HERPESVIRUS

Fred Rapp, Organizer

April 8 — 13, 1984

Plenary Sessions:

April 9:
Pathogenesis of Herpesvirus Infections 167
Herpesvirus Latency 169

April 10:
Structure and Function of the Virus Genome 170
Herpesvirus Gene Expression 172

April 11:
Viral Immunology 174
Models for Treatment of Herpesvirus Infections 176

April 12:
Vaccines 177

April 13
Treatment of Herpesvirus Infections 179

Poster Sessions

April 9:
Pathogenesis and Latency
Poster Abstracts 1306 — 1331 181

April 10:
Molecular Genetics
Poster Abstracts 1332 — 1362 190

April 11:
Defenses Against Viruses
Poster Abstracts 1363 — 1400 199

Pathogenesis of Herpesvirus Infections

1277 NATURAL HISTORY OF GENITAL HSV INFECTION, Lawrence Corey, Department of Laboratory Medicine, University of Washington, Seattle, Washington 98195.

Major gaps in the knowledge of the natural history of genital HSV infection still exist, especially as to the chronicity and risk factors associated with recurrence of disease. Recent studies of the natural history of recurrent genital herpes shows great variability in the rate of recurrence. In a recent cohort of patients with frequently recurring genital herpes followed over an 8 month period of time, less than 30% of patients had a stable rate of recurrence over the first four as compared to the second four months of disease. In fact, 25% of patients had a 50% decrease and 26% a 50% increase in the first as compared to the subsequent four months of investigation. Viral type, host immune processes and duration of disease appear to influence the recurrence rate of disease. Recent evidence from several groups have indicated that primary genital HSV-1 infection recurs much less frequently than primary HSV-2. Among a cohort of 160 patients with primary HSV-2 infection followed over a 12 month period of time, 88% recurred, with a median time to first recurrence of approximately 125 days. In contrast, among a cohort of 30 patients with primary genital HSV-1 infection, only 55% recurred within the first year of therapy and the median time to their first recurrence was more than 300 days. In addition, the rate of recurrence over time was 0.97 recurrences per month in patients with primary genital HSV-1 compared to 0.35 recurrences in patients with primary genital HSV-2 ($p < .01$). To evaluate whether reinfections with different HSV-2 strains is a major factor of recurrent disease we have evaluated sequential HSV isolates from 40 patients who had contact with new sexual partners during the course of follow-up. Among the 153 isolates from these patients, all were identical by restriction endonuclease analysis of viral DNA using 5 enzymes per isolate (BamH-1, Kpn-1, XHO, SAL-1 or PST-1 and Hpa-1. The mean number of recurrences between isolates in this cohort was 15.8 and the mean number of months between isolates was 22.8. Thus in heterosexual white upper middle class patients the frequency of reinfection with different HSV-2 strains appears to be quite low.

Despite a large body of experimental data in animals, evidence for specific host factors that influence subsequent recurrence of disease are meager. Among immunocompetents evidence for host factors influencing subsequent recurrence of disease include, 1) the median time to the first clinical recurrence after first episode primary genital HSV infection, is 126 days compared to 46 days between recurrent genital HSV-2 infection. These data suggest that larger antigenic exposure to HSV may boost host factors to higher levels than the shorter antigenic exposures seen with recurrences of disease. In addition, recent studies using continuous antiviral chemotherapy suggest a "rebound" phenomenon immediately after drug is discontinued; again suggesting waning of host factors over time. Recent studies evaluating local immune responses and viral strain factors as they relate to viral shedding and reactivation of genital HSV infections will be discussed.

1278 THE PATHOGENESIS OF VARICELLA-ZOSTER VIRUS INFECTIONS, Ann M. Arvin, Department of Pediatrics, Stanford University School of Medicine, Stanford, CA 94305

With effective vaccines for other viruses, varicella has emerged as the childhood exanthem with the highest risk of serious complications.(1,2) Varicella infection in the normal host provides a unique opportunity to relate viral replication to the immune response during a primary herpes viral infection in which the incubation period, the phase of acute illness and the recovery phase can be defined precisely. A possible sequence of events with primary varicella is inoculation of respiratory mucosa and replication in regional lymph nodes (Day 0-3), primary viremia and replication in the liver (Day 4-6) and secondary viremia and skin lesions (Day 12-16). By solid phase radioimmunoassay, we have found that IgG and IgM antibodies are detectable in subjects tested 1-3 days after the onset of the varicella rash. Sixty percent of subjects tested within 1-5 days also had IgA antibody to VZV. When these sera were analyzed by the immune transfer method, it was apparent that IgM antibodies in the early sera reacted with a much broader range of infected cell proteins than did IgG antibodies. The severity of varicella in children with congenital and drug-induced cellular immunodeficiency indicates that cellular immunity is important in restricting VZV replication. Only 40% of normal subjects with varicella that we have tested had VZV specific T-lymphocyte transformation within 3 days. The cytotoxicity mediated by natural killer cells and T-lymphocytes that has been described recently in patients with reactivation of VZV may be important in cellular immunity during primary VZV.(3,4) Our data have shown that normal subjects with varicella have elevated serum interferon which may enhance NK activity. In addition, we have found that T-lymphocytes from subjects with varicella have a marked increase in the expression of HLA DR antigen which may be involved with T-lymphocyte mediated cytotoxicity. The skin infection with varicella suggests that viremia is important in pathogenesis. Although VZV has been isolated from the blood of immunocompromised children and we have isolated VZV from peripheral blood mononuclear cells of two normal children, viremia has been difficult to demonstrate.(5) Using fluorescein-conjugated monoclonal antibodies to VZV proteins, we have detected VZV antigen on circulating monocytes from subjects with acute varicella which suggests that the virus may be carried to skin target cells by this means. It has been assumed that immunity after primary varicella confers resistance to re-infection with VZV. However, we have found that immune healthy adults have immunologic evidence of re-infection after household exposure to VZV. This boost in immunity with exposure to exogenous virus could be important in restricting the replication of latent endogenous VZV in immune subjects.(6)

1. Preblud, S.R., Pediatrics, 68:14, 1980.
2. Feldman, S., Hughes, W. et al, Ped. 56:388,1975.
3. Ihara, T. et al, Abst.395, ICAAC, 1983.
4. Cooper et al,Abst.394, ICAAC, 1983.
5. Myers, M.J.,J Infect Dis 140:229,1979.
6. Arvin, A.M. et al, JID 148:200, 1983.

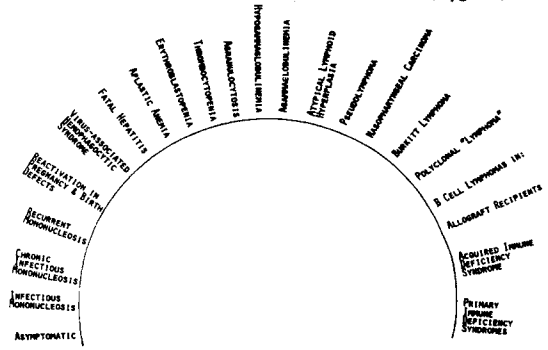
1279 THE PATHOGENESIS OF EPSTEIN-BARR VIRUS INFECTION. Ronald Glaser, Department of Medical Microbiology and Immunology and Comprehensive Cancer Center, The Ohio State University College of Medicine, Columbus, OH 43210

The Epstein-Barr virus (EBV) is a human oncogenic herpesvirus which has been associated with the self-limiting lymphoproliferative disease, infectious mononucleosis (IM). The EBV has also been implicated in the etiology of nasopharyngeal carcinoma (NPC), Burkitt's lymphoma (BL), and B-cell lymphoma associated with cellular immune depression, as for example, in Acquired Immune Deficiency Syndrome. Patients with NPC routinely have high IgG antibody titers against EBV early antigen (EA), particularly the diffuse (D) component, and virus capsid antigen (VCA). High antibodies to both of these antigens are also found in BL patients, except that antibody to EA is generally to the restricted (R) component of the EA complex. It is also known that antibody titers to EBV antigens generally decline gradually after effective therapy so that EBV serologic tests have been used to monitor successful treatment. Nasopharyngeal carcinoma patients at diagnosis, and often throughout the course of disease, have high antibody titers to VCA IgA as well. This is not the case for patients with IM and BL, and it is not clear as to why this antibody is somewhat specific for NPC. Studies from our laboratory have shown that EBV is also capable of inducing a virus-specific DNase, and that NPC patients, when compared to normal EBV positive individuals, IM and other cancer patients show a high frequency of strong anti-EBV DNase antibody reactivity at the time of diagnosis. Antibody titers to the EBV DNase not only have been found to be closely associated with NPC patients at diagnosis, but have also been shown in one study to be predictive for survival of juvenile NPC patients.

Since EBV has been associated with IM and BL, two diseases involving B-lymphocytes, it was surprising that when NPC biopsies were examined by in situ hybridization, the localization of EBV DNA was in the epithelial cells of the tumor. Normally, this virus infects only B-lymphocytes of human and certain nonhuman primates. Any investigation of the association between EBV and NPC faces the difficulty associated with the laboratory host range of this virus. How EBV infects normal human nasopharyngeal epithelial cells is still unclear. However, there are data from our laboratory and others which have shown that EBV can infect human epithelial cells (tumor), and normal epithelial cells of squirrel monkeys. These data strongly support the hypothesis that normal human nasopharyngeal epithelial cells could be directly infected with EBV. It is possible that interaction between EBV and/or EBV-infected cells with one or more co-factors (e.g., dietary carcinogens) could result in malignant disease.

1280 EPSTEIN-BARR VIRUS INDUCED DISEASES IN IMMUNE DEFICIENT PATIENTS, David T. Purtilo, Department of Pathology and Laboratory Medicine, University of Nebraska Medical Center, Omaha, NE 68105

Since 1975 many EBV-associated diseases beyond nasopharyngeal carcinoma, Burkitt lymphoma, and infectious mononucleosis have been documented (Figure).



SPECTRUM OF EBV-ASSOCIATED DISEASES

Depending on the type and degree of immune deficiency, various outcomes of EBV infections occur. Aproliferative and lymphoproliferative expressions of EBV infections occur as described in X-linked lymphoproliferative syndrome (XLP). EBV uniquely infects the immune system itself (B-cells). In XLP it can induce destruction of thymic epithelium which accelerates the immune defects. Anomalous cytotoxic T cell and natural killer cells activity can produce fatal hepatitis. Aproliferative phenotypes probably result from the deranged immune responses to EBV. We have identified EBV genome in the cases using molecular hybridization. Specific cytogenetic alterations are associated with conversion from polyclonal to monoclonal malignancy. Similar events may transpire owing to breakdown in immunologic surveillance in viral-induced Kaposi's sarcoma, hepatocellular, cutaneous squamous cell and cervical carcinomas. Detection and prevention of immune deficiency and treatment of viral infections by immuno- and anti-viral therapies may obviate the malignancies.

