessee 38101

A variety of deletion mutants (defective-interfering or DI mutants) of negative strand RNA viruses have been described. In almost every case where the genetic contents of the DI RNA species have been determined, the deletions have been in those regions of the standard virus genomes which code for the most abundant proteins. In contrast, we report here that several spontaneous DI mutants of Sendai virus have retained information for these proteins. RNAs from deletion mutants of Sendai virus were analyzed by polyacrylamide gel electrophoresis in 6M urea. Each mutant exhibited a unique pattern of multiple subgenomic RNA species ranging in molecular weight from about 8×10^{5} to $< 3 \times 10^{6}$. To determine the gene contents of the mutants, we used the 18S class of virus-specific mRNA as the labeled probe. This class contains transcripts representing 60% of the genome, starting at the 3' end (5 of the 6 virus genes). RNA species from each of the 8 mutants hybridized to this probe. At saturation, 73%, 85%, and 40% of the labeled 18S virus mRNA was hybridized by mutants 1, 8, and 11, respectively, compared to 95-100% when hybridized to standard virus genomes. These results suggest that all of these isolates contain some information originating from the 3' end of the genome, in contrast to most previous findings with both vesicular stomatitis virus and Sendai virus. These observations suggest that the generation of DI particles from the most abundantly transcribed region of Sendai virus is not a rare event and that this event may take place at a different step in virus genome replication than previously supposed.

660 THE BINDING OF THE PHOSPHOPROTEINS AND GENOMES OF THE ENDOGENOUS PRIMATE TYPE C VIRUSES: POSSIBLE MODULATION OF ENDOGENOUS VIROGENE EXPRESSION. Arup Sen and George J. Todaro, Laboratory of Viral Carcinogenesis, National Cancer Institute, Bethesda, MD

The major phosphoprotein of RD-114 and of the various isolates of baboon type C viruses bind to viral RNAs of each other but not to the RNA of other mammalian and avian type C viruses. The RNA genome-phosphoprotein binding data obtained using the proteins and 70S viral RNAs from these viruses indicate that regions coding for the genome-associated phosphoproteins as well as their specific RNA-binding sites have been conserved in these genetically transmitted type C viruses of primates and cats (see table below).

Phosphoprotein from:	Phosphoprotein molecules bound 70S viral RNA molecule of:				
	PP-1	M28	RD-114	Mouse	Avian
Papio papio (PP-1)	7-9	6-8	6-8	<1	<1
Papio cynocephalus (M28)	7-9	6-8	5-7	<1	<1
Felis catus (RD-114)	7-9	6-8	6-8	<1	<1

Certain primate species, from which transmissible type C viruses have not been isolated, express type C viral RNA and viral structural proteins. The ability of the endogenous baboon viral phosphoproteins to bind to transcripts from related endogenous primate viruses might, thus, be valuable in studying the expression of as yet unidentified persistent viruses in primate cells that do not release complete, infectious virions.

Mechanisms of Persistence, Genetic Expression, DNA Containing Animal Viruses

661 CHARACTERIZATION OF HSV 1 DNA IN TRANSFORMED CELLS, Douglas F. Moore and David T. Kingsbury, University of California at Irvine, Irvine, California 92717

The physical state and extent of transcription of Herpes simplex virus DNA in two lines of cells biochemically transformed by HSV were examined. Cell lines 139 and 171 which have been transformed by uv irradiated HSV 1 contain viral DNA representing approximately 15% and 30% of the viral genome respectively. Studies using the "network" technique of Varmus et al. as modified by Bellett indicated that there is a complex relationship between cellular and viral DNA. In both cases a fraction of the viral DNA sequences were integrated, the remainder being present in a nonintegrated state. The network studies have been corroborated by experiments using alkaline sucrose centrifugation to fractionate cellular DNA. In both types of experiments DNA-DNA hybridization was used to locate the viral sequences.

RNA-DNA hybridization of total cellular RNA indicated that only a very small fraction of the viral DNA present in these cell lines was transcribed.

662 EPSTEIN-BARR VIRION ASSOCIATED ENZYMES, Wendy Clough and Carol Prezyna, Dept. of Biological Sciences, University of Southern California, Los Angeles, 90007; and Steven R. Goodman, Biological Laboratories Harvard University, Cambridge, Mass 02138.

Epstein-Barr virus (EBV) is an excellent example of a virus that is capable of persistently infecting host cells. Many stable lymphocyte lines exist which carry multiple copies of the viral genome and express certain viral antigens, but which produce no or few viral particles in culture. The induction and maintenance of this persistent state is not currently understood. We are examining certain aspects of the early stages of EB viral infection to learn more about the development of persistent infection. We have shown that EB virions contain a DNA polymerase activity that may well be a structural component of the virion, and which is different from any previously reported host cell or virus induced enzymes. Some of its characteristics have lead us to postulate a repair function for this polymerase. Such a function may be necessary at an early stage of infection because of the nicked and gapped state of mature herpes virus DNA. We are currently investigating virions for possible nuclease and ligase functions. Ultimately, we hope to apply information from these enzyme studies to learning more about the initiation of EBV infection.

663 TRANSIENT INTEGRATION OF VIRAL DNA AS A MODIFIER OF INTEGRATION SITE SPECIFICITY, Robert F. Baker, Gerald Edelman, and David Larocca, Department of Biological Sciences University of Southern California, Los Angeles 90007.

We are studying modification of integration site specificity and host range of herpes viruses (EBV and HSV) and transfected SV40-recombinant DNA. Modification of individual viral genomes result from recent transient integration events of provirus forms of viral DNA genomes. We have developed a method for isolation of terminal fragments of linear viral DNA having host cell-viral DNA junctions near termini; this allows isolation of restriction fragments bearing junctions even when fragment lengths are heterogenous and do not form electrophoretic bands on gels. Junction-bearing fragments are being compared, mapped by restriction enzymes, and, as hybridization probes on Southern gels, used to investigate the fate of persistently-integrated and transiently-integrated viral DNAs. We have also used recombinant SV40 early gene DNA pieces attached in vitro to procaryotic DNA or to sea urchin histone genes to follow changes in sequence at termini of proviral SV40-recombinant DNA after transfection and during multiple passages of recombinant DNA in permissive and non-permissive cell line. One reasonable interpretation is that upon excision and reintegration of individual viral DNA molecules, there is exchange of host genome sequences flanking termini of proviral DNA segments. We are forming a model for transient and persistent infections resulting from variations in nucleotide sequence flanking termini of the linear form of individual virus genomes. Exchange of short host DNA sequences at termini of linear viral DNAs can result in variations in position of integration at middle repetitive sites; these variations could account for transposon-like characteristics as well as ability to persistently infect cells. This is being investigated in virus-nonproductive Raji and virus-productive P3HR1 cell lines carrying EBV genome.

664 ACUTE AND LATENT INFECTION OF MACROPHAGES BY MURINE CYTOMEGALOVIRUS, Frank J. Dutko, Alan R. Brautigam and Michael B. A. Oldstone, Scripps Clinic and Research Foundation, La Jolla, CA 92037

Murine cytomegalovirus (MCMV) can infect macrophages in vitro. Peritoneal exudate cells from C3H/St mice were adhered to plastic and then washed vigorously. The adherent population of cells were 90% macrophages as evidenced by phagocytosis of zymosan particles, as well as by staining with Giemsa. MCMV replicated in adherent peritoneal cells as shown by: 1) an increase in plaque forming units (PFU) of virus in the supernatant, 2) infectious center assay, which indicated 30% of the cells produced at least 1 PFU, and 3) in situ hybridization, which indicated 85% of the cells contained viral DNA. MCMV could replicate to the same extent in thioglycollate stimulated as in unstimulated peritoneal cells.

MCMV could also be shown to be present in macrophages of latently infected adult mice (injected i.p. with 100 PFU of MCMV within 24 hours of birth) by: 1) allogeneic and syngeneic cocultivation of adherent peritoneal cells with mouse embryo fibroblast cells, and 2) quantitation of the number of viral genome equivalents by DNA:DNA hybridization reactions. MCMV could be recovered from macrophages from mice 3 months to 2-1/2 years of age.

We conclude that macrophages are permissive for the replication of MCMV. Furthermore, MCMV can be harbored in and recovered from macrophages of latently infected mice. Supported by USPHS grants NS-12428, AI-07007, and Research Fellowship No. 1 F32 AI 05596.

665 EXPRESSION OF SURFACE RECEPTORS FOR THE Fc FRAGMENT (IgG) IN FRESHLY INFECTED, PERSISTENTLY INFECTED AND SUPERINFECTED HUMAN LYMPHOID CELL LINES BY HERPES SIMPLEX AND EPSTEIN-BARR VIRUSES, José Menezes and Angelo E. Bourkas, Lab. Immunovirology, University of Montreal and Ste. Justine Hospital, Montreal, Quebec, Canada.

Herpes simplex virus (HSV: types 1 and 2) and Epstein-Barr virus (EBV) were used to investigate the ability of these herpesviruses to induce surface receptors for the Fc fragment of IgG in freshly infected, persistently infected and superinfected human lymphoid cell lines (HLCL). We found that while persistent infection by HSV was characterized by the expression of Fc-receptors, persistent infection by EBV was not. HSV infection of HLCL led to induction of Fc-receptors, while superinfection of chronically HSV-infected cells (HSV-Raji) did not increase the percentage of Fc-receptor positive cells. Contrary to HSV, EBV seems to lack the ability of inducing Fc-receptors after infection of these cells. 85-90% of the HSV persistently infected HSV-Raji cells showed intracellular HSV antigens as detected by indirect immunofluorescence using rabbit anti-HSV antibody and FITC-goat anti-rabbit IgG. 54% of HSV-Raji cells expressed Fc-receptors as shown by means of EA-rosettes, using ox-erythrocytes coated with rabbit 7S IgG. In comparison, the parent, uninfected Raji HLCL expressed no HSV antigens and less than 1% of the cells had receptors for Fc. Differences between the percentage of antigen positive and Fc-bearing cells of HSV-Raji could be attributed to the possibility that (a) only a portion of persistently infected cells express Fc-receptors or (b) the EA-rosetting technique may not be sensitive enough for detecting all of the Fc-receptor-bearing cells. Furthermore, preliminary studies using inhibitors of virus replication, would suggest that HSV-DNA replication is necessary for Fc-receptor induction following infection.