Ref. Purtilo, D.T. (1984). Editor "Immune Deficiency and Cancer: Epstein-Barr Virus and Lymphoproliferative Malignancies", Plenum Press, New York.

Herpesvirus Latency

1281 MOLECULAR BIOLOGY OF HSV-1 LATENCY IN A MOUSE MODEL SYSTEM, Nigel W. Fraser, The Wistar Institute, Philadelphia, PA 19104

The conventional interaction of viruses with susceptible host cells is a lytic cycle of infection, in which virus replicates in the host cells and multiple copies of viral progeny are produced. However, viruses can also reside in the host cell in a latent state, in which no infectious virus is recovered unless reactivation occurs. HSV-1 is a classic example of a virus that is capable of latent infection and this is easily demonstrated in mouse ganglia. However, the small amount of DNA available from mouse ganglia limits the usefulness of this system in molecular studies of HSV-1 latency. Examination of the anatomical distribution of HSV-1 DNA by both C_0T and Southern blot analysis has revealed latent viral DNA not only in the trigeminal ganglia but also in the brain of animals inoculated by corneal scarification. However, on screening total mouse brains, only 50% were found to contain latent HSV-1 DNA as compared with 100% when brain stems alone were screened. This indicates enrichment of latent viral DNA in the brain stem. In situ hybridization studies have confirmed these findings, and have served to more precisely define the location of HSV-DNA during latency. The availability of an enriched source of latent HSV-1 DNA and thereby an increased percentage of latently infected animals in which this DNA can be demonstrated provides the tools to aproach several molecular biology questions previously inaccessible to experimental study. Perhaps the most interesting and straightforward experiments are those dealing with the physical state of the latent viral DNA. Thus, using restriction enzyme digestion and Southern blot analysis, we have demontrated that the latent viral DNA 1) differs from the classical HSV-1 defective particle which consists only of the right-hand half of the prototype genome, including the uniques short region; and 2) differs from the linear virion DNA in its lack of free ends. The results of CsCl buoyant density gradient centrifugation experiments strongly suggest that the vast majority of the latent HSV-1 DNA molecules exist in an episomal state. Thus, latent HSV-1 DNA appears similar in many respects to latent EBV DNA.

1282 SUCCESSIVE APPROXIMATION OF HUMAN HERPES SIMPLEX VIRUS LATENCY BY IN VITRO MODELING, Brian Wigdahl[1], Carol A. Smith[1], Erik De Clercq[2], and Fred Rapp[1], The Pennsylvania State University College of Medicine[1], Hershey, PA 17033, and Katholieke Universiteit Leuven[2], Leuven, Belgium

Herpes simplex virus (HSV) is most probably maintained in the ganglion neurons of the peripheral nervous system of humans in a latent form that can reactivate producing recurrent disease. To examine the mechanisms of HSV latency and reactivation, we have constructed several in vitro model systems (1) to provide information complementary to that obtained with animal models. Because the neuron plays a central role in HSV latency and recurrent infection, the in vitro systems were designed to approximate this cell-virus interaction. We initially described HSV type 1 (HSV-1) infections of human embryo lung fibroblast (HEL-F) cells and rat fetus sensory neurons wherein virus latency was established by blocking productive virus replication with combined (E)-5-(2-bromovinyl)-2'-deoxyuridine (BVDU; 30 μM) and human leukocyte interferon (IFN-α; 125 IU/ml) treatment. In both systems, virus latency was maintained after inhibitor removal by increasing the incubation temperature from 37 to 40.5°C, and virus replication was reactivated by decreasing the incubation temperature from 40.5 to 37°C. As determined by DNA blot hybridization, the latently infected HEL-F cell and neuron populations contained detectable quantities of most, if not all, HSV-1 HindIII, XbaI, and BamHI DNA fragments. Furthermore, there was no detectable change in size or molarity of the HSV-1 terminal or junction DNA fragments obtained by HindIII, XbaI, or BamHI digestion of latently infected neuron or HEL-F cell DNAs, compared with digestion of a reconstruction mixture of purified HSV-1 virion and HEL-F cell DNAs. These data suggest that the predominant form of the HSV-1 genome in either latently infected cell population was nonintegrated, linear, and unit-length DNA. We have now constructed a model system using neurons isolated from human fetus dorsal root ganglia as the host cell type. Neurons were partially purified by treatment with mitotic inhibitors, and shown to be neuronal in origin by the detection of the neurotransmitter substance P and neuron plasma membrane antigen A2B5 by indirect immunofluoresence using primary monoclonal antibodies, a biotinylated secondary antibody, and either rhodamine or fluorescein-conjugated avidin. Virus latency was established in human fetal neurons by combined treatment for 7 days with BVDU and IFN-α and maintained by increased incubation temperature after inhibitor removal. This model will permit, for the first time, the biological and molecular analysis of HSV latency in a primary human neuron.

References

1. Wigdahl, B., Scheck, A.C., Ziegler, R.J., De Clercq E., Rapp, F. J. Virol. in press, 1984.

1283 HUMAN CMV-HUMAN LYMPHOCYTE INTERACTIONS, Michael B. A. Oldstone, Department of Immunology, Scripps Clinic and Research Foundation, La Jolla, CA 92037

The molecular basis of cytomegalovirus (CMV) latency, activation and disease production in man is unknown. Observations of virus associated immunosuppression and activation of virus during transplantation suggest a direct role for CMV-lymphocyte interactions. Our initial approach to analyzing this problem was the use of an experimental model, mouse CMV (MCMV) infection. Such studies resulted in four important observations. First, a subset of splenic B lymphocytes harbored the virus latently. This virus was activated during subsequent allogeneic stimulation or when such B lymphocytes were cultured with the mitogen, lipopolysaccharide. Second, resident peritoneal macrophages harbored MCMV in a latent form, but thioglycollate stimulated macrophages released the virus. Third, MCMV resided latently in cells of the male (sperm) and female (follicular cells) reproductive systems. Fourth, utilizing a model of latent and dimethylacetamide driven activation of MCMV in teratocarcinoma cells, the molecular block was localized at the level of MCMV RNA in the nuclei of infected cells. Only limited RNA transcripts were made.

With this background we evaluated human CMV infection of human lymphoid cells. We report here that CMV can infect lymphocytes. Using recent viral isolates, positive selection with monoclonal antibodies, the fluorescent activated cell sorter and molecular probes for CMV, we found that a subset of T lymphocytes expressed immediate-early antigens but not late antigens of CMV. Further, CMV infection altered several of the expected specialized functions of lymphocytes. Thus, we conclude that CMV can directly infect human lymphocytes; this infection is abortive. Further in the course of infection, CMV turns off some important differentiated functions of lymphocytes.

Structure and Function of The Virus Genome

1284 GENOMES OF THE EQUINE HERPESVIRUSES: MOLECULAR STRUCTURE, REGIONS OF HOMOLOGY AND DNA SEQUENCES ASSOCIATED WITH TRANSFORMATION. Dennis O'Callaghan, Ray Baumann, Donna Sullivan, Gretchen Caughman and John Staczek, Department of Microbiology, University of Mississippi Medical Center, Jackson, Mississippi 39216.

The equine herpesviruses (EHV) are excellent models for study of the biological and biochemical properties of herpesviruses. Restriction enzyme/Southern blot and electron microscopic analyses of EHV linear double-stranded DNA molecules have elucidated the following: The EHV-1 genome is a 92 megadalton (md) molecule comprised of two components, a fixed long(L) region (72.8 md) covalently linked to an invertible short (S) region (19.2 md). 6.4 md inverted repeats (IRs) bracket a unique S sequence and enable the S region to invert, thus generating two isomers of EHV-1 DNA. Similarly, the 96.2 md EHV-3 genome is comprised of a 73.3 md fixed L region and a 22.9 md invertible S region containing 8 md IRs and, like EHV-1, exists as two isomers. The EHV-2 (cytomegalovirus) genome is a 126 md molecule that has internal areas of homology but does not appear to invert. The genome of EHV-1 defective interfering (DI) particles has been shown to be similar to EHV-1 standard (STD) DNA in size, but to be comprised of repetitive DNA sequences originating solely from S region sequences (0.77-1.00 map units) and the L terminus (0-0.05 map units). Reassociation analyses indicate that EHV-1 and EHV-2 share 1.8-3.7 md, EHV-2 and EHV-3 share 1.2-1.9 md, and EHV-3 and EHV-1 share 7.6-8.2 md. Hybridization studies using cloned EHV-1 DNA fragments as probes indicate these regions of homology between EHV-1 and EHV-3 are dispersed throughout their genomes. Cloned EHV-1 probes hybridized to defined regions of the EHV-3 genome in a predominantly colinear arrangement, and shared sequences mapped in both the L and S regions, including the IRs. Reciprocal experiments using cloned and electroeluted EHV-3 probes confirmed these results. All three EHVs have oncogenic potential which has allowed the development of five distinct models of transformation. UV-irradiated EHV-1 and EHV-1 enriched for DI particles have been used to transform permissive hamster embryo(HE) cells, and STD EHV-1 has been used to transform nonpermissive mouse embryo cells. Similarly, EHV-2 has been used to transform semipermissive HE cells and EHV-3 to transform nonpermissive HE cells. Analysis of EHV-1 transformed and tumor HE cell lines indicates that a defined viral DNA sequence (0.32-0.38 map units) is stably integrated and selectively retained in all cell lines. This sequence is transcribed and at least two viral proteins can be detected by immunoprecipitation. Additional experiments are in progress to ascertain whether EHV-2 and EHV-3 transformed cells retain DNA sequences homologous to the EHV-1 0.32-0.38 sequence. DNA transfection/transformation studies and protein analyses are being conducted to determine whether EHV DNA homologies reflect functional similarities.

1285 MAPPING VARICELLA-ZOSTER VIRUS FUNCTIONS, Richard W. Hyman[1], Linda Kudler[1], Hugh F. Maguire[1], and Charles Grose[2], Department of Microbiology[1], The Pennsylvania State University College of Medicine, Hershey, PA 17033, and Department of Microbiology[2], University of Texas Health Science Center, San Antonio, TX 78284.