666 HERPES SIMPLEX VIRUS DNA AND mRNA IN ACUTELY AND CHRONICALLY INFECTED MOUSE GANGLIA. Alvaro Puga, Joel D. Rosenthal, Harry Openshaw and Abner L. Notkins, Laboratory of Oral Medicine, National Institute of Dental Research, National Institutes of Health, Bethesda, MD 20014.

Trigeminal Ganglia of mice infected with Herpes Simplex Virus were examined for the presence of virus-specific DNA and mRNA sequences at both acute and chronic stages of the infection. Ganglionic DNA and RNA were mixed at vast mass excess with a trace of high specific activity, 125-I-labeled viral DNA and the reassociation kinetics of the probe was followed. Viral DNA sequences are detected at a level of 1.2-2.0 genome equivalents per cell during the acute stage and at a level of 0.11±0.003 genome equivalents per cell during the chronic stage. Viral mRNA is found at the level of 0.1-0.2 RNA equivalents during the acute stage, but cannot be found during the chronic stage by techniques that would have detected the presence of one equivalent per two thousand cells. These results suggest that the virus genetic information is not expressed during the chronic stage of the infection and support the hypothesis that during this stage the viral genome exists as a non-replicating, truly latent entity.

667 PERSISTENCE OF HERPES SIMPLEX VIRUS IN HEMIC CELL LINES, Charles R. Rinaldo, Bonnie S. Richter, Paul H. Black and Martin S. Hirsch, Massachusetts General Hospital and Harvard Medical School, Boston, MA 02114

Herpes simplex virus (HSV) has been recognized for its ability to infect human leukocytes and lymphoreticular cells. Data from this laboratory has previously demonstrated that HSV, type 1, has the capacity to replicate in a variety of cell lines with B, T, or myeloid properties. The present results indicate that HSV persists in certain hemic cell lines for many months after the acute infection. HSV has persisted for 205 days post-infection in CEM (T) cells, with titers of virus ranging from 5.0 - 6.6 $\log_{10}$ plaque-forming units (PFU)/ml in cell lysates. An average of 99% of the virus was cell-associated, approximately 3% of the cells produced infectious virus as assayed by infectious centers, and an average of 21% of the cells were positive for HSV antigen as detected by immunofluorescence. The cell concentration and viability of the cells remained comparable in both the uninfected and HSV-infected CEM cells. In contrast to the CEM cells, NC37 (B) and K562 (myeloid) cell lines have supported viral replication for shorter time periods post-infection. Titers of virus reached peak values of greater than 6.0 $\log_{10}$ PFU/ml in both cell lines, with the majority of virus being cell-associated. The persistence of HSV in hemic cell lines provides an in vitro model for the study of chronic HSV infection and the effect of HSV on the properties of lymphoid and myeloid cells.

668 PERSISTENT HERPES SIMPLEX VIRUS INFECTION: PATHOGENESIS AND CONTROL, B.K. Murray, P.S. Morahan, E.N. Kitces and W.J. Payne, Medical College of Virginia, Richmond, VA 23298

Oral inoculation with herpes simplex virus (HSV) in mice was used to establish acute and persistent neuronal infections. During acute infection, HSV could be isolated from primary lesions on the lip, ipsilateral sensory ganglia (trigeminal), cerebellum, contralateral sensory ganglia, and the cerebrum. From 90-100% of the trigeminal ganglia were infected, while infectious HSV could be recovered from the brains of 95% of the animals. However, deaths due to herpetic encephalitis ranged from 0-100%, concomitant with 100% ganglionic infection. Infectious HSV could be recovered from explanted ganglia 15 months after infection, but virus could only be recovered for 12 days after infection from ganglion homogenates. Infectious virus could not be recovered from the brain beyond 14 days after infection, but viral antigens and histological evidence of HSV pathology could be detected beyond this time. Persistent ganglionic infection could be controlled by prior immunization with a HSV vaccine in up to 100% of the immunized animals. Heat aggregation of the vaccine preparation did not affect its ability to protect the host from acute ganglionic infection or from HSV encephalitis, but protection from latent ganglionic infection was reduced from 98% to 19%. Glutaraldehyde polymerization of the vaccine likewise did not affect its ability to protect the host from acute ganglionic infection or HSV encephalitis, but protection from latent ganglionic infection was decreased from 90% to 58%. At the time of oral challenge with HSV, the level of serum neutralizing HSV antibody was lower in animals immunized with the polymerized vaccines than in animals immunized with the non-polymerized vaccines (<10 units vs 20 units). Passive transfer of immune sera offered partial protection from oral and neural infection with HSV. (DE 04700)

669 PERSISTENCE OF SV40 VIRAL SUBGENOMES IN RECIPIENT CELLS. Mary Pat Moyer, Rex C. Moyer, Mary Gerodetti, and George Lipotich, Trinity University, San Antonio, Tx 78284.

Transfection of permissive cells with defined viral genome fragments provides an interesting model for persistent viral infections, as in vivo recombination events might periodically induce virus production. Permissive African green monkey cells were transfected with EcoRI/HapII restriction endonuclease-generated SV40 DNA subgenomes. The regions comprising 74% of the genome (early genes) and 26% of the genome (late genes) could be separately maintained in the permissive cells in a potentially biologically active form. Fusion of cells containing the complementary subgenomes, or transfection of DNAs from fragment-containing cells into permissive cells, yielded infectious SV40. Comparable results were obtained when rat cells which had received the EcoRI/HapII 74% SV40 subgenome (Abrahams, et al.) were used in conjunction with the 26% subgenome-containing monkey cells. In addition, complete SV40 genome equivalents could be maintained in some permissive cell cultures which were co-transfected with both the 74% and 26% SV40 DNA fragments. Some of these cultures spontaneously produced virus. Others did not produce virus, but SV40 could be induced by treatment with iododeoxyuridine. Support by the Bettye Thorman Cancer Research Trust and the Morrison Trust is gratefully acknowledged.

670 PERSISTENCE OF SV40 AND BK PAPOVAVIRUSES IN TISSUE CULTURE CELLS OF THEIR NATURAL HOSTS, P. Upcroft, H. Skolnik, K. Takemoto* and G. Fareed, Molecular Biology Institute, UCLA, Los Angeles, Ca. and *NIAID, Bethesda, Md.

The interaction of two papovaviruses with tissue culture cells of their natural hosts, SV40 in a continuous line of rhesus monkey kidney cells (LLC MK_2) and the human papovavirus BK in primary human fetal brain cells, has been investigated. In both cases, a persistent infection was established for 15 months with SV40 and approximately 3 years with BK virus. The cultures shed virus continually during these periods with an average of 20% T-antigen positive cells for SV40 and 1-5% for BK. The BK viral genomes persisted without incurring rearrangements during this 3 year period. No defective genomes were detected until 4 months after the establishment of persistence by SV40, with approximately 5-10% of the genomes being smaller than wild-type for the next 11 months. The virus produced from both cell populations was predominantly wild-type as assessed by plaque formation and restriction endonuclease analysis of the genomes. Cloned lines of BK persistently-infected human cells have been obtained which are T-antigen negative and have both free and integrated forms of BK genomes.

Cloned sublines of the infected rhesus cells have ranged from clones carrying no detectable free viral DNA and being resistant to superinfection by SV40, to clones carrying many copies of viral DNA and shedding virus. One type of clone appears to reflect the modulation in latency of the original culture in that no virus was produced for one month after cloning, followed by one month of virus shedding with considerable cell death, followed by a latent period with no virus production. During the latent period the cells carried many wild-type viral genomes with approximately 10% being shortened, rearranged species.

671 HERPES SIMPLEX VIRUS TYPE 1 (HSV-1) PRODUCTION IN SOMATIC CELL HYBRIDS BETWEEN A NON-PERMISSIVE CHINESE HAMSTER CELL LINE AND HUMAN CELLS: REQUIREMENT FOR THE PRESENCE OF SPECIFIC HUMAN CHROMOSOMES, Uta Francke and Bertold Francke, Department of Pediatrics, University of California, San Diego and Tumor Virology Laboratory, Salk Institute for Biological Studies, La Jolla, California 92037.

Several established cell lines derived from Chinese hamsters have been found to be non-permissive for productive infection by HSV-1. One of these, the HPRT deficient cell line 380-6 derived from V79, was fused with human cells carrying different translocations. Fourteen hybrid clones from 6 independent fusion experiments were analyzed simultaneously for human chromosome content and for their ability to support productive HSV-1 infection. The results indicate that human chromosome 11 is necessary and sufficient to support HSV-1 production in these hybrid cells. Among the human chromosomes that could unambiguously be excluded was chromosome 3. This chromosome has previously been found to be essential for HSV-1 production in hybrid cells made with a non-permissive Chinese hamster DON line (Carritt and Goldfarb, Nature 264: 256, 1976), implying that the defects leading to non-permissivity in the two Chinese hamster cell lines may not be identical.