One fundamental problem in working with human varicella-zoster virus (VZV) is that the virus barely grows in cell culture. Stable, authentically cell-free, high-titer VZV stocks are currently unobtainable. Therefore, the classical approach to virus genetics, the isolation of conditional-lethal mutants and their arrangement into complementation groups, is clearly untenable. For a dominant VZV gene product, direct mapping by marker rescue can be attempted. However, this approach is inherently limited to only a few genes.

Employing recombinant DNA technology, we have undertaken an alternative approach to VZV genetics. Recombinant VZV DNAs in conventional E. coli plasmid vectors were recloned into mammalian cell selection vectors. These latter vectors contained selectable markers for mammalian cells. Thereby, stable mouse and monkey cell lines expressing VZV proteins were selected, developed, and studied.

The proteins of the cell lines were radiolabeled with [^{35}S]methionine, [^{3}H]glucosamine, or [^{32}P]orthophosphate to label protein, glycoprotein, and phosphoprotein, respectively. VZV-specific proteins were immunoprecipitated with one or more of the following reagents: human zoster convalescent sera, polyclonal antisera prepared in Hartley strain-2 guinea pigs, and mouse monoclonal antibodies. The immunoprecipitated proteins were denatured and resolved by electrophoresis through polyacrylamide gels. The gels were dried and subjected to fluorography or autoradiography. VZV-specific protein bands could then be assigned to the VZV DNA resident in the transfected cell.

This work was supported by grants CA 16498 and CA 18450 from the National Cancer Institute and grant AI 14604 from the National Institute of Allergy and Infectious Diseases.

1286 REGULATION OF HUMAN CYTOMEGALOVIRUS GENE EXPRESSION, Mark F. Stinski and Richard M. Stenberg, Department of Microbiology, University of Iowa, Iowa City, IA 52242

The first genes expressed after reactivation from latency or after primary infection presumably code for a viral regulatory protein(s) that controls subsequent viral gene expression. These genes are hypothesized to be immediate early (IE) viral genes which are expressed independently of any preceding viral protein synthesis. Although, IE RNA originates from a region (0.660 to 0.751 map units for Towne strain) in the large unique component of the viral genome, only the viral RNA originating between 0.709 and 0.751 map units (Towne strain) is mRNA that can be translated in a rabbit reticulocyte lysate (1). In this region, there are three IE coding regions that have been designated regions one, two, and three. IE region one codes for the most abundant viral RNA molecule, a 1.95 kb species which is transcribed from right to left on the prototype arrangement of the viral genome. The viral mRNA originates from a region of approximately 2.8 kb (0.739 - 0.755 map units). The viral RNA is a spliced molecule containing a 3' terminal exon of 1341 nucleotides. Upstream of the major body of the mRNA are three small exon sequences of 185, 88, and 121 nucleotides. Based on the DNA sequence, the viral mRNA molecule has one open reading frame which begins within the second exon and extends for 491 amino acid residues (2). In vitro translation of mRNA selected by hybridization to IE region one demonstrated that this region codes for the predominant 72,000 dalton IE protein found in the infected cell within one hour after infection (1). It is hypothesized that this viral gene codes for the major regulatory protein controlling transcription of the viral genome at early times.

In vivo or in vitro, IE region one is highly transcribed by RNA polymerase II relative to IE region two or three. DNA sequence analysis of the upstream promoter-regulatory region of IE region one detected two distinct repeats of 19 and 18 nucleotides, both being repeated four times. The potential secondary structure and the repeat sequences in the regulatory region of IE region one are presumably related to the high level of transcription of this IE gene. The structure of the genes in IE region two and three require further investigation.

1. Stinski, M. F., D. R. Thomsen, R. M. Stenberg and L. C. Goldstein. 1983. Organization and expression of the immediate early genes of human cytomegalovirus. J. Virol. 46:1-14.
2. Stenberg, R. M., D. R. Thomsen and M. F. Stinski. Structural analysis of the major immediate early gene of human cytomegalovirus. J. Virol. (In press).
3. Thomsen, D. R., R. M. Stenberg, W. F. Goins and M. F. Stinski. The promoter-regulatory region of the major immediate early gene of human cytomegalovirus. Proc. Natl. Acad. Sci. U.S.A. (In press).

Herpesvirus Gene Expression

1287 SYNTHESIS AND PROCESSING OF HERPES SIMPLEX VIRUS GLYCOPROTEINS. Richard J. Courtney and Teresa Compton, Department of Microbiology, University of Tennessee, Knoxville, TN 37996.

Herpes simplex virus type 1 (HSV-1)-specific glycoproteins are designated gB, gC, gD and gE. Recent studies within our laboratory and others have focused on the synthesis, processing and localization of the HSV-specific glycoproteins and their partially glycosylated precursors within the infected cell. With regard to synthesis of the HSV glycoproteins, we have used an HSV-1 temperature sensitive (ts) mutant, tsA1 (kindly provided by Dr. Priscilla Schaffer, Harvard Medical School), to define possible differences in the control mechanisms operative for the synthesis of these glycoproteins. Cell cultures infected with tsA1 at the nonpermissive temperature (39°C) synthesized no detectable gC or any precursors of this glycoprotein; in contrast, gB and gD synthesis was nearly equivalent to that detected in wild type virus-infected cells. Since it has been reported that the mutation in tsA1 is located at 0.385-0.398 map coordinates (1) which is quite distinct from the map location of the gC gene, these results suggest that the expression of an additional gene function(s) may be required for synthesis of gC but is apparently not required for the synthesis of gB or gD. Temperature shift-up studies of tsA1-infected cells have also suggested that an HSV-1 gene function other than gC may be required continuously to achieve wild-type levels of gC synthesis. With regard to the processing and localization of the precursors to HSV-1 glycoproteins, we have previously reported that the high mannose precursors $pgB_{(110)}$, $pgC_{(105)}$ and $pgD_{(52)}$ are the major glycoprotein species associated with the nuclear fraction of HSV-1 infected cells (2). In recent studies focused on the nonglycosylated forms of these proteins, we have found that HSV-1 infected cells cultured at a lower temperature (34°C) tend to accumulate significant amounts of the nonglycosylated proteins and these are primarily associated with the nuclear fraction. The apparent temperature dependence on the processing of the HSV-glycoproteins has provided an approach to follow the synthesis, localization and the eventual processing of these precursor glycoproteins in the absence of inhibitors of glycosylation.

1. Weller, S. B., K. J. Lee, D. J. Sabourin and P. A. Schaffer. 1983. Genetic analysis of temperature-sensitive mutants which define the gene for the major herpes simplex virus type 1 DNA binding protein. J. Virol. 45:354-366.
2. Compton, T. and R. J. Courtney. 1984. Virus-specific glycoproteins associated with the nuclear fraction of herpes simplex virus type-1 infected cells. J. Virol. In press.

1288 STRUCTURE AND FUNCTION OF VIRAL THYMIDINE KINASES, Saul Kit, Malon Kit, and Haruki Otsuka, Division of Biochemical Virology, Baylor College of Medicine, Houston, TX 77030

In recent years, viral thymidine kinase (tk) genes have become increasingly important in molecular biology and medicine for: (i) studies on the regulation of gene expression and the cotransfection of foreign genes into eukaryotic cells; (ii) the construction of viral cloning vehicles; and (iii) investigations on the pathogenesis of neurotropic herpesvirus infections. Seven viral tk genes have already been cloned in bacterial plasmids and sequenced, namely, the tk genes of type 1 and type 2 herpes simplex viruses (HSV-1, HSV-2), marmoset herpesvirus (MarHV), pseudorabies virus (PRV), vaccinia virus, variola virus, and monkey pox virus. Although all of the dThd kinase enzymes induced by the viruses exhibit native molecular weights of about 80,000, it now appears that the herpesvirus dThd kinase enzymes are composed of 40K dalton subunits, while the orthopoxvirus dThd kinase enzymes are composed of 20K dalton subunits. The seven viral-induced enzymes also exhibit individual differences in many biochemical and immunological properties, differences that will ultimately be understood when the crystallographic structures of the enzymes have been determined. In the interim, the amino acid sequences predicted from the nucleotide sequences permit interesting comparisons. Studies of the predicted amino acid sequences of orthopoxvirus dThd kinase polypeptides by J. J. Esposito (personal communication) have disclosed that these amino acid sequences are remarkably similar. The predicted sequences of vaccinia virus, monkey pox virus, and variola virus dThd kinase polypeptides differ in only eight amino acids. In contrast, the HSV-1 (Clone 101) and HSV-2 (strain 333) dThd kinase polypeptides, which have 376 and 375 amino acids, respectively, differ in 99 amino acids. When the predicted amino acid sequences of the HSV-1 and HSV-2 dThd kinase polypeptides are aligned, it may be seen that the carboxy-terminal residue and residue 270 are deleted from the HSV-2 polypeptide, while an amino acid has been added at residue 60. The predicted MarHV dThd kinase polypeptide (376 amino acids) shows only limited sequence homology to that of the HSV-2 dThd kinase polypeptide, with the amino terminal residue of the latter polypeptide displaced by 40 amino acid residues from the amino terminal residue of the MarHV dThd kinase polypeptide. The PRV dThd kinase differs from the HSV and MarHV enzymes in that the PRV enzyme does not efficiently utilize dCyd, acyclovir, and BIOLF-62 as nucleoside substrates. We have recently cloned the PRV tk gene, prepared a series of hybrid plasmids with deletions in the PRV DNA, and carried out marker rescue experiments to delineate the approximate boundaries of the PRV tk gene. These studies and sequencing analyses suggest that the PRV tk gene is about 1.7 kb or less. Nucleotide sequencing has been completed on 1345 bp of the PRV tk gene DNA. A deletion in the coding sequence in the PRV tk gene has also been transferred to pseudorabies virus. The latter PRV tk deletion mutant is being used for analyses of the role of the PRV tk gene in the pathogeneses of virus infections.

1289 ORGANIZATION AND EXPRESSION OF THE GENOME OF HUMAN CYTOMEGALOVIRUS STRAIN AD169. Deborah H. Spector[1], Sherrol H. McDonough[1], Silvija I. Staprans[1], Joyce C. Tamashiro[1], Theodore Friedmann[1,2], and David Filpula[2]. [1]University of California, San Diego, La Jolla, CA 92093; [2]Genex Corporation, Gaithersburg, MD 20877.

The use of recombinant DNA technology has greatly facilitated detailed analysis of the organization and expression of human cytomegalovirus (HCMV). HCMV (strain AD169) has a genome structure similar to that of herpes simplex virus -1 (HSV-1) and -2 (HSV-2). The double-stranded DNA genome, 240 Kbp in length, consists of covalently linked long (L) and short (S) segments each bounded by inverted repeats. The region where the L and S components meet is termed the L-S junction, and the L and S segments can invert relative to this junction point, establishing the potential for four sequence orientations. At both termini of the genome is a direct repeat, designated the "a" sequence. DNA sequence analysis has revealed that the 550 bp HCMV "a" sequence is bounded by a 25 bp direct repeat and contains many dispersed direct repeats as well as a specific 24 bp segment which appears to be conserved in the HSV-1 and HSV-2 "a" sequences. Heterogeneity has been observed at the L-S junction and termini of HCMV and appears to be due in part to a variation in the number of these "a" sequences.

In an earlier study on viral gene regulation, we used our set of cloned subgenomic fragments of HCMV as hybridization probes to characterize the patterns of transcription in permissively infected human fibroblasts. As a result of these experiments we were able to localize transcriptionally active regions of the HCMV genome at immediate early, early, middle, and late times in the infection. Recently we have focused on transcripts synthesized from two regions of the genome. One region includes the repeats bordering the long unique segment and represents a major site of transcription during the entire infection except at the immediate early time point. With the use of the S1 nuclease assay we were able to map the 5' terminus of the 2.7 Kb polyadenylated RNA encoded by this region to a site within EcoRI fragment O approximately 600 nucleotides from the junction of EcoRI fragments O and W. The 3' terminus of the RNA was localized to a site in fragment W approximately 1850 nucleotides from the RI site and approximately 1500 nucleotides upstream from the start of the "a" sequence. This transcript appears not to be spliced. The second region of RNA transcription examined includes EcoRI fragments d and R in the long unique segment. This region is transcribed at early and middle time points of the infection but not at later times. Preliminary mapping studies indicate that the 2.2 Kb polyadenylated RNA species encoded by this region is spliced and that the direction of transcription proceeds from fragment R to fragment d. From DNA sequence analysis it appears that a long open reading frame for this transcript would include the 50 bp sequence in EcoRI fragment R which shares homology with the anti-sense strand of the 5' coding exon of the avian retrovirus oncogene v-*myc*.

1290 SYNTHESIS, STRUCTURE AND FUNCTION OF CYTOMEGALOVIRUS MAJOR NONVIRION NUCLEAR PROTEIN, Wade Gibson, Department of Pharmacology and Experimental Therapeutics, The Johns Hopkins University, School of Medicine, Baltimore, Maryand 21205

Human fibroblast cells infected with primate strains of cytomegalovirus (CMV) contain large amounts of DNA-binding protein, which is not present in the mature virus particle (1). One- and two-dimensional separations of this protein in denaturing polyacrylamide gels have established that (i) its size is strain dependent, varying between about 50K (Old World simian CMVs) and 53K (human CMVs) (2), and that (ii) it has a net basic charge $\geq$ 8. Immunological experiments using monospecific, polyvalent antisera have shown that the counterpart proteins of human and simian strains are cross-reactive, and that such sera do not react with noninfected cells. Studies based on the use of selective inhibitors of DNA and protein synthesis, *in vitro* protein synthesis, as well as metabolic and "pulse-chase" radiolabeling, have demonstrated the following. First, synthesis of this protein species requires the preceeding synthesis of both viral protein(s) and DNA. Second, infected-cell RNA will direct the synthesis of this protein species *in vitro*. And third, this protein is phosphorylated (3) -- a modification that appears to slow its electrophoretic mobility.

Efforts to determine the function of this protein have focused on establishing its intracellular localization and macromolecular associations. These experiments have shown that this protein partitions with the nuclear fraction following treatment of infected cells with NP-40 or a mixture of deoxycholate/Tween 40, and is not removed from that fraction by hydrodynamic shear (i.e., sedimentation through 2M sucrose) or by freeze-thaw disruption of the remaining nuclear "membrane." The protein can be released from such nuclei, however, by increasing the salt concentration to approximately 0.4M or by using more vigorous techniques of physical disruption (e.g., sonication). Rate-velocity sedimentation analyses of the salt-released protein indicate that it is in a monomeric form. The protein recovered from nuclei either by sonication or salt extraction, will bind to DNA-sepharose when subjected to affinity chromatography. Further, this protein is enriched for when DNA-binding proteins are recovered from infected cells either by polyethyleneimine precipitation or chromatin isolation. These and other results will be discussed as they bear on the involvement of this protein in the CMV infection process, and out hypothesis that it may serve a histone-like role in binding to DNA.

1. Gibson, W., T. Murphy and C. Roby. 1981. Virology 111:251-262.
2. Gibson, W. 1983. Virology 128:391-406. (see Fig. 5)
3. Weiner, D. and W. Gibson. 1983. Virology. 129:155-169. (see Fig. 2)

1291 ONCOGENIC TRANSFORMATION BY HERPESVIRUSES, Denise A. Galloway, Jay A. Nelson, and James K. McDougall, Fred Hutchinson Cancer Research Center, Seattle, Washington 98104

There are many suggestions that herpesvirus are in some way associated with human malignancies. Most frequently cited is the association of Epstein Barr virus (EBV) with Burkitt's lymphoma and nasopharyngeal carcinoma; herpes simplex virus type 2 (HSV-2) with cervical carcinoma; and cytomegalovirus (CMV) with Kaposi's sarcoma. In general the association has been based on seroepidemiological data and finding viral antigens and nucleic acids in tumors. An important question is to define the oncogenic potential of these viruses. We have chosen to study HSV-2 and CMV in detail, to precisely define the viral sequences that are capable of transforming rodent cells to a malignant phenotype in vitro. These experiments have identified a single fragment of HSV-2 DNA (1,2) and a single fragment of CMV DNA (3,4) with transforming activity. In both cases the viral fragments do not appear to encode a protein suggesting a novel mechanism of transformation not previously described for DNA tumor viruses must occur. A complete molecular description of the transforming fragments will be presented as well as possible models to explain their action.

(1) Galloway, D.A. and J.K. McDougall J. Virol. 42:530-537 (1982).
(2) Galloway, D.A. and J.K. McDougall Nature 302:21-24 (1983).
(3) Nelson, J.A., B. Fleckenstein, D.A. Galloway, J.K. McDougall. J. Virol. 43:550 -57. (1982).
(4) Nelson, J.A., B. Fleckenstein, G. Jahn, D.A. Galloway, and J.K. McDougall. J. Virol. (in press) (1984).

Viral Immunology

1292 HUMAN B-CELL SPECIFIC TRANSFORMATION ANTIGENS ANALYZED USING EPSTEIN-BARR VIRUS INFECTION AS A MODEL SYSTEM, David A. Thorley-Lawson, Department of Pathology and Medicine, Tufts University School of Medicine, Boston, MA 02111

Epstein-Barr virus (EBV) infection, like antigenic or mitogenic stimulation, of resting, small peripheral blood B lymphocytes drives them to differentiate into proliferating lymphoblasts characterized by the expression of two B cell differentiation antigens BLAST-1 and BLAST-2 (1). EBV is unique, however, as it fixes the B cells at the lymphoblastoid stage causing them to proliferate indefinitely (2). This process has been termed "immortalization" and is characterized by operproduction of the BLAST-1 and BLAST-2 antigens. These antigens are similar in that they are both derived from 43,000 dalton precursor polypeptides and are readily detected on EBV and PWM driven lymphoblasts, on cells in the germinal centers of lymph nodes (wherein reside the B lymphoblasts) and on chronic lymphocytic leukemia cells of B cell origin. A number of pieces of evidence suggest, however, that the antigens are distinct:

a) Monoclonal antibodies (1,2,3,4,5) against the two antigens do not cross-react in preclearing immunoprecipitation experiments.
b) The isoelectric points of the two native antigens (after neuraminadase treatment) are different, the pI of BLAST-1 being 7.1 and of BLAST-2 being 3.5.
c) The biosynthetic processing of the 43,000 dalton precursor polypeptides is different. In the case of BLAST-1, the molecule acquires carbohydrate, probably of the O-linked but, not N-linked form and has mature molecular weight of 44-45,000 in association with a second chain of 63,000. The BLAST-1 antigen is then stably expressed at the cell surface with a half life of about 16 hours. BLAST-2 on the other hand acquires only N-linked sugar, and has a mature molecular weight of 45-47,000 but, is quickly shed from the cell surface as a stable 35,000 dalton soluble polypeptide.
d) The BLAST-1 and BLAST-2 antigens are expressed at different times. More detailed studies, currently underway, on the exact correlation between the time of expression of BLAST-1 and BLAST-2 and the process of transformation will also be presented.

1. Thorley-Lawson, D.A. et.al. (1982) Cell 30:415-425.
2. Henle, W. et.al. (1967) Science 157:1064-1065.
3. Kintner, G. and Sugden, B. (1981) Nature 296:458.
4. Slovin, S. et.al. (1982) Proc. Natl. Acad. Sci. 79:2649-2653.
5. Rowe, M. et.al. (1982) Int. J. Cancer 29:373.

1293 THE ROLE OF NATURAL DEFENSE MECHANISMS IN THE RESISTANCE TO HERPESVIRUS INFECTIONS, Carlos Lopez, Laboratory of Herpesvirus Infections, Sloan-Kettering Institute for Cancer Research, New York, NY 10021.

Natural resistance mechanisms are those aspects of host defense which do not require presensitization in order to be operative and are thus ready immediately to respond to an invading pathogen. In contrast, adaptive immunity requires prior exposure to antigen and usually is not operative until several days after the infection commences. Although many barriers and other cell types can participate in natural defense, this discussion will concentrate on Natural Killer (NK) cells and the rapid generation of interferon-alpha because recently developed data suggests that these mechanisms play very important roles in resistace to herpesvirus infections. NK cells are effectors which effecientl lyse a number of tumor targets, virus-infected cells, and certain normal cells. Studies with murine and human effector cells indicate that NK cells are heterogeneous and that different subpopulations can be evaluated by using different target cells in the assay. A major role for NK cells in resistance to herpesvirus infections has been suggested in mice genetically resistant to HSV-1 and cytomegalovirus. In man, NK has been found to be deficient in patients with or susceptible to severe virus infections (1). NK heterogeneity appears to be important since NK with HSV-1 infected fibroblasts was deficient but with K562 tumor cells was normal in some of these patients. Early production of interferon has been shown by Kirchner's group (2) to also correlate with genetic resistance to HSV-1 in the mouse. This group has recently suggested that the early interferon might act to activate macrophages so that they will sequester HSV-1 replication. In man, a deficiency of interferon-alpha generation was found to be the best correlate of susceptibility to opportunistic infections in patients with AIDS (3). These studies suggest that natural defense mechanisms are required for resistance against herpesvirus infections and deficiencies can be associated with severe infections.