672 MAPPING OF STRUCTURAL GENE SEQUENCES IN ADENOVIRUS 2 DNA BY HYBRID ARRESTED CELL-FREE TRANSLATION, Bruce M. Paterson*, Bryan E. Roberts+, and Michael B. Mathews#. *Laboratory of Biochemistry, NCI-NIH, Bethesda, MD 20014, +Dept. Biology, Brandeis University, Waltham, MA 02154, #Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724.

We have developed an approach to directly correlate structural gene products with their corresponding DNA (or RNA) sequences and have used the procedure to map the viral polypeptides expressed late in adenovirus 2 infected HeLa cells within the viral genome. Such an approach would be directly applicable to the identification of viral specific polypeptides involved in persistant viral infections. The procedure and various approaches are to be described.

673 EXPRESSION OF EARLY ADENOVIRUS GENES; PROCESSING OF RNA BY CUTTING AND BIGHTING? Geoffrey R. Kitchingman and Heiner Westphal, Laboratory of Molecular Genetics, National Institutes of Health, Bethesda, Md. 20014

We have studied the in vivo transcription of early adenovirus genes, including those persistently expressed in transformed cells. Separated strands of viral DNA were complexed with nuclear or cytoplasmic RNA, and the hybrids observed in the electron microscope. While large nuclear RNA molecules often form a continuous duplex comprising the entire gene block in three of the four early regions of the adenovirus genome, many of the shorter nuclear and cytoplasmic RNAs bridge loops of "intervening" single-stranded DNA while hybridizing to the DNA sequences flanking the loop. Examples of RNA's observed in early region 2, coding for the 72K DNA-binding protein, are shown here.

All three RNA's have common end points, but internal sequences, complementary to the "intervening" DNA loops, are missing in two of them, as indicated by the white bars. Accurate maps of early transcripts in all four regions will be presented. It is tempting to assume that internal sequences are removed from primary viral transcripts during mRNA maturation. Experiments probing such precursor-product relationship are in progress.

674 BIOCHEMICAL AND IMMUNOCHEMICAL CHARACTERIZATION OF THE U-ANTIGEN OF CELLS TRANSFORMED BY SIMIAN VIRUS 40. R. Schmidt-Ullrich, W. S. Thompson and D.F.H. Wallach, Tufts-New England Medical Center, Radiobiology Div. 171 Harrison Ave., Boston, MA 02111.

In purified plasma membranes of SV40-transformed hamster lymphocytes (GD248) we have identified a SV40-specific ∿100,000 D, pI 4.7 protein which reacts immunochemically with sera, positive for U- but not for T-antigen by indirect immune fluorescence. (Schmidt-Ullrich, R., Thompson, W.S., Lin, P.-S. and Wallach, D.F.H., Simian Virus 40(SV40)-specific proteins in the membranes of SV40-transformed hamster and mouse cells. Proc. Natl. Acad. Sci.USA 74,5069-5072, 1977). Assignment of this protein as the perinuclear U-antigen is now further supported by the facts that Triton X-100 extraction of highly purified GD248 nuclei suppresses their U-antigen reactivity and that these Triton extracts contain a pI 4.7, ∿100,000 protein identical to the plasma membrane component. A six-fold concentration of this protein in the nuclear envelope (in comparison to the plasma membrane) and about equal concentrations of the SV40-specific transplantation (surface) antigen (TSTA, TSSA) in both groups of membranes cannot be explained by cross-contamination, during cell fractionation. Metabolic labeling with [^{14}C] glucosamine and susceptibility to neuraminidase treatment in isolated plasma membranes identify the perinuclear U-antigen as a sialoglycoprotein. This fact distinguishes U- from T-antigen. Therefore, the large functional dissimilarities of these antigens (site affinity, heat lability and immunologic reactivity) may arise from different (possibly competing) mechanisms of host cell processing of the SV40 protein A, i. e. glycosylation and/or phosphorylation.

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675 UV-RADIOLOGICAL ANALYSIS OF HSV-1 TRANSCRIPTION: EVIDENCE FOR PROMOTER SWITCH, Robert Millette, Sue Talley-Brown, Rosemary Klaiber, Margaret Pedersen and Tom Beck, Dept. of Immun. & Micro., Wayne St. Univ. Sch. of Med., Detroit, MI 48201

The transcriptional organization of the HSV-1 genome has been analyzed by studying the sensitivity of viral polypeptide synthesis to UV-irradiation of the infecting virus. HSV-1 was irradiated with 0 to 400 erg/mm^2 of UV light and used to infect Xeroderma pigmentosum (X.p.) cell monolayers. Early (alpha) polypeptides were analyzed by adding cycloheximide from 0 to 8 h p.i., removing the drug, and pulse labeling proteins for 45 min with ^{35}S-methionine. Proteins were separated by electrophoresis in polyacrylamide slab gels and quantitated by densitometry of gel autoradiograms. The UV-inactivation cross section data for polypeptide synthesis indicates that the alpha protein genes are removed from their promoters by about 1500 to 4200 base pairs. Thus the mRNA for these proteins appears to be derived from larger precursors.

To analyze the transcriptional units of the late viral proteins, adenosine arabinoside (100 μM) was used to effectively block replication of the infecting, irradiated genome. Although this concentration of the drug had little effect on the overall rate of protein synthesis, the synthesis of certain late (γ) polypeptides was strongly inhibited. In addition, the normal turn off of certain α and β polypeptides was blocked. X.p. cells were infected with UV-irradiated HSV; AraA was present from 1 to 8.75 h p.i. Cells were labeled with ^{35}S-met at 8h and the polypeptides quantitated as above. The results indicate that the genes for many of the late proteins are adjacent to their promoters. Evidence for promoter switch from early to late transcription will be presented.

676 IMMUNOHISTOCHEMICAL DETECTION OF HEPATITIS TYPE B ANTIGEN IN INFECTED TISSUES, Gordon R. Dreesman, Guy A. Cabral and Ferenc Gyorkey, Baylor College of Medicine and Veterans Administration Hospital, Houston, TX 77030

Liver samples obtained from humans infected with hepatitis B virus (HBV) were examined for HB surface antigen (HBsAg) and core antigen (HBcAg) particles by light, immunofluorescent and electron microscopy. HBsAg and HBcAg particles were observed by electron microscopy in the cytoplasm and nuclei of infected hepatocytes, respectively. Biopsy material and liver specimens embedded in paraffin were examined in a comparative study using specific anti-HBs or anti-HBc sera in direct and indirect immunofluorescence and immunoperoxidase techniques. Essentially similar results were observed when biopsy material was studied by the above procedures. However, immunoperoxidase techniques, employing horseradish peroxidase conjugated to antibody or to Fab fragments, proved to be more sensitive in detecting virus-specific antigens in formalin-preserved, paraffin-embedded liver sections. In further studies HBsAg was localized in blood vessel walls. In contrast to the liver studies, HBs and HBc particles and HBV antigens were observed by electron microscopy and by immunoperoxidase staining in the mesangium of a kidney obtained from an individual in renal failure. Numerous Dane particles and high levels of "e" antigen and DNA polymerase were found in the sera of this patient. Additional studies have centered on examination of infected tissues for the presence of viral proteins using antisera specific for individual polypeptides derived from purified HBsAg particles.

677 Characterization of SV40 U-antigen in Ad2$^+$SV40 hybrid virus infected and in SV40 transformed cells, Wolfgang Deppert, Max Planck Institut für Biophysikalische Chemie, 3400 Göttingen, Federal Republic of Germany.

Human adenoviruses abortively infect monkey epithelial cells, with the restriction of viral replication occuring at a step prior to the production of "late" viral capsid proteins. This interaction of human adenovirus with nonpermissive monkey cells may result in a persistent adenovirus infection. The abortive as well as the persistent adenovirus infection can be changed to a fully lytic one by superinfection of the monkey cells with simian virus 40 (SV40). It has been shown that this "helper" function of SV40 is controlled by the early region of the SV40 genome, and the protein responsible for the helper function in Ad2$^+$ND1 infection has been identified.

This report describes the relationship of this protein to SV40 U-antigen in Ad2$^+$SV40 hybrid virus infected cells and to SV40 U-antigen in SV40 transformed cells as analyzed by biochemical cell fraction procedures and immunofluorescence studies. It was found that this protein specifically associates with the nuclear matrix and carries U-antigen determinants. In SV40 transformed cells, U-antigen also is associated with the nuclear matrix fraction, indicating that SV40 U-antigen in hybrid virus infected HeLa cells and in SV40 transformed cells is immunologically and functionally related.

The association of SV40 U-antigen with the nuclear matrix may be important for understanding the SV40 helper function, since the nuclear matrix seems to play an important role in the synthesis, processing, and transport of RNA.

678 THE ROLE OF TWO EARLY GENES OF POLYOMA AND SV-40 IN TRANSFORMATION, Michele M. Fluck and Thomas L. Benjamin, Harvard Medical School, Boston, Mass. 02115

We have compared the properties of ts-a/A mutants of Polyoma and SV-40 as well as those of hr-t mutants of Polyoma and viable deletion mutants of SV-40 with cordinates .54-.59,analoguous to those of the hr-t mutants. Ts-a/A mutants of both viruses induce an efficient abortive transformation, while hr-t and dl viable deletion mutants are defective in this process. In both viral systems, the two classes of mutants (hr-t/dl and ts-a/A) can complement each other to induce stable cell transformation.
Stable clones of ts-a/A transformed cells have been derived using the selection of anchorage dependence as well as that of release from density regulation of growth. The properties of such clones have been compared with respect to various parameters. The results of these experiments will be reported.

679 EXPRESSION OF MURINE CYTOMEGALOVIRUS. J.K. Chantler, University of British Columbia, Vancouver, B.C. V6T 1W5.

Three systems have been examined: (1) a productive infection of mouse embryo cells in vitro; (2) a non-productive infection of G-1 phase 3T3 cells, and (3) a latent infection of embryos after transplacental transmission of the virus in vivo.

1. During productive infection 3 proteins can be detected at 0-4h.p.i. and these appear to be immediate-early proteins as they are also synthesized after removal of a cycloheximide block. An early nuclear membrane alteration has also been detected at this time by immunofluorescence. No further viral proteins have been detected until after the initiation of viral DNA synthesis at 8-10h.p.i. at which time structural proteins start to accumulate in the cell.

2. When 3T3 cells arrested in the G-1 phase of the cell-cycle are infected incomplete viral replication occurs unless the cells are stimulated to traverse the cell-cycle (Muller and Hudson, J. Virol. 22: 267-272, 1977). In this system only the immediate-early proteins are made. No viral DNA synthesis occurs and neither structural proteins nor progeny virus can be detected.

3. Mice which have been infected intraperitoneally with MCMV have been found to produce abnormal embryos several months after inoculation. No infectious virus has been found in the embryos or in cell-cultures derived from them but the presence of latent MCMV can be shown by immunofluorescence and 'in-situ' hybridisation.

680 EXPRESSION OF CMV-SPECIFIC PROTEINS IN PRODUCTIVE AND ABORTIVE INFECTIONS, S. St. Jeor, L. Hernandez, C. C. Chua and M. Tocci, Dept. of Micro., The Milton S. Hershey Medical Center, The Pennsylvania State Univ. College of Medicine, Hershey, PA 17033. Human cytomegalovirus (CMV) differs from other members of the herpesvirus group in that it has an extremely narrow host range but abortively infects a wide variety of cell types. In addition, the virus has an extended latent period with virus DNA synthesis occurring 24 hrs post-infection. The studies to be reported were initiated to determine the sequence of CMV structural and nonstructural proteins in abortive and productive infections. Virus specific proteins were identified using either antiserum prepared specifically against virus capsid proteins or CMV acute human serum. Cells were examined for nonstructural and capsid antigens using immunofluorescence and immunoprecipitation. Virus specific proteins purified by immunoprecipitation were further examined using polyacrylamide gel electrophoresis. The results of these studies indicated that virus DNA synthesis was a prerequisite for capsid protein synthesis. In permissive cells, no capsid proteins were detected if virus DNA replication was blocked with fluorodeoxyuridine or cytosine arabinoside. Cells abortively infected with human CMV contained virus-specific early antigens but no detectable virus capsid proteins were produced. In addition, virus DNA synthesis did not occur. It appears that control of capsid protein synthesis in cytomegalovirus infected cells is similar to that observed with both adeno and SV40 virus. Additional experiments with confluent and nonconfluent cells indicated that the physiologic state of the cells affected the sequential appearance and amount of both nonstructural and capsid proteins.

681 EXPRESSION OF THE MURINE CYTOMEGALOVIRUS GENOME. Vikram Misra and J.B. Hudson. Department of Microbiology, University of British Columbia, Vancouver, B.C. V6T 1W5.

Transcription of the murine cytomegalovirus genome was examined during productive and non-productive infections caused by the virus.

During productive infections, approximately 25 percent of the genome was represented as stable transcripts in the cell at 6 hours post infection, i.e., before the onset of viral DNA synthesis, whereas RNA transcribed from 35 to 40 percent of the DNA was present in the cells in the later stages of infection. RNA sequences corresponding to 6 h (early) transcripts would be detected in the cell throughout the infectious cycle. Both 'early' and 'late' RNA comprised two RNA classes differing about 7 to 10 fold in concentration.

Viral DNA synthesis in the host cell was required for the expression of 'late' genes since in the presence of inhibitors of protein and DNA synthesis only 'early' transcription occurred.

Control was also exerted on the transport of transcripts from the nucleus to the cytoplasm of infected cells. Although RNA extracted from the nuclei of infected cells arose from 25 (early) and 35 (late) percent of the viral genome, transcripts from only 11 (early) and 15 (late) percent of the DNA were detected in the cytoplasm.

Cells of mouse origin (3T3 cells), arrested in the G_1 phase of the cell cycle, retained the viral genome in a non-replicating state, but could be induced to enter the lytic cycle by serum activation. Transcripts from 19 percent of the genome were observed in G_1 arrested, MCMV-infected cells. Viral RNA in these cells comprised only one abundance class, which was similar to the scarce class in 'early' RNA from infected exponentially growing cells.

682 IN VITRO SPLICING OF AD 2 mRNA, Charles Lawrence, and Tony Hunter, Salk Institute, San Diego, Calif. 92112, Heiner Westphal, National Institutes of Health, Bethesda, Md. 20014.

An activity has been detected which activates internal initiation sites for protein synthesis in adenovirus 2 mRNA of high molecular weight in vitro. These new mRNA activities are associated with RNA species of lower molecular weight suggesting that they arose by functional processing of the high molecular weight RNA. Two lines of evidence suggest that Ad 2 RNA is processed in vitro by a mechanism which involves splicing of 5'- and 3'-sequences.

683 EXTENT OF TRANSCRIPTION OF THE E STRAND OF POLYOMA DNA DURING THE EARLY PHASE OF INFECTION. Nicholas H. Acheson, Dept. of Virology, ISREC, 1066 Epalinges, Switzerland.

Both strands of polyoma DNA are transcribed over most or all of their length late during lytic infection. However, hybridization of saturating amounts of early nuclear polyoma RNA with labeled E or L strands of polyoma DNA suggested that only one-half of the E strand, and none of the L strand, is transcribed during the early phase. It remained possible that the entire E strand is transcribed, but transcripts from the half of this strand which does not code for early messenger RNAs are rapidly degraded. To test this hypothesis I have hybridized pulse-labeled early RNA, which should contain detectable amounts of all transcription products, with restriction endonuclease fragments of polyoma DNA which lie either wholly within the region coding for early mRNAs, or wholly outside this region. The results show that a small but significant fraction of pulse-labeled nuclear RNA, but almost no labeled cytoplasmic RNA, hybridizes with Hpa II fragments 1 and 3, which lie outside the "early" region of polyoma DNA. The proportion of polyoma-specific RNA which hybridizes to these fragments is independent of the labeling time. These results suggest that occasional read-through past a terminator of transcription at the end of the early region may occur on the E strand. Alternatively, a low level of L strand transcription may be taking place. This alternative is being tested by hybridization of pulse-labeled RNA with separated strands of the fragments in question.

684 CYCLIC DISSOCIATION OF TEMPLATE, PRODUCT, AND QB REPLICASE DURING MDV-1 RNA SYNTHESIS, Carl Dobkin, Donald R. Mills, Fred Russell Kramer, and Sol Spiegelman, Institute of Cancer Research, Columbia University, New York, N.Y. 10032

The more MDV-1 RNA template there is relative to Qβ replicase in an in vitro reaction, the fewer replicase molecules there are on any template strand, When MDV-1 RNA is in great excess, mono-enzyme complexes are the replicative intermediate. Mono-enzyme replicative complexes, like active complexes formed at lower template-to-enzyme ratios, synthesize single-stranded MDV-1 RNA. By varying the MDV-1 RNA template used to construct them, we investigated the way mono-enzyme replicative complexes dissociate at the end of the first cycle of replication. In an excess of pure MDV-1 (-) RNA, mono-enzyme replicative complexes produced virtually pure MDV-1 (+) RNA during both the first and second cycles of replication indicating that the replicase does not cling to the product strand and then use it as the next template. When mutant MDV-1 RNA was added to a reaction in which all active replicase had just begun its first cycle of replication on wild-type MDV-1 RNA, the second cycle of replication yielded both mutant and wild-type MDV-1 RNA. This implies that at the end of each cycle of replication, the replicase dissociates from the template as well as from the product RNA and goes on to choose a new template strand. Although the replicase does not favor the just-produced strand in its subsequent choice of template, it does show a preference for the just-used template strand. This suggests that the release of the product strand from the replicative complex preceeds the dissociation of the template and the replicase.

685 STUDIES ON THE EXPRESSION OF EARLY SV40 PROTEINS (T ANTIGENS), Hawley K. Linke, Tony Hunter and Gernot Walter, Tumor Virology Laboratory, The Salk Institute, San Diego, Ca. 92112.

Prior to the initiation of SV40 DNA synthesis, the virus has been shown to induce two antigenically related proteins defined as a 100,000 molecular weight T antigen (100k T) and a 17,000 molecular weight T antigen (17k T). Recent experiments in our laboratories have analyzed the ^{35}S methionine labelled tryptic peptides of the 100k T and ordered such peptides along the early gene region of SV40. (Mann, et al, 1977, Journal of Virology 24:151-169) The data presented here is a similiar analysis of the 17k T and a closer study of the mode of expression of both proteins. Two-dimensional peptide analysis of proteins translated in vivo and in vitro demonstrate: a) that the 17k T, like the 100k T, is SV40 coded; b) the two proteins contain both extensive overlapping peptides and peptides unique to each; c) the precise regions of early DNA which express the 17k T. Size analysis and subsequent in vitro translation of SV40 RNA has identified the most likely messenger species for both proteins. Formyl methionine labelling of in vitro products established the independent initiation of both proteins.

In toto the data impose a unique model of variable expression of a single gene region. Such a model will be presented.