1. Lopez, C., Kirkpatrick, D., Read, S.E., et al. J. Infect. Dis. 147:1030, 1983.

2. Engler, H., Zawatzky, R., Goldbach, A., et al. J. Gen. Virol.55:25, 1981.

3. Lopez, C., Fitzgerald, P.A., and Siegal, F.P. J. Infect. Dis.(in press).

1294 IMMUNOLOGICAL REACTIVITY AND SPECIFICITY OF HERPES SIMPLEX VIRUS PROTEINS, Bodil Norrild, Institute of Medical Microbiology, University of Copenhagen, Copenhagen, Denmark

Herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2) genomes encode more than 50 proteins. The structural proteins make up only half of the proteins synthesized during a productive infection, the other proteins being either regulatory or specifying enzyme functions.

During a primary or recurrent HSV-infection most proteins induce the production of specific antibodies or the activation of T-lymphocytes. Among the HSV-proteins identified from both HSV-1 and HSV-2 are several glycoproteins which are present in the viral envelope and are inserted in the plasma membrane. The immunogenicity of these proteins has been studied in great detail and it is well known that each of the gA/B, gC, gD and gE glycoproteins from the two herpes virus types specifies a characteristic set of antigen determinants. The glycoproteins of HSV-1 and HSV-2 map colinearly on the gene map, with the exception of gF of HSV-2, and the corresponding glycoproteins from the two virus types share antigen determinant sites although type-specific determinants are also present. The analysis of the immunologic specificity of the glycoproteins was studied by use of both hyperimmune rabbit sera and monoclonal antibodies made to the proteins.

The development of the hybridoma methodology in combination with the use of the immunoblotting technique made it possible to study the immunological reactivity of HSV-proteins not detected by the methods used previously. The identification and the immunological specificity of these insoluble HSV-proteins showed that some of these were strong immunogens during infection of the human host.

Analysis of HSV-proteins made it possible also to study the spectrum of antibodies made in human sera during infection. The frequency of antibodies raised to the individual HSV-proteins and the variability from individual to individual will be discussed.

1295 SPECIFIC IMMUNE RESPONSES OF CHILDREN WITH CONGENITAL CMV INFECTION, Robert F. Pass, William J. Britt, Sergio Stagno and Charles A. Alford, Department of Pediatrics, University of Alabama in Birmingham, Birmingham, Alabama 35294

Congenital cytomegalovirus (CMV) infection is present in around one percent of infants born in the U.S. Most infected infants escape without sequelae, but around 10 to 20% will have clinically evidence infection, which results in damage to the central nervous system or organs of perception [1]. Although almost all infants with congenital CMV infection have vigorous humoral immune responses to the virus, as indicated by serum antibody levels measured with infected cell or crude virion antigen preparations, there is evidence that they are unable to terminate active viral replication. Damage caused by the infection may progress during the first two years of life, and viral shedding in urine usually persists beyond five years.

Investigations of CMV specific cellular immune responses in these children have revealed impaired blastogenesis and lymphokine production, compared to results obtained in seropositive adults or adults with recently acquired infection. These impairments have not been consistently associated with abnormal responses to mitogens, decreased numbers of T lymphocytes, derangements in the ratio of helper/suppressor T cells in peripheral blood or the presence of viremia detectable by viral isolation. Impaired blastogenesis (usually no detectable response) is specific for CMV; as patients with congenital CMV infection who have acquired herpes simplex virus have developed a blastogenic response to the latter [2]. Deletion of T8+ lymphocytes from cultures has failed to improve the poor blastogenic. Substitution of parental macrophages (plastic adherent) similarly did not change the absent or impaired responses of the infected child's T lymphocytes to CMV. The specific impaired blastogenic response is significantly associated with the persistence of viruria. Detectable lymphocyte responses have not consistently appeared until after five years of age. Although the mechanism for the abnormal cellular immune response to CMV in children with congenital infection remains obscure, it is clearly virus specific and not a result of a generalized cellular immune defect.

1. Alford, C.A., Stagno, S. and Pass, R.F.: Natural history of perinatal cytomegaloviral infection. In: Perinatal Infections (Ciba Foundation Symposium), Excerpta Medica (Amsterdam), 1980, pp 125-147. (Presented at The Ciba Foundation, London, November 26 - 29, 1980).
2. Pass, R.F., Stagno, S., Britt, W.J. and Alford, C.A.: Specific cell mediated immunity and the natural history of congenital infection with cytomegalovirus. J. Infect. Dis., 1983, in press.

Models for Treatment of Herpesvirus Infections

1296 ANTIVIRAL AGENTS, ANTI-INFLAMMATORY AGENTS AND MODELS OF HERPES SIMPLEX IN THE MOUSE T.J. Hill*, W.A. Blyth*, D.A. Harbour*, A.B. Tullo**, D.L. Easty**, and C. Shimeld**, Departments of Microbiology* and Ophthalmology*, University of Bristol, U.K.

Animal models used to test the effects of drugs on infection with HSV should, where possible, allow assessment of these effects on the acute, latent and recurrent phases of the disease. In the mouse ear model (1), systemic treatment with acyclovir (ACV) before or soon after inoculation with HSV can virtually prevent the growth of virus in the skin and the invasion of the nervous system (and hence reduce mortality and latent infection). A recent modification of this model uses the ability of HSV to cause zosteriform spread of lesions. Mice are infected in the neck in the dermatome (C2, C3) which includes the pinna where lesions can occur in up to 100% of animals 5 or 6 days after the original inoculation. With this model topical treatment of the pinna with 5% acyclovir cream from the 3rd day after inoculation of the neck with HSV drastically reduced the incidence of zosteriform spread of lesions to the ear and halved their duration. ACV given in the drinking water from 1 day after inoculation prevented death and the appearance of lesions on the ear and almost completely prevented establishment of latency. When treatment was started on the 2nd or 3rd day after inoculation increased incidences of lesions on the ear were seen but animals were still protected from death. The zosteriform spread model and the original mouse ear model have the added advantage that recurrent cutaneous lesions can develop spontaneously in latently infected animals (1) and can be induced by various stimuli (3,4). After one such stimulus, stripping the pinna with cellophane tape, recurrent lesions occur 3-6 days after stripping in 25-40% of animals. Systemic treatment with ACV was effective in preventing such recurrent disease but did not eliminate latent infection from the sensory ganglia (5). Moreover, if applied frequently enough topical treatment of the pinna with 2% acyclovir cream greatly reduced the incidence of recurrent lesions (6). Treatment of existing recurrent disease with 2% or 5% ACV cream shortened the duration of lesions but again did not eliminate latency.

The inappropriate topical treatment of human herpetic keratitis with corticosteroids can lead to long term damage in the cornea. In a mouse model of herpetic keratitis (7) such treatment caused prolonged shedding of virus from the eye and an increase in the extent of latent infection in the trigeminal ganglion.

Refs. (1) Hill, T.J. et al. (1975). J. gen. Virol. 28, 341. (2) Hill, T.J. et al. (1983). Prog. Brain Res. 59, 173. (3) Hill, T.J. et al. (1978). J. gen. Virol. 39, 21. (4) Harbour, D.A. et al. (1983). J. gen. Virol. 64, 1491. (5) Blyth, W.A. et al. (1980). J. gen. Virol. 48, 417. (6) Hill, T.J. et al. (1982). Antiviral. Res. 2, 135. (7) Tullo, A.B. et al. (1983). Arch. Ophthal. 101, 961.

1297 HERPETIC EYE DISEASES IN ANIMALS AS MODELS FOR THERAPEUTIC STUDIES OF ACUTE AND LATENT HERPESVIRUS INFECTIONS, W. J. O'Brien, Departments of Ophthalmology and Microbiology, The Medical College of Wisconsin, Milwaukee, WI 53226

Infections by members of the herpesvirus family are common causes of debilitating eye diseases. Herpes simplex viruses and herpes zoster may infect the conjunctiva, cornea, iris, and uvea. Cytomegalovirus, in addition to herpes simplex virus, has been found to infect the retina in both immunocompromised hosts and apparently normal hosts. Models for the acute stages of ocular infections by HSV may be produced in many species of animals, however, the most useful models include infections of rabbits, mice, and monkeys. The establishment of latent viral infections by colonization of neural tissues develops in a large percentage of the acutely infected animals. Reactivation characterized by shedding of virus in tears can be induced, but recrudescences characterized by recurrent forms of ocular pathology have not been reproducibly induced. The rabbit and mouse models appear to be most useful and practical, however, all of the models suffer from high cost and long duration.

The model of acute herpetic keratitis in rabbits induced by various strains of HSV has played an important role in the development of four drugs which are currently available to treat this disease in humans; 5-iodo-2'-deoxyuridine, 5-trifluoro-2'-deoxyuridine, 9-β-D-arabinofuranosyladenine, and 9-(2-hydroxyethoxymethyl) guanine. Numerous new nucleoside analogs have been shown to be efficacious in this model. Natural and synthetic interferons have shown efficacy within and across species lines when used in a prophylactic manner. Vaccines and other forms of immunotherapy have been shown to be beneficial in studies utilizing a mouse model of herpetic keratitis. In addition to efficacy, the models for acute herpetic eye disease have provided a means of assessing therapeutic dose responses, drug delivery systems, and toxicity.

Studies of therapeutic efficacy in the treatment of recrudescent forms of herpetic eye diseases have established that no currently available antiviral agent is capable of eliminating virus from the nervous system. Treatment with therapeutic agents before or within a few hours of infection have been shown to reduce the frequency of ganglial infection and, thereby, may have prophylactic potential. Forms of therapy which involve high dosage over extended periods of time have not been successful in reducing the frequency of ganglial infection. Furthermore, due to the lack of an adequate model of recrudescence, little progress has been made in the assessment of therapeutic agents for treatment of recurrent herpetic disease.

Studies of eye diseases produced by herpesvirus infections have contributed to the general understanding and assessment of factors which are important in the therapy of infections. The development of models of recrudescent forms of herpetic diseases will provide the key to the development of forms of therapy which will eliminate or control recurrent attacks.

Vaccines

1298 CLONING, EXPRESSION, AND IMMUNOLOGICAL PROPERTIES OF HERPES SIMPLEX GLYCOPROTEIN D-RELATED RECOMBINANT PROTEINS, R. J. Watson and L. W. Enquist, Molecular Genetics, Inc., 10320 Bren Road East, Minnetonka, MN 55343; R. J. White, C. J. Heilman, C. P. Cerini, D. Steinberg and D. W. McCoy, Medical Research Division, American Cyanamid Company, Lederle Laboratories, Pearl River, NY 10965.