686 ADENOVIRUS 2 CYTOPLASMIC RNAs FROM THE LEFT 11% OF THE GENOME, D. Spector, M. McGrogan, H.J. Raskas, Wash. Univ. Med. Sch., St. Louis, MO 63110

Hybridization-selection with specific DNA fragments was employed to map cytoplasmic RNAs specified by the left 11% of the adenovirus 2 genome. Selected RNA was eluted and then fractionated by size. Analysis of early and late RNAs identified five different transcripts. The predominant early species (13S) maps within map positions 0-6; its synthesis is shut off at late times. The other four transcripts include 22S RNA, a second 13S RNA and two 9S RNAs. These four RNAs share common sequences, and the appearance of each is temporally regulated. The 22S RNA accumulates primarily at early times and can be selected by DNA fragments from 2.9-10.7. The 13S RNA is synthesized throughout infection and can be selected with all fragments except 7.8-8.7. This appears to be a "spliced" RNA. The 9S RNAs are synthesized at late times. One species can be selected with the 8.7-10.7 fragment; the other small RNA can be selected by fragments 2.9-5.9 and 5.9-7.8. The results suggest the following structural relationships between these four RNAs: The 13S RNA contains 5' and 3' sequences of the 22S RNA, and each 9S RNA sequence is common to half of the 13S RNA. The 13S species is the only viral RNA identified thus far that appears to be made in the same form both early and late. At least one of these RNAs, the 22S size class, hybridizes to sequences within 0-2.9 on the genome, well to the left of the body of the message. This result suggests that 5' "leader" sequences may be present in this molecule.

687 DETECTION OF VIRAL NUCLEIC ACID SEQUENCES USING IN SITU CYTOLOGICAL HYBRIDIZATION, James K. McDougall and Denise A. Galloway, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724

In situ hybridization methods have been used to detect and localize satellite DNA species, globin mRNA sequences in differentiating cells, viral DNA in productively infected cells and tumor cells and viral mRNA in virus-transformed cells. We are investigating the method as a means of detecting viral RNA sequences in human tumors and in non-neoplastic tissues. From many studies on DNA virus-induced tumors it is clear that partial sets of viral genomes are sufficient and necessary to induce the neoplastic transformation process. In the cases of SV40 and adenovirus the fragment of viral DNA with transforming potential is approximately equal to 5.3 kilobases. This is equivalent to about 1.6% of a herpesvirus genome and, with present methods, would be undetectable as DNA. Detection of RNA is a more attractive possibility as the target is amplified and can be localized by in situ methods to particular cell types.

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688 DETECTION OF CITRUS EXOCORTIS VIROID COMPLEMENTARY RNA IN INFECTED HOST TISSUE, L. K. Grill and J. S. Semancik, Dept. Pl. Path., Univ. of Calif., Riverside, CA 92521

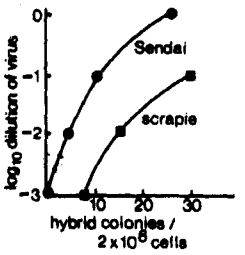
Molecular hybridization with ^{125}I-labelled citrus exocortis viroid RNA has been utilized to survey nucleic acid preparations from *Gynura aurantiaca* for viroid complementary molecules. A differential hybridization effect was detected between nucleic acid extracts from healthy and infected tissue. Significant RNase resistant ^{125}I-labelled citrus exocortis viroid resulted in hybridization studies with the infected tissue extracts. Characterization studies indicated that RNA from the infected tissue was involved in the formation of a duplex molecule with the citrus exocortis viroid RNA and had properties of an RNA-RNA hybrid. Subcellular fractionation of infected tissue indicates that complementary molecules are present in both nuclear and soluble RNA fractions. The complementary RNA may represent an intermediate molecule involved in the replication of the viroid and/or a pathogenic expression of the viroid infection.

689 MEMBRANE-FREE SCRAPIE ACTIVITY, T. G. Malone, R. F. Marsh, R. P. Hanson, and J. S. Semancik, Dept. Plant Pathology, Univ. of Calif., Riverside, CA 92521.

The association of scrapie infectivity with membrane fractions of infected cells has been well established. Studies of the distribution of the agent have concentrated on membrane-rich subcellular fractions, however no exclusive associations between scrapie infectivity and a specific subcellular membrane have been found. In light of the seemingly ubiquitous distribution of scrapie activity, determinations of scrapie infectivity in subcellular fractions from infected hamster brains through the course of infection were made. Bioassay revealed the presence of increasing scrapie infectivity in the 100,000 x g supernatant fractions as the asymptomatic course of infection progressed. Electron microscopic examination of infected material from supernatant fractions revealed no virus-like particles or discernable membrane structures. Vigorous homogenization of membrane-rich fractions was found to release infectivity into the supernatant fraction, suggesting that the increase of scrapie activity in the 100,000 x g supernatant fractions observed in vivo is not necessarily due to maturation of less dense particles, but rather to dissociation of membranes during the process. Determinations of the stability of the scrapie agent following treatments with various enzymes and detergents indicate that infectivity in both membrane-rich and "membrane-free" fractions remains equally resistant to degradation. The membrane hypothesis assigns scrapie activity to an assemblage of macromolecules bound in a membrane complex, however, on the basis of these date, the importance of membrane structures in the transmission of the scrapie agent must be questioned and await a careful resolution of which components of the scrapie agent complex are critical to the infectious process.

690 CELL FUSION INDUCED BY THE SCRAPIE AGENT, Marie-Claude Moreau, Chev Kidson, David M. Asher, Paul W. Brown, Hayden G. Coon, D. Carleton Gajdusek, and Clarence J. Gibbs, Jr. National Institutes of Health, Bethesda, Maryland 20014

Sendai and many other enveloped viruses induce fusion of cells in vitro. A preliminary study suggests that the scrapie agent may have the same property. A standard method was used to demonstrate and quantify cellular fusion. Two cell lines, one defective in the enzyme hypoxanthine guanine phosphoribosyl transferase and the other in thymidine kinase, produce hybrid cells able to grow under conditions that select against both parents. Mixtures of the two cell lines were incubated in the presence of Sendai virus, scrapie virus, normal mouse brain or plain medium. Cells were plated and grown for 2 to 3 weeks in slective medium containing aminopterin; colonies were then counted. Inactivated Sendai virus and several preparations of scrapie mouse brain produced hybrid colonies, while controls did not. Rates of fusion were proportional to dilutions of both viruses (fig). The two curves (averages of 3 experiments) are similar, although fusion rates for scrapie were consistently lower; this might be due to differences in fusing abilities of the 2 agents, or simply to different concentrations. If found to correlate well with infectivity, fusion could provide a rapid assay for scrapie. Further studies of fusion induction by the viruses of scrapie, kuru and Creutzfeldt-Jakob disease are in progress.



691 STUDIES OF THE SPONGIFORM ENCEPHALOPATHY AGENTS IN TISSUE CULTURE. Richard T. Yanagihara, David M. Asher, D. Carleton Gajdusek, and Clarence J. Gibbs, Jr., National Institutes of Health, Bethesda, Maryland 20014

The agents of scrapie, kuru and Creutzfeldt-Jakob disease are known to persist and replicate in cultured cells derived from infectious tissues. However, many lines of cells lose infectivity after several subcultures. Of ten cell lines derived from scrapie-virus infected mouse brains, one had spontaneous rapid growth; a murine oncorna virus was detected in that line, which no longer transmitted scrapie to mice. Eight cell lines superinfected with SV40 virus readily acquired rapid growth, altered cellular morphology and SV40-T antigen; one cell line still contained the scrapie agent when assayed at the 12th subculture, while the other seven did not. The infected line contained about 1 LD_{50} of scrapie per 10^4 cells. Lines of cells derived from brains of chimpanzees with kuru and Creutzfeldt-Jakob disease were also serially propagated and superinfected with SV40; two such lines assayed at 50th subculture levels, as well as SV40-transformed cells from the two lines at similar levels, were not found to contain the kuru and Creutzfeldt-Jakob disease agents. We conclude that in the spongiform encephalopathies only a minority of culturable cells in brain may be infected, and that such cells can disappear from lines of cultured cells. The persistence of the infectious agents in occasional cultures at higher passage levels suggests that cells other than neurons may support infection.

692 Experimental Creutzfeldt-Jakob disease, Elias E. Manuelidis, M.D., Edward J. Gorgacz, V.M.D., Ph.D., Laura Manuelidis, M.D., Yale University, New Haven, Conn., 06510.

Creutzfeldt-Jakob disease of man was serially transmitted to guinea pigs, mice and hamsters. Characteristic slow incubation periods were observed. Clinical symptomology in some mice and hamsters resembled that seen in experimental scrapie. Light and electron microscopic studies revealed typical changes of a spongiform virus encephalopathy; no conventional virus particles were observed.

Experiments utilizing these convenient animal models demonstrated infectivity of extraneural tissues, viremia, and transmission via various routes of inoculation. Preliminary data on subcellular fractionation of infectivity will also be presented.

693 PROGRESS IN THE PURIFICATION OF THE SCRAPIE AGENT, Robert G. Rohwer, Franklin Hamilton, D. Carleton Gajdusek, National Institutes of Health, Bethesda, Md. 20014

Scrapie infected and uninfected mouse brains have been processed through a sequence of differential velocity sedimentations and sedimentations to equilibrium under conditions previously characterized for the distribution of the infectious agent. These gradients have been monitored for their mass distribution of nucleic acids, proteins and lipids and those fractions expected to contain the scrapie agent have been analyzed for their comparative complexity by means of highly resolving gel electrophoresis systems for both proteins and nucleic acids. We are attempting to discriminate those differences which are the artifactual consequences of the massive pathology of the scrapie brain vs. those directly related to the agent itself.

694 CHARACTERIZATION OF NORMAL GLIAL CELLS IN CONTINUOUS CULTURE, D. Van Alstyne and V. K. Singh, Children's Hospital, Immunology Unit, Vancouver, B.C. Canada.

A continuous tissue culture cell line of glial cells from adult rat brain has been established. The procedure involves the induction of a highly selective neuronal degredation localized intracerebrally by neurochemical means (Singh and McGeer, Brain Res.1977, in press), dissection of the lesioned area of the brain, mincing of the tissue in the presence of growth medium and distribution of the dissociated tissue in glass petri dishes. Neuronal degredation was achieved by injection of picamole amounts of kainic acid which is thought to induce gliosis following formation of the lesion. Tissue culture medium used is MEM containing 10% fetal calf serum, 0.2% glucose, 5 units/ml penicillin and 5 µg/ml streptomycin. These cells form complete monolayers and exhibit contact inhibition. They are routinely detached using 0.2% trypsin and subcultured into petri dishes at a cell density of 10^3 cells per cm^2. Although all cells attach to plastic tissue culture flasks they do not subsequently divide, necessitating the use of glass petri dishes. Several morphologically distinct cell types are present, including astrocytes and oligodendrocytes, thus it is unlikely that malignant cell growth has resulted from a kainic acid induced activation of a latent, transforming virus. The *in vivo* signal to end gliosis is apparently not operative *in vitro* since cells continue to divide on subculture despite previous contact inhibition of growth. Further characterization of this cell line with regards to glial-specific biochemical markers and its ability to replicate rubella virus is in progress.

695 DISCOVERY OF TREATMENTS THAT ENHANCE OR SUPPRESS SCRAPIE INFECTION, R.H. Kimberlin Institute for Research on Animal Diseases, Compton, Newbury, England.

A single i.p. injection of 1mg of MER (methanol extraction residue of BCG; kindly provided by Professor D.W. Weiss) given just before an i.p. injection with scrapie reduced incubation time in mice by up to 19 days; this effect is equivalent to increasing the dose of scrapie agent by 80-fold. A similar injection of 3.5mg of MER in hamsters reduced the i.p. incubation time by 28 days. However the enhancing effects of MER on scrapie infection in mice only occurred when MER preceded scrapie agent by ½ to 4 hours. This suggests that MER may have a direct effect on peritoneal cells, either by reducing inactivation of injected scrapie or by stimulating the infection of cells. The table shows the effect of 9 daily injections of 1mg of HPA 23 (ammonium 5-tungsto-2-antimoniate; kindly provided by Dr. J.C. Chermann) on the incidence of scrapie in mice, infected i.p. after the second injection. HPA 23 is a potent viral inhibitor which appears to reduce the efficiency of scrapie infection by at least 90% and to extend the incubation times to the dose-response end point of Chandler scrapie in Compton white mice. The discovery of two treatments with opposite effects on scrapie could be of great value in studies on the cellular and molecular events of scrapie infection.

Dil^n of scrapie brain	Control		HPA 23		Delay in I.P.
	% cases	I.P.	% cases	I.P.	
10^{-2}	100	172±2	100	201±2	29
10^{-3}	100	180±2	88	222±6	42
10^{-4}	100	194±2	22	219	25

I.P. = mean incubation period in days ± SEM

696 PASSIVE SERUM IMMUNOTHERAPY DIRECTED AGAINST FLV STRUCTURAL ANTIGENS, Fred Sanfilippo, Richard S. Metzgar and Jeffrey J. Collins, Duke Univ. Med. Ctr., Durham, N.C. 27710

DBA/2 mice injected with a leukemogenic dose of Friend leukemia virus (FLV) can be protected against splenomegaly, viremia and non-specific immunosuppression by systemic treatment with chimpanzee anti-FLV or anti-FLV gp71 antisera administered after virus inoculation. Protection in this system directly correlates with the production of anti-gp71 antibody by treated mice, as measured by RIA and cytotoxicity to FLV-infected mouse cells. The possibility that the transferred antibody binds to viral determinants and enhances their immunogenicity resulting in host protection has been ruled out since 1) no detectable host anti-chimp γ-globulin antibody has ever been detected in protected mice, and 2) mice made specifically tolerant to chimp γ-globulin can still be protected by immune chimp sera. Titration and kinetic studies indicate that treatment of mice with chimp anti-FLV antiserum at various times before inoculation of FLV can provide protection even when the amount of residual circulating transferred chimp antibody is below the protective endpoint. Furthermore, spleen cells from mice treated with chimp anti-FLV serum with or without subsequent FLV inoculation can transfer protection to lethally irradiated recipients challenged with FLV. These results suggest that some type of serum-immune cell interaction occurs in mice receiving the xenogeneic immune sera. However, the observation that nude (athymic) mice can be protected by transfer of immune serum (with the subsequent production of anti-gp71 antibodies) suggests that this process may not require a T-cell-dependent response. In vitro assays of the antibody forming cell response to goat RBC suggest that elimination of a suppressor cell population by treatment with heterologous anti-FLV antisera may be involved in the protection against FLV-induced disease.

697 PASSIVE SERUM IMMUNOTHERAPY DIRECTED AGAINST VIRUS-ASSOCIATED NON-STRUCTURAL ANTIGENS, Jeffrey J. Collins, Gary Roloson, Darrow E. Haagensen, Jr., Peter J. Fischinger, Ronald C. Montelaro, and Dani P. Bolognesi, Duke Univ. Med. Center, Durham, N.C. 27710 and National Cancer Institute, National Institutes of Health, Bethesda, Md. 20014

The ascites form of a mouse mammary adenocarcinoma (AD755a) originally arising in a C57Bl mouse has been used to establish a passive serum immunotherapy model system in which the transfer of as little as 5 µl of syngeneic anti-AD755a antiserum IP can protect against challenge with at least 10^3 LD_{100} of the tumor cells. The ability to transfer protection with immune serum depends on the strain of the recipient mouse and the genetic control of this strain specificity is currently under investigation. While the precise mechanism responsible for this potent serum transfer protection has not yet been determined, it is clear that an early interaction occurs in the recipient mouse after administration of immune serum between tumor cells and normal monocytes (possibly macrophages). With respect to the specificity of the antigens involved, we have found that the AD755a cells are actively releasing a C-type oncornavirus (denoted ADV) which has the host-range and serological characteristics of a Friend-Rauscher-type virus. Analysis of the serological reactivity of the anti-AD755a antiserum and of the antigenic expression on the AD755a cells indicates the presence of at least two virus-associated antigens: 1) FLV-type gp71 and 2) ADV-induced non-structural antigen. Absorption studies indicate that only antibodies directed against the latter are capable of mediating the serum transfer protection, although mice can be made resistant to direct AD755a cell challenge by immunization with FLV. The detailed molecular and biochemical characteristics of the ADV-associated surface antigens on the AD755a cells are under active study.

698 IN VIVO AND IN VITRO MODELS OF DEMYELINATING DISEASES: PERSISTENT AND TEMPERATURE SENSITIVE REPLICATION OF MOUSE HEPATITIS VIRUS AND MEASLES VIRUS IN CELLS OF NEURAL ORIGIN. Wayne Flintoff, Alexandra Lucas, Marion Coulter, Robert Anderson, Vincent Morris, Dean Percy, and Samuel Dales, Dept. Microbiol. and Immunol., U.W.O., London, Ont. Can., N6A5C1

Intraperitoneal infection of mice with the neurotropic JHM strain of murine hepatitis virus causes demyelinating lesions resulting from an infection of the oligodendroglia. Such lesions were not observed in mice inoculated with the MHV_3 strain. The replication of these viruses has been investigated in several cell lines of neural origin. A rat Schwannoma cell (RN2) functions as a descriminating host for the JHM virus. In these cells, this virus readily establishes a persistent infection characterized by a cyclical rise and fall in titer with an accompanying cytopathology. Furthermore, the RN2 cells confer a thermal lability which the virus does not demonstrate in the fully permissive host cell, L-2. Infection of the RN2 cells with MHV_3 is aborted immediately. In C6 rat astrocytomas, both viruses are completely aborted, whereas in murine gliomas, a temperature sensitive persistence is readily established with both viruses. In murine neuroblastomas, both viruses establish a short term temperature sensitive persistence. Measles virus (Halle and Edmonston strains) readily establishes persistent infections in the rat RN2 and C6 cell lines. These cells confer temperature sensitivity on the viruses which they do not possess when replicating in Vero cells. After several days under nonpermissive conditions, viruses can be recovered from both the mouse hepatitis and measles infected cultures by shift down to the permissive temperature. Experiments are in progress using specific cDNA probes to determine the state of the viral genomes at the nonpermissive temperature. (Supported by the MRC, MS Society and USPHS).

699 PERSISTENT INFECTION OF AN APPARENTLY HEALTHY BULL WITH BOVINE VIRAL DIARRHEA VIRUS, Manuel F. Coria and Arlan W. McClurkin, National Animal Disease Center, Ames, IA 50010
The natural spread of Bovine Viral Diarrhea Virus (BVDV) has been hypothesized to be due to inapparently infected animals. During routine surveillance of calves born within the enteric diseases of cattle project, a non-cytopathic (N-CPE) strain of BVDV was isolated from buffy coat cells of a newborn bull calf. At various intervals during 3½ years, N-CPE BVDV was isolated from buffy coat, lacrimal and synovial fluids, semen, and nasal swabs. At the same intervals antibodies to BVDV, IBR, Bovine Parvovirus, and Bovine Adenovirus Type 7 were not detected. Antibodies to PI-3 and Chlamydia spp that were detected in 2-week post-colostrum serum samples, subsequently declined over 6 and 11 months of testing. Lymphocyte stimulation studies indicated that the bull was immunosuppressed. At 2 years of age the bull was given a triple dose of killed BVDV vaccine, and responded with insignificant levels of antibodies at 4 and 5 months post-vaccination. Isolated virus was given to 2 susceptible calves that developed pyrexia and intermittent diarrhea and high levels of BVDV antibody. Breeding studies with the bull produced healthy non-infected calves from immune and susceptible dams. The bull was killed at 3½ years of age, when it developed laminitis and became inappetent and lethargic. Histological examination of the bull's kidneys revealed that they were apparently normal, but some of the glomular membranes were positive by immunofluorescence, as were various other tissues that yielded N-CPE BVDV. Recently 3 cows persistently infected with N-CPE BVDV were obtained from a herd vaccinated 3 times with a killed BVDV vaccine. Two cows have given birth to BVDV-infected calves that died 1 to 3 days later.