Glycoprotein D isolated from cells infected with herpes simplex virus type 1 or 2 has been shown to elicit the formation of type-common neutralizing antibodies in mice and protects against subsequent lethal challenge.[1,2]. As part of a collaborative program aimed at developing a recombinant derived subunit vaccine, DNA fragments encoding herpes simplex virus type 1 and type 2 glycoprotein D (gD-1 and gD-2, respectively) have been inserted into plasmid vectors and expressed under the transcriptional control of the Escherichia coli lac promotor-operator.[3] The proteins expressed in this system comprised gD sequences fused to a small bacteriophage λ cro leader (i.e. cro-gD). Such cro-gD fusion proteins were found to be intrinsically unstable in E. coli and accumulated to low levels only. We found that fusion of this cro-gD coding sequence to the 5' end of a sequence encoding β-galactosidase (β-gal), resulted in high levels of synthesis of cro-gD-β-gal fusion proteins provided that certain carboxy-terminal gD coding sequences were deleted. These cro-gD-β-gal proteins accumulated as insoluble intracellular aggregates to levels comprising approximately 10% of the total cell protein.[4] The ability of purified cro-gD-β-gal fusion proteins to immunize mice against herpes simplex type 2 infections was tested in an intraperitoneal vaccination/lethal intraperitoneal challenge model. Certain of these chimaeric proteins (related to gD-1 or gD-2) produced significant protection in this model. Surprisingly, Freund's complete adjuvant had a negative effect on this protection. No correlation could be demonstrated between the protection elicited by chimaeric proteins in this model and serum antibodies as analyzed by neutralization, immunoprecipitation, and ELISA methodology.

1. W. L. Chan. Immunology 49 (1983) 343.
2. D. Long, T. J. Madera, M. Ponce de Leon, G. H. Cohen, P. L. Montgomery, R. J. Eisenberg. Infection and Immunity (In press). February 1984 issue.
3. R. J. Watson, J. H. Weis, J. S. Salstrom, L. W. Enquist. Science 218 (1983) 381.
4. J. H. Weis, L. W. Enquist, J. S. Salstrom and R. J. Watson. Nature 302 (1983) 72.

1299 PROGRESS TOWARDS PREVENTION AND MODIFICATION OF HERPES GENITALIS BY VACCINATION, Gordon R.B. Skinner, Department of Medical Microbiology, University of Birmingham, UK

This study describes the preparation and efficacy of inactivated vaccine AcNFU$_1$(S$^-$) MRC towards prevention and modification of herpes genitalis. Vaccine is prepared by high multiplicity infection of serum-starved human embryo lung cells (MRC-5) with type 1 herpes simplex virus. Cell nuclei are removed and the cytoplasmic extract treated with detergent and formaldehyde. Virus particles are removed by ultracentrifugation and virus proteins precipitated by cold acetone. The vaccine contains all the virus polypeptides represented in virus-infected cells, including glycoprotein D which is consistently detected by immuno-precipitation. Virus particles are not detected and nucleic acid hybridisation studies indicate levels of below 5 ng of viral and 500 ng of cellular DNA in a standard vaccine dose (2×10^7 cell equivalents). There is evidence that the vaccine will protect a number of animal species from primary type 2 herpes virus infection and reduce the incidence of herpes virus-induced cervical cancer in mice. Vaccination has been offered to two groups of patients:

(1) Subjects at risk of the disease, namely, consorts of patients with recurrent herpes genitalis; in these patients the rate of transmission has been reduced from 30% in unvaccinated to <1% in vaccinated subjects during an 18 month period of follow-up.

(2) Patients who had experienced one clinical episode of herpes genitalis; in these patients vaccination has reduced the proportion of patients experiencing recurrent episodes of disease from approximately 90% in unvaccinated to 25% in vaccinated patients within one year of the initial clinical episode.

In a follow-up period of 3 years, there has been no evidence of significant local or general side-effects.

It is intended to submit the findings to the scrutiny of a randomised placebo-controlled double-blind trial in collaboration with the Centre for Applied Microbiological Research, Porton Down, UK.

1300 A LIVE VARICELLA VACCINE. Michiaki Takahashi. Research Institute for Microbial Diseases. Osaka University. Suita, Osaka, Japan.

A live varicella vaccine has recently been developed. Oka strain of varicella zoster virus(VZV) was attenuated by serial passage in human embryonic lung cells at 34°C followed by passage in guinea pig embryo cells, which was then propagated in human diploid cells for production of vaccine.[1] Vaccine virus differed from wild viruses in the following respects : First, the ratio of infectivity of vaccine virus in guinea pig embryo cells and human embryo cells is more than 10 times higher than that of wild viruses. Second, a distinct difference can be observed between vaccine virus DNA and that of wild-type viruses in the migration pattern in agarose gel electrophoresis after digestion with HpaI endonuclease. The results of these two marker tests agreed well for all the ten isolates from vesicles of varicella or zoster that developed in vaccinees and the conclusions reached were consistent with the clinical diagnosis.

The most striking feature of the varicella vaccine is that it could be used to high risk children.[2] Approximately 1,500 children with underlying diseases including those who have been receiving steroid therapy have successfully been vaccinated with minimal clinical reactions. Among them, approximately 250 acute leukemic children have been vaccinated under the following conditions : 1)the patients should be in complete remission, 2)cell mediated immunity assessed by skin test with PHA or other regents in vivo or in vitro should be normal, 3)anticancer theraphy should be suspended 1 week prior to 1 week after vaccination with continuation of 6-MP treatment. Under the avobe conditions, clinical reactions were observed in about 20 percent of the vaccinees but the symptoms were generally mild. Immune responses and protective effect have been satisfactory.

In the follow-up study, incidence of zoster in the vaccinated leukemic children and in nonvaccinated leukemic children with a history of varicella, all receiving the same kind of chemotherapy were compared. In one study group, 7 of 50 vaccinated children (14.0%)and 10 of 59 nonvaccinated children(6.9%)developed zoster : the clinical symptoms were mild in all. In another study group, 4 of the 44 vaccinated children(9.0%)developed mild zoster, while 8 of 37 nonvaccinated children(21.6%)developed zoster, two of whom developed severe pain. Although the number of case is not large enough, there is no indication, at present, that immunization with the varicella vaccine would increase the frequency or severity of zoster in comparison with natural varicella. The current available data suggest that the live varicella vaccine will be a potent measure to prevent varicella in immunocompromized childre.

1)Takahashi M. et al., Lancet 2 1288, 1974
2)Takahashi M. et al., in"The Human Herpesviruses"edit. Nahmias A.S. et al.Elsevier 1981

1301 PREVENTION OF HUMAN CYTOMEGALOVIRUS DISEASE, Stanley A. Plotkin, Infectious Diseases Division, Children's Hospital of Philadelphia, Philadelphia, PA, 19104

A virus isolated in WI-38 human embryo fibroblasts from the urine of a congenitally infected infant named Towne was passaged in WI-38 Towne virus 125 times with 3 clonings by plaque isolation before experimental pools were prepared for use as an attenuated vaccine. Studies of vaccination in normal volunteers showed that: 1)Serologic responses typical for primary infection appeared by the 4th week post-vaccination, including ACIF, CF, and neutralizing antibodies. 2)In vitro lymphocyte proliferation responses specific to CMV antigen developed in all vaccinees. These responses were not strain-specific, i.e., antigens prepared from all laboratory and wild strains tested elicit proliferation of lymphocytes. 3)No CMV was isolated from urine, throat secretions, buffy coat, semen, or cervical secretions after vaccination of any normal subject. 4)The only clinical reaction was a local erythema and induration at the injection site developing during the 2nd week post-vaccination and then disappearing. 5)Altered T cell helper/suppressor ratios or diminished Con A responses, which occur commonly in natural CMV infection, did not develop.

A trial in renal transplant candidates (RTC) was organized on a controlled basis. RTC were randomly distributed to receive either vaccine or placebo 8 weeks or more prior to transplantation. Serologic and cellular immune responses in vaccinated RTC did not occur as uniformly as in normal subjects, and the responses that did occur were weaker.

The first 91 patients were analyzed by the original seological status of donors (D) and recipients (R) prior to vaccination. The overall infection rate was almost 100% in the originally seronegative recipients who received a kidney from a seropositive donor. The vaccination status in this group of 32 patients had no effect on virus excretion post-transplant.

When the patients in the D+R- group were grouped into those who were asymptomatic after transplant, those who had illnesses scoring 1-6, and those who had more severe illnesses with scores of 7 or greater, there was a different distribution in the vaccinees and placebo recipients. Whereas about half of the placebo recipients had scores of 7 or greater, only 1 of the vaccinees fell into this group. This difference was significant at the $p=<.05$ level. The mean scores for the placebo group revealed an average of more than twice the clinical severity compared to the vaccine group (5.67 vs 2.70, respectively).

Towne vaccine did not prevent infection with CMV, but did mitigate the disease that resulted from the infection. Vaccinees who received a kidney from a seronegative donor did not excrete virus, despite immunosuppression. CMV strains recovered from vaccinees transplanted with a kidney from a seropositive donor were shown by restriction endonuclease analysis to be different than the vaccine strain. Thus the Towne vaccine virus has little or no ability to become latent in the vaccinee, at least in a complete genomic form.

Treatment of Herpesvirus Infections

1302 BIOCHEMISTRY, PHARMACOLOGY AND CLINICAL EFFICACY OF ACYCLOVIR, Gertrude B. Elion, Wellcome Research Labs., Burroughs Wellcome Co., Research Triangle Park, NC 27709

Acyclovir (9-(2-hydroxyethoxymethyl)guanine, ACV) in an acyclic nucleoside analog with a high activity and selectivity for herpes simplex viruses, types 1 and 2 (HSV-1, HSV-2) and varicella zoster virus (VZV). This selectivity is due to the specific phosphorylation of ACV by the viral thymidine kinases induced by HSV-1, HSV-2 and VZV, a reaction not catalyzed to any extent by cellular kinases. The ACV monophosphate (ACV-MP) is converted to the triphosphate (ACV-TP) which is a potent inhibitor of the viral DNA polymerases. The relationship between the amount of ACV-TP formed and the inhibition constant (K_i) for the particular DNA polymerase is indicative of the potency of the effect of ACV on DNA replication. Although Epstein-Barr virus (EBV) does not specify its own thymidine kinase, the EBV DNA polymerase is extremely sensitive to even the small amount of ACV-TP formed. Extensive investigation of the fate of ACV in animals and in man have shown that it is metabolically stable, being excreted largely unchanged in the urine. The drug penetrates tissues well and has a plasma half-life in man of approximately 3 hours. In patients given ACV by intravenous infusion, the concentration of drug in the cerebrospinal fluid has reached concentrations one-half the plasma levels. Oral absorption of ACV is species-dependent. In man the oral bioavailability is approximately 20% at therapeutic doses. ACV has a very favorable chemotherapeutic index and shows little or no toxicity at clinically effective doses. The compound was non-carcinogenic in lifetime studies in rodents and had no effect on reproductive processes or prenatal, perinatal, or postnatal development of offspring in rats, rabbits and mice. It does not inhibit immunological responses, of either the cell-mediated or antibody type.