700 IN VITRO AND IN VIVO STUDIES ON A VIRAL-LIKE AGENT OBTAINED FROM CERTAIN HUMAN CELL CO-CULTIVATION PROCEDURES, C. W. Godzeski and R. J. Boyd, The Lilly Research Labs, Inc., Indianapolis, IN, 46206.
Co-cultivated tissue culture systems are reportedly more sensitive to the presence of latent viruses than other indicator techniques. The propagation of fibroblasts from human synovectomy samples in direct culture procedures gave no indication of viral presence. When certain synovial samples were co-cultivated with human embryonic cells (WI38, HES, HEB), a slowly developing cytopathogenicity was observed. Microfoci, which took 30-70 days to develop, appeared in the culture. Electron microscopic examination of the affected cultures gave sporadic evidence of cell damage and virus-like particles (VLP's) both in thin sections and in negative stained preparations. The VLP's in negative stained samples were 92-97 nm in size while thin sections varied in the 110-160 nm range. The VLP's and the noted C.P.E. were confined to co-cultivated cells obtained from patients with rheumatoid arthritis and no such effects were noted with osteoarthritic or "normal" trauma cell samples similarly treated. Cell cultures from these systems have been passaged 5 times in suckling mice with increasing virulence, a general viremia, and 100% mortality within 7-15 days post injection. No etiological relationship has been established or implied by these studies.

701 IMMUNITY AND IMMUNOGENETICS IN GUAMANIANS WITH AMYOTROPHIC LATERAL SCLEROSIS AND PARKINSONISM-DEMENTIA, Paul M. Hoffman, Deanna S. Robbins, Minerva T. Nolte, Clarence J. Gibbs, Jr., and D. Carleton Gajdusek, NINCDS, NIH, Bethesda, Md. 20014
Immune responses and HL-A phenotypes were studied in amyotrophic lateral sclerosis (ALS) and Parkinsonism-dementia (PD), two disease of suspected viral etiology. An increased incidence of HL-A BW16 at the second locus was present in PD (Lancet 2,717,1977). Diminished skin test responses, lymphopenia, low percent and total T cells, and diminished mitogen responses were seen in ALS and PD but not in other neurologic diseases on Guam. Significantly elevated serum IgA and IgG levels were present in ALS and PD as compared to age and sex matched normals. Among ALS and PD cases, two subgroups were apparent. One group was anergic or markedly hyporesponsive to skin tests, had low percent and total T cells, lymphopenia, and an increased incidence of BW-35 (50%). A second group had the most normal skin test reactivity, significantly fewer instances of lymphopenia and low percents and total numbers of T cells, and had a low incidence of BW-35 (6%). IgA and IgG levels were not different between groups. Multiple abnormalities in cellular immunity were more common early and intermediate in the course of ALS and PD than in long term survivors in the total group studied. These two subgroups will be followed to determine if any of these immune and immunogenetic factors influence survival in ALS and PD.

702 LYMPHOCYTIC CHORIOMENINGITIS VIRUS: PERSISTANCE AND CNS PATHOLOGY; ROBERT N. HOGAN AND ANDREW A. MONJAN, JOHNS HOPKINS UNIVERSITY, BALTIMORE, MD 21205

In the rat, neonatal infection with lymphocytic choriomeningitis virus (LCMV) can result in an acute destructive lesion of the retina or cerebellum. Aside from these early necrotic pathologies, which are immune-mediated, there is a marked retardation of general somatic and brain growth, as well as a slowly evolving lesion within the dentate gyrus of the hippocampus. The etiology of this lesion is still under study, but the following facts have been determined. Attenuation of the granule cells of the area dentata only occur following intracerebral inoculation with LCMV during the first 5 postnatal days. By 6 to 7 months, the specific loss of these cells is marked, resulting in a layer of 1-2 cells as compared to a normal thickness of 16-20 cells. The earliest manifestations of the pathology may be seen around one month after infection and consist of a mononuclear infiltrate associated with focal areas of cell loss. Golgi-silver staining methods have demonstrated that dendritic condensation of granule cells occur prior to cell loss as well as in the residual neurons. In addition, there are alterations in the patterning of mossy fiber axon terminations in the pyramidal cells within adjacent fields. Immunosuppression (ALS and/or neonatal thymectomy) augments rather than abrogates the development of the pathology: more cells are infected, more cells are lost, and virus persists for up to 7 months in the remaining granule cells. Thus, in the immunologically compromised host, LCMV may exert, within some brain areas, a direct or indirect virus-lytic effect upon neurons as well as to induce alterations in neuronal circuitry. (Supported in part by PHS grants NS 09779 & HD 08490)

703 CHRONIC ADENOVIRAL INFECTION OF THE URINARY TRACT IN CHIMPANZEES, David M. Asher, Herbert L. Amyx, Ludmila V. Shavrina Asher, John J. Hooks, Keerti V. Shah, Clarence J. Gibbs, Jr., and D. Carleton Gajdusek, National Institutes of Health, Bethesda, Md. 20014, and the Johns Hopkins University School of Hygiene and Public Health, Baltimore, Md. 21205

Chimpanzees in a research colony had spontaneous intermittent excretion of an adenovirus, of a new type called Pan 11, in the urine for periods longer than a year. The Pan 11 adenovirus elicited undifferentiated tumors in hamsters inoculated as newborns; cultured cells derived from a primary tumor produced rhabdomyosarcomas in hamsters. T antigens of the virus were found to be related but not identical to those of simian adenovirus SA7 by indirect immunofluorescence. Pan 11 virus has been isolated only from the urine of chimpanzees and not from secretions or tissues outside the urinary tract; however the exact anatomic sites of infection remain unknown. Specific neutralizing antibodies to Pan 11 virus were found in the urine of infected chimpanzees; antibodies were primarily IgG with some IgA also present. Indirect evidence indicated that antibodies did not enter the urine from the serum, but were locally produced. Most of the chimpanzees had no evidence of renal dysfunction, however chronic interstitial nephritis was found in one animal and acute bacterial nephritis in another. This persistent adenoviral infection of chimpanzees resembles those of mice and dogs, in which chronic adenoviral nephritis predisposes to experimentally-induced acute bacterial nephritis. Animal caretakers accidentally infected with Pan 11 adenovirus had no evidence of persistent infection or urinary abnormalities.

704 PRELIMINARY EVIDENCE AGAINST THE THEORY OF ACTIVATION OF LATENT ENDOGENOUS ONCORNOVIRUS BY ALLOGENIC REACTION. Richard C. Hard, Jr. and Sue Shipley Cross, Medical College of Virginia, Richmond, Virginia 23298

Host Versus Graft (HVG) syndrome is the fatal complex of lesions which may follow the perinatal inoculations of F_1 hybrid spleen cells expressing infectious ecotropic murine leukemia virus (MuLV). $(T_6 \times RFM)F_1$ hybrid thymus cells, which are histoincompatible but do not express MuLV, do not cause the disease in RFM hosts. Tests for the presence of MuLV in the lymphoid tissues of RFM hosts inoculated with $(T_6 \times RFM)F_1$ hybrid spleen cells expressing MuLV show low levels of oncornovirus expression which could be of either donor or host origin. However, no virus is detected following inoculation of thymus cells. It would seem, then, that reaction to histocompatibility antigens is insufficient to activate endogenous virus in the RFM host.

In other work, it has been shown that neither virus alone, nor virus inoculated with thymus cells causes HVG disease. The implication of these results are that both the histocompatibility and viral antigens must be on the surface of the same cell to induce the aberrant rejection phenomenon we have observed.

705 LATENCY AND IMMUNE RESPONSES IN MICE AFTER VAGINAL OR SYSTEMIC INFECTION WITH HERPES SIMPLEX VIRUS TYPE 2. Mary C. Breinig, Paul F. Cline and Page S. Morahan, Medical College of Virginia, Richmond, VA 23298
The temporal relationships among humoral and cell mediated immune responses (CMI) following vaginal or systemic intravenous infection with a clinical isolate of herpes simplex virus type 2 (HSV-2) have been established in individual BALB/c mice relative to the pathogenesis of the acute infection. The association of immune responses with establishment and maintenance of latent virus infection is currently being determined. Systemic infection induced prolonged delayed type hypersensitivity and splenic proliferative CMI responses as well as a serum neutralizing antibody response. In contrast, vaginal infection was associated with transient CMI responses which appeared during the acute infection, and with the rare appearance of a serum neutralizing antibody response. The dorsal root ganglia of surviving mice were removed between 24 and 45 days following primary infection, and were cocultivated with susceptible indicator cells for detection of latent HSV-2. Our present results indicate that the incidence of latency in survivors of the vaginal HSV-2 infection was very low, while there was a 22% incidence of latency in survivors of the systemic infection. Latent infection was almost exclusively associated with the concomitant presence of a systemic neutralizing antibody response in the mice. Observations are being extended to include the effects of the mouse strain and virus isolate on latency and immunity. (Supported in part by CA 16193, AI 70863 and AI 05431).