ACV is highly effective as an ophthalmic ointment in the treatment of herpetic keratitis. Treatment with a water-soluble topical ointment of ACV reduced the period of viral shedding, the duration of pain and the time to healing in initial genital herpes infections. In recurrent genital herpes, the oral and intravenous forms of the drug were more effective than the topical treatment. Prophylaxis with intravenous infusions or with oral ACV prevented recurrences of HSV infections in immunocompromised patients with bone marrow or heart transplants as well as in patients with frequent episodes of genital herpes. The intravenous drug caused a more rapid disappearance of viral shedding, acute pain and new lesion formation in HSV and VZV infections in immunosuppressed individuals, as well as in normal patients with herpes zoster. Studies are in progress to study the effect of ACV in herpes encephalitis, neonatal herpes and severe mononucleosis.

1303 MOLECULAR BASIS FOR TREATMENT OF HERPESVIRUS INFECTIONS, William H. Prusoff, Department of Pharmacology, Yale University School of Medicine, New Haven, CT 06510

An understanding of the molecular basis for the antiviral activity of those compounds that are clinically efficacious, not only is of importance for their intelligent use, but also for the development of possibly more effective agents. As of today there are only four compounds that have been approved by the FDA for use against herpesvirus infections in man: 5-iodo-2'-deoxyuridine, 5-trifluoromethyl-2'-deoxyuridine, 9-β-D-arabinofuranosyl adenine and 9-(2-hydroxyethoxymethyl)guanine. The molecular basis for their activity will be discussed as well as the limitations associated with their use. The approaches that have been made to circumvent these limitations as well as those approaches that may merit investigation will also be discussed.

References

1. Prusoff, W.H., Otto, M.J., Lin, T.-S., Mancini, W.R., Chen, M.-S. and Lee, J.J. (1983). Present Status and Future Developments in Antiviral Chemotherapy In "Medical Virology II". Eds. L.M. dela Maza and E.M. Peterson, pp. 273-306. Elsevier Biomedicine, North Holland, Amsterdam.
2. Prusoff, W.H., Lin, T.-S., Mancini, W.R., Otto, M.J., Siegel, S. and Lee, J.J. (In Press). Overview of the Possible Targets for Viral Chemotherapy. In "Targets for the Design of Antiviral Agents". Eds. R. Walker and E. De Clercq. NATO Advanced Study Institutes Series.
3. Prusoff, W.H. and Otto, M.J. (1983). Problems in the Pharmacology and Pharmacokinetics of Antivirals. In "Problems of Antiviral Therapy". Beacham Colloquium. Academic Press, London.

1304 THERAPEUTIC POTENTIALS OF BROMOVINYLDEOXYURIDINE (BVDU) IN THE TREATMENT OF HERPESVIRUS INFECTIONS, Erik De Clercq, Rega Institute for Medical Research, Katholieke Universiteit Leuven, B-3000 Leuven, Belgium.

BVDU [(E)-5-(2-bromovinyl)-2'-deoxyuridine] is a highly potent and selective antiherpes agent. It is particularly active against herpes simplex virus type 1 (HSV-1), varicella-zoster virus (VZV), pseudorabies virus, bovid herpesvirus type 1, herpesvirus platyrrhinae, and simian varicella zoster virus. Its minimal inhibitory concentration for these viruses falls within the range of 0.002 to 0.01 μg/ml, that is 10,000 fold lower than the concentration required to affect normal cell metabolism. BVDU is also effective against herpes simplex virus type 2 (HSV-2), Epstein-Barr virus (EBV), and, according to some studies, cytomegalovirus (CMV), but only at a concentration exceeding 1 μg/ml. Because of its discriminating behavior toward HSV-1 and HSV-2, BVDU can be advocated as a useful probe for the identification of HSV-1 and HSV-2 strains in clinical isolates. The selectivity of BVDU as an antiviral agent depends primarily upon a specific phosphorylation by the viral deoxythymidine (dThd) kinase which restricts the action of the compound to the virus-infected cell. The HSV-1 encoded dThd kinase is also endowed with dTMP kinase activity, capable of converting BVDU successively to its 5'-monophosphate and 5'-diphosphate. The HSV-2 specified dThd kinase would not be able to convert BVDU 5'-monophosphate onto its 5'-diphosphate, and this may be related to the relatively weak activity of BVDU against HSV-2. The active form of BVDU would correspond to its 5'-triphosphate, and in this form it could act as both inhibitor and alternate substrate of the DNA polymerase. The antiviral action of BVDU may be primarily based upon its incorporation into viral DNA which would render the DNA more apt to degradation as well as less functional for transcription. Animal experiments have demonstrated the efficacy of both topical and systemic (i.e. oral) BVDU in the treatment of herpetic eye infections (keratitis, iritis), herpetic encephalitis, and cutaneous and orofacial HSV-1 infections. In monkeys BVDU has proven efficacious in the therapy of simian varicella virus infection, even if treatment was delayed until clinical symptoms appeared. From phase I trials in humans BVDU appears to offer great promise for the topical treatment of herpetic keratitis (if administered as 0.1 % eye drops) and for the systemic treatment of severe HSV-1 and VZV infections in immunocompromised patients, including bone-marrow recipients and leukemic children (if administered orally at 7.5 mg - 15 mg/kg/day for 5 days). In the majority of the patients treated orally with BVDU, the VZV infection, whether varicella or zoster, or localized or disseminated, was arrested within 2 - 3, if not 1 day, after BVDU treatment was started. The absorption of BVDU following oral administration is virtually complete, but the compound is rapidly degraded by phosphorolytic cleavage to its free base, BVU [(E)-5-(2-bromovinyl)uracil]. This rapid degradation may obviously alter the clinical efficacy of BVDU. Measures have been devised to prevent or reverse the conversion of BVDU to BVU.

1305 STRATEGIES FOR TREATMENT OF HUMAN HERPESVIRUSES, Thomas C.Merigan, M.D. Stanford University School of Medicine Stanford, California 94305

Today there are a number of human herpesviruses, in which acute chemotherapy or prophylaxis can be achieved with either substituted pyrimidine nucleotides or immunobiologics. For example, either zoster immune globulin or cytomegalovirus hyperimmune globulin can prevent initial infection in high risk susceptables. It seems likely that such reagents will ultimately be replaced by one or a combination of appropriate human (or mouse) monoclonal antibodies. Natural alpha interferon protects against CMV reactivation in renal transplant patients and be used therapeutically in both varicalla and zoster infections in immunosuppressed individuals. Several substituted pyrimidine antagonists have been shown to be useful therapeutically in varicella zoster and herpes simplex infections including adenine arabinoside and acyclovir and bromovinyldeoxeuridine is promising. Other than the recent information that acyclovir, when used in treatment of initial herpes genitalis decreased incidence of recurrences, there is no evidence these agents will prevent the long term course of these infections including colonization of ganglia and subsequent potential for recurrence. On the other hand, cellular events seem to be important in the genesis of viral reactivation. Hence, immune prophylaxis which could be given at any time might be more practical than agressive early treatment. Our recent findings suggest that gamma interferon producing helper T cells play an important role in recurrent herpes labialis. Interleukin 2 and other immune regulators which have become available from biosynthetic sources are therefore important to evaluate for their influence on selectively driving lymphocyte subpopulations that are crucial in modulating clinically apparent sequalae of herpes simplex reactivation. It is even possible that combinations of specific immunotherapy with clinical antivirals will provide long term, specific control over herpesviral disease.

Pathogenesis and Latency

1306 TRANSFER AND PERSISTENCE OF VIRAL ANTIBODY PRODUCING CELLS IN BONE MARROW TRANSPLANTATION, Britta Wahren, Annika Linde, Per Ljungman and Vivi-Anne Sundqvist, National Bacteriological Laboratory and Huddinge Hospital, Stockholm, Sweden

Antiviral humoral reactivity was studied after bone marrow transplantation. Antiviral IgG determined by monoclonal immunoassays and reflecting the presence of functional B-cells, was found for a substantial time after transplantation. However, most patients unexpectedly ceased to produce antibody after an extended period of observation (>3-12 months). This was so, whether the viral reactivity was of donor or recipient origin. The transferred viral antibody-producing cells thus usually persisted for only a moderate length of time and seemed to have a finite life. The findings indicate that both donor and recipient memory cells are susceptible to the conditioning and/or post-transplantation treatment. In a few cases transferred immunity of long duration was seen, indicating occasional engraftment of memory cells.

1307 ZOSTERIFORM SPREAD OF HERPES SIMPLEX VIRUS AS A MODEL OF THE RECRUDESCENT LESION : STUDIES ON THE PATHOGENESIS AND IMMUNE REGULATION OF 'RECRUDESCENCE'

Anthony Simmons and Anthony A. Nash, Cambridge University Dept. of Pathology CB2 1QP

When inoculated into the midflank of certain strains of mice, HSV replicates in the skin and also spreads to the sensory ganglion supplying the dermatome. Return of virus to the skin along different axons results in an ipsilateral, band-like, zosteriform eruption. The emergence of virus into normal epidermis via nerves is analogous to the final step in recrudescence, and forms a useful model in which to study immune processes acting upon virus reaching the skin in this way. We have investigated the effect of both cellular and humoral immunity in an adoptive transfer system. The results indicate that 'recrudescent' lesions appear in the presence of T-cell populations normally associated with rapid clearance of virus from the site of infection. Cytological studies show that virus is at first highly localised in the epidermis. In the absence of tissue damage 'immune' cells are not recruited to the site of infection. Once cell breakdown occurs, inflammation, and subsequently a mononuclear infiltrate, can be seen. In contrast we find that certain monoclonal antibodies are able to suppress the re-emergence of virus from the nervous system, and thus protect against the development of a lesion, provided that they are present before the zosteriform eruption occurs.

We therefore present a model in which to study both the pathogenesis of the recrudescent lesion and the relative roles of cell mediated and humoral immunity in its prevention.

1308 KINETICS AND QUANTITATION OF INDUCED HSV-1 OCULAR SHEDDING. J.M. Hill, J.B. Dudley, Y. Shimomura and L.P. Gangarosa. Medical College of Georgia, Augusta, GA 30912.