706 ISOLATION OF C-TYPE VIRAL ANTIGEN FROM HUMAN TISSUE, John T. Reynolds, Mark Sawyer, Nathan E. Nachlas, Jr., Roberta J. Black and Sandra Panem, Depts Microbiology and Pathology, University of Chicago, Chicago, Ill 60637
C-type viral antigen was detected in human tissue using immunofluorescence and an antiserum to HEL-12 virus. Antigen was first demonstrated in over 60 patients with systemic lupus erythematosus (SLE) complexed with antiviral antibody but was absent in a comparable number of patients with non-SLE immune complex diseases. We now identify a similar antigen in 30 of 30 term placentas from normal women and those with SLE. The reactive material has been isolated from SLE immune complexes and from placenta. The isolation procedures utilize high salt and low pH elution followed by sequential chromatography on Sephacryl 200 and Con A- Sepharose. A fraction enriched in a 70,000 mol wt glycoprotein has been isolated from placenta and SLE immune complexes. These fractions are compared with purified HEL-12 virus, SiSV, BaEV, R-MuLV and SR-RSV for (1)their ability to block reaction of anti-HEL-12 virus serum with SLE kidney, placenta or virus infected cells; and (2)mobility under varying conditions of acrylamide gel electrophoresis. The data suggest that a protein similar to a primate C-type viral envelope protein is routinely expressed in pregnancy and during the pathogenesis of SLE.

707 ENHANCEMENT OF E-ROSETTES WITH INTERFERON, A. Khan, J. Boettcher, O. Garrison, A. Antonetti, S. Graham, N.O. Hill, Wadley Institutes of Molecular Medicine, Dallas, Texas, 75235
It has been shown that interferon may be immunosuppressive. We investigated the effect of interferon on E-rosettes *in vitro*. The interferon was added to the E-rosette test at different concentrations as shown in the table. Significant increases in the E-rosettes as well as the E-rosette scores were observed with all the concentrations used. The E-rosette scoring technique was utilized which gives a semiquantative measurement of the E-rosette receptors on the lymphocytes by taking into consideration the number of erythrocytes in each rosette (Khan, A., Thometz, D., and Hill, J.M., Wadley Medical Bulletin 5:297, 1975).

INCREASE IN E-ROSETTES
(*in vitro*) WITH
INTERFERON

UNITS/ML	T SCORE	T CELLS %
Control	102	54
10	133	76
100	133	75
1000	137	77

708 PROPERTIES OF THE NAEGLERIA AMEBAE CYTOPATHOGEN, T.H. Dunnebacke and F.L. Schuster, Viral and Rickettsial Disease Laboratory, State of California Department of Health, Berkeley, CA 94704 and Department of Biology, Brooklyn College, Brooklyn,NY 11210

A cytopathogen has been found in the amebae of the genus Naegleria, the causative agent of the human disease primary amebic meningoencephalitis. Designated NACM for Naegleria ameba cytopathogenic material, it presents a quandary of questions, many of which are still unanswered. NACM is passageable in tissue culture cells, indicating that it is not degraded by nuclease as the viroids, it is resistant to inactivation by UV in the same range as the scrapie agent, yet differs from scrapie in heat sensitivity, and it has properties of a protein. NACM causes a cytopathic response in a variety of mammalian and avian cells. Our new studies have found that the response is highly specific; some lines such as rabbit kidney (RK13) are totally refractory, developing neither cytopathology nor producing passageable material, whereas hamster cells (0853) become cytopathic in 5-6 days and yield passageable material. Continuing purification studies utilizing isoelectric focusing show that the cytopathogen behaves as an acid protein. Inoculations into mice indicate the response is long termed, in the order of 120 days. The classification of NACM must await more information about its properties.

709 SECONDARY STRUCTURE IN POTATO SPINDLE TUBER VIROID. A. Hadidi* and J. N. Vournakis,** *Plant Virol. Lab., PPI, ARS, U.S.D.A., Beltsville, MD 20705; **Dept. Biol., Syracuse Univ., Syracuse, NY 13210.

Secondary structure of pure, uniformly ^{32}P-labelled circular and linear forms of potato spindle tuber viroid (C- or L-PSTV) was investigated by limited hydrolysis with pure, single-strand-specific S_1 nuclease. Enzyme reactions were performed at a constant enzyme/substrate ratio, while temperature and ionic strength were systematically varied. The digestion kinetics of C- or L-PSTV revealed that both forms were resistant to S_1 nuclease at high ionic strength. Resolution of the low ionic strength-high temperature digestion products on 20% polyacrylamide 7 M urea slab gels showed that both forms were converted to very similar sets of six specific fragments, ranging in size from 38 to 114 nucleotides. The sum of all fragments was 345-355 nucleotides. Approximately 10% of the original nucleotides in C- or L-PSTV were converted to mononucleotides by the S_1 digestion. The relative proportions of the S_1-resistant fragments were altered by varying the temperature of the digestion. A comparative study of the secondary structure of PSTV and 5s rRNA from tomato (host of PSTV) was also made. A secondary structure model consistent with the existence of several helical regions having different stabilities is proposed for PSTV.

710 *IN VIVO* SYNTHESIS AND PERSISTENCE OF POTATO SPINDLE TUBER VIROID. A. Hadidi* and J.M. Keith.** *Plant Virol. Lab., ARS, U.S.D.A., Beltsville, MD 20705; **Lab. of Biol. of Viruses, NIAID, NIH, Bethesda, MD 20014.

De novo synthesis of ^{32}P-pulse-labelled circular and linear forms of potato spindle tuber viroid (C- and L-PSTV) was studied in leaves of systemically infected tomato plants. In all experiments, C-PSTV synthesis preceded the formation of L-PSTV. Fingerprint patterns of RNase T_1-resistant oligonucleotides of uniformly ^{32}P-labelled C- and L-PSTV are very similar and differ from that of 5s rRNA of tomato. These results suggest that C- and L-PSTV are two forms of a single RNA species and that L-PSTV arises *in vivo* from C-PSTV. The nucleotide composition of the newly formed linear PSTV is the same as that of the circular form and contains 1-1.2 mole % modified nucleotides. Subsequently, the nucleotide composition of linear PSTV undergoes a slow and specific change which involves the formation of modified nucleotides. At the end of this alteration process, the majority of the linear PSTV molecules are resistant to RNases T_1, T_2, and A and nuclease P_1. Alkaline hydrolysis of these molecules and separation of nucleotides by paper electrophoresis at pH 3.5 revealed that more than 90% of the ^{32}P label migrated as four distinct spots ahead of Up. Pi was released from the eluate of each spot upon digestion with alkaline phosphatase. No change in the nucleotide composition of circular PSTV was observed. These results suggest that extensive modification of PSTV could provide a mechanism for persistence of viroids in the infected cells. To our knowledge, this is the first report of extensive modification of an RNA.

711 SUBACUTE SCLEROSING PANENCEPHALITIS: AN ABORTIVE INFECTION BY A MEASLES-LIKE VIRUS, James C. Ramsey, Larry Eron, Judy Sprague, Paula Bensky, Delphine Jones, Ruth Dunlap, and Paul Albrecht, Bureau of Biologics, Bethesda, MD. 20014

A measles-like virus, IP-3, isolated from the brain of a child with subacute sclerosing panencephalitis (SSPE) remains cell-associated in tissue culture and produces a chronic encephalitis in monkeys that resembles histologically and clinically SSPE in man. Comparison of IP-3 with measles virus reveals substantial differences between the virions. The IP-3 infectious particle possesses a lighter buoyant density than measles (1.21 vs. 1.24 g/cc). While it is composed of nucleocapsid and a glycoprotein similar in size to measles, IP-3 apparently lacks the measles membrane and phosphoproteins. While their nucleocapsids cross-react antigenically and are the same size, their primary structures are slightly different. The IP-3 genome is 50S and its nucleocapsid is 200S, the same as measles non-defective particles, different from measles defective particles (18S and 130S, respectively). These data are consistent with an abortive infection by IP-3, and not with infection by defective particles, as the cause of SSPE. The abortive infection may be the result of a misassembly of the virion particle due to alterations or absence of viral structural proteins, which might allow the virus to persist within the cell. The mechanism by which the expression of virus information is altered is under investigation and may be applicable to other slow virus infections.

712 DEMYELINATION INDUCED BY TS MUTANTS OF MOUSE HEPATITIS VIRUS. Martin V. Haspel, Peter W. Lampert and Michael B. A. Oldstone, Scripps Clinic and Research Foundation, La Jolla, CA 92037

We report that selection for temperature-sensitive (ts) mutants of mouse hepatitis virus type 4 resulted in an amelioration of the acute fatal encephalomyelitis with the frequent and reproducible induction of demyelination. Intracranial inoculation of 4 week old BALB/c St mice with 10,000 plaque units (PFU) of ts mutant 8 (3% fatalities) or ts mutant 11 (30% mortalities) produced demyelination in 58% and 75% of the survivors, respectively. In contrast, inoculation of 0.6 PFU (18% mortalities) or 2 PFU (50% fatalities) of the wild-type (wt) virus induced demyelination in 11% and 33% of the survivors, respectively. Selection for a ts^+ revertant resulted in the acquisition of full virulence. There was no clear relationship, unlike the wt virus, between lethality of the virus dose and the frequency of demyelination among the survivors. Ts mutant 7 (25% mortalities) produced demyelination in 22% of the survivors while demyelination was observed in 5% of mice infected with ts mutant 15 (16% fatalities). These differences among the mutants suggest the possibility of elucidating the molecular events leading to demyelination. Furthermore, the study of virus-induced human demyelinating diseases has been limited by the unavailability of a suitable animal model. This experimental system using ts mutants should provide this much needed animal model.
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