Iontophoresis(Ionto) of 6-hydroxydopamine(6-HD) to the rabbit eye, followed by topical instillation of 2% epinephrine(EPI), induces ocular shedding of HSV-1 reliably and with a high frequency in latently infected rabbits. Rabbit eyes were inoculated with HSV-1 (McKrae strain), showed dendritic lesions indicative of acute HSV infection, and subsequently shed virus spontaneously at least once during 20 to 40 days postinoculation (P.I.). Two Ionto conditions were employed. Group A (4 rabbits, 62 days P.I.) received Ionto of 1.0% 6-HD at 0.75 mAmps for 3 min. Group B (4 rabbits, 65 days P.I.) received Ionto of 0.1% 6-HD at 0.5 mAmps for 8 min. Following Ionto treatment, 2% EPI was instilled topically once on the day of Ionto and twice daily for 4 consecutive days. In group A, 88% (7/8) of the eyes shed virus. The average duration of shedding was 3.6 days. The titers, determined from the tear film, ranged from 2.0 to 7.7 x 10^4 PFU/eye. The highest daily average, 1.6 x 10^4 PFU/eye, occurred on day 6. In group B, 75% (6/8) of the eyes shed virus. The average duration of shedding was 5.5 days. The viral titer of the tear film ranged from 2.0 to 1.4 x 10^5 PFU/eye. The highest daily average titer, 4.2 x 10^4 PFU/eye, also occurred on day 6. There was no statistical difference in the kinetic and quantitative results of HSV-1 shedding between the two Ionto conditions. The production of ocular adrenergic supersensitivity provides a shedding model which is simpler and more reliable than adrenergic stimulation alone (i.e., EPI Ionto daily for 3 days). This new model for viral reactivation and induction of HSV-1 ocular shedding should assist in the elucidation of mechanisms associated with HSV-1 latency and reactivation. The ability to quantitate HSV-1 shedding adds another dimension to the usefulness of this model.

1309 MURINE CYTOMEGALOVIRUS (CMV) INFECTION OF THE EYE, James F. Bale, Jr., Marsha O'Neil, R. Nick Hogan and Earl R. Kern, University of Iowa, Iowa City, IA 52242

Infection with human CMV has been associated with several ocular disorders. These occur most commonly as a result of congenital CMV infection, but can accompany acquired infections, particularly in immunosuppressed patients. To study CMV infection of ocular structures, three week old Swiss-Webster mice were inoculated ip with 2 x 10^4 plaque forming units (pfu) of murine CMV (MCMV), a 0 to 20% lethal inoculum. MCMV was recovered from homogenates of eye tissues 3, 5 and 7 days after infection. Peak virus titers, mean of 2.93 ± 0.67 log pfu of MCMV per gm of ocular tissue, occurred on day 5. In addition, MCMV was isolated from eye or optic nerve explant cultures obtained 14, 21, 60, 90 and 120 days after infection. Isolation of MCMV from explanted tissues was confirmed by immunofluorescence staining and transmission electron microscopy. MCMV was also recovered from intraocular fluids 4, 7, 9, 11, 14, 21, 60 and 90 days after ip infection. Intraocular fluid infection occurred most frequently on days 11, 14 and 21, when MCMV was recovered from 14 of 17 animals (82%). Peak titer of MCMV, mean of 2.30 ± 1.65 log pfu of MCMV per ml of intraocular fluid, was observed on day 21. Light microscopic studies on days 14 and 21 disclosed mild inflammatory changes at the angles of the anterior chamber in occasional animals. These experiments demonstrate that MCMV infects the ocular tissues of mice during acquired systemic MCMV infection and indicate that MCMV persists in ocular structures for as long as 120 days. These studies should be directly relevant to ocular disorders which occur during acquired CMV infections of humans and suggest that ocular tissues may be a site of CMV persistence or latency.

1310 LATENT HERPES SIMPLEX VIRUS INFECTION OF THE BRAIN TRIGEMINAL AND OLFACTORY SYSTEMS. W.G. Stroop, VA Medical Center and Univ. of Utah Medical School, Salt Lake City, UT; D.L. Rock, Iowa State Univ., Ames, IO; N.W. Fraser, The Wistar Institute, Philadelphia, PA

It has long been known that herpes simplex virus, type 1 (HSV-1) can establish a latent infection of sensory ganglia. However, it has only been recently shown that HSV-1 can establish a latent infection of the brain. We hybridized a ^{3}H-labeled HSV-1-specific probe prepared by nick translation of virion DNA to brain and ganglionic tissues *in situ*. This technique allowed us to observe the spread of HSV-1 from a peripheral site, the eye, to the ganglion, and subsequently to the brain. Forty-three infected mice representing the acute and latent stages of infection and 15 uninfected mice were examined. During the acute phase, both HSV-1 DNA and RNA were detected in the nuclei and cytoplasm of cells, but only HSV-1 RNA was detected during latency. HSV-1 nucleic acids were found in neurons and small supporting cells in the trigeminal ganglion, cerebral cortex and in the sensory portion of the trigeminal system in the pons-medulla during the acute phase, but only in neurons during the latent phase. Latent HSV-1 was detected predominantly in the descending nuclei and mesencephalic nucleus of the trigeminal system. Latent HSV-1 was also discovered in the olfactory bulb, entorhinal cortex, parahippocampal gyrus and/or hippocampus consequent to an adventitious infection of the nasal mucosa. These studies raise the possibility that reactivation of these latently infected neurons might be related to the pathogenesis of herpes encephalitis of human adults.

1311 BLOCK IN THE TRANSPORT OF MURINE CYTOMEGALOVIRUS (MCMV) RNA IN LATENTLY INFECTED UNDIFFERENTIATED CELLS, Frank J. Dutko, Michael B.A. Oldstone*, Jeff R. Marks#, John A. Mercer# and Deborah Spector#, Sterling-Winthrop Research Institute, Rensselaer, NY, *Scripps Clinic and Research Inst., La Jolla, CA and #University of California, San Diego, CA.

We have previously shown that undifferentiated mouse PCC4 cells infected with MCMV are a model for a latent infection. Here we report that undifferentiated cells infected with MCMV produced a high amount of viral RNA which was retained in their nuclei. Undifferentiated cells were separated into nuclear and cytoplasmic fractions with NP-40 at 16 hrs after infection with MCMV. RNA was isolated from the nuclear fraction by homogenization with guanidinium thiocyanate and centrifugation through CsCl. Poly A+ RNA was isolated from the cytoplasmic fraction. Uninfected cells and control positive mouse embryo cells were processed similarly. The RNA samples were dotted onto nitrocellulose membranes and hybridized with recombinant DNA fragments of the MCMV genome. Using probes representing most of the MCMV genome, the nuclear fraction but not the cytoplasmic poly A+ fraction from the undifferentiated PCC4 cells contained viral RNA. In contrast, in MCMV-infected mouse embryo cells, the cytoplasmic poly A+ fraction contained more viral RNA than the nuclear fraction. These results show that productively infected mouse embryo cells quickly transport MCMV RNA from the nuclei to the cytoplasm resulting in low steady-state levels of virus RNA in the nuclei. In contrast, MCMV-infected undifferentiated cells did not transport viral RNA to the cytoplasm and showed a high steady-state level of viral RNA in their nuclei. The block in the nuclear-to-cytoplasm transport of MCMV RNA may be due to a defect in viral RNA processing (splicing) of viral RNA. We are currently examining this possibility

1312 MOLECULAR ASPECTS OF HERPES SIMPLEX VIRUS TYPE 1 (HSV-1) INDUCED RETINITIS, Sally S. Atherton and J. Wayne Streilein, UT Health Science Ctr, Dallas, Texas 75235

It has been shown that within one week after inoculating HSV-1 into the anterior chamber of one eye of a BALB/c mouse an acute destructive inflammatory reaction develops in both the injected and the uninjected eye. The cornea of the injected eye is destroyed completely but the retina of that eye is spared; surprisingly, the opposite pattern of destruction (retina destroyed, cornea spared) is seen in the uninjected (contralateral) eye even though the virus spreads to this eye. Since the retina of the injected eye is spared from destruction, we propose that a local factor(s) is produced within the injected eye to protect the retina. To gain insight into the virus-dependent aspects of this pathology, we have examined the post-inoculation distribution of HSV-1 quantitatively through time. At intervals after inoculating $\sim 1X10^4$ $TCID_{50}$ into the anterior chamber of one eye, animals were sacrificed and total DNA was extracted from both optic nerves, the optic chiasma, trigeminal ganglia, brain, lenses, and the anterior and posterior halves of each eye of each animal. The DNAs were quantitated, dot-blotted, and hybridized with nick-translated HSV-1 DNA. By 10-14 days after inoculation, significantly more viral DNA was present in the posterior half of the uninjected eye than in the corresponding portion of the injected eye. These data suggest that viral replication may be responsible in part for the retinal destruction observed in the uninjected eye and that continuing replication of the virus in the injected eye is down-modulated in situ by a specific factor such as interferon or defective-interfering particles. (Supported by USPHS Grant EY 05165)

1313 EXPRESSION OF HERPES SIMPLEX VIRUS (HSV)-SPECIFIED DNA-BINDING PROTEINS IN EPIDERMOID CARCINOMA OF THE GENITALIA, Gordon R. Dreesman, Kenneth L. Powell, Dorothy J.M. Purifoy, David L. Bronson, Valda N. Kaye, Elwin E. Fraley, Ervin Adam, Raymond H. Kaufman, Joseph L. Melnick; Baylor College of Medicine, Houston, TX 77030; University of Leeds, Leeds, UK; University of Minnesota Medical School, Minneapolis, MN 55455

Cancers of the cervix, vulva and penis are similar in that they are epidermoid (squamous cell) carcinomas of the genitalia. An association of these tumors with prior HSV infections has been made on the basis of seroepidemiological data. Previously we reported that an HSV-specified DNA-binding protein, infected-cell-specific protein 34/35 (ICSP34/35), is expressed in tumor tissue derived from women with cervical or vulvar carcinoma. This antigen was detected in the cytoplasm of the tumor cells by utilizing a rabbit polyclonal anti-ICSP antiserum. In this study we have extended this earlier observation and have found, by indirect immunoperoxidase staining, ICSP34/35 in tumor tissue from 5 of 6 men with epidermoid cancer of the penis. The identity of the antigen(s) was investigated with a panel of monoclonal antibodies prepared to ICSP34/35. The 5 tissues that reacted with the polyclonal antibody also gave a positive reaction with 2 of the 6 individual monoclonal antibodies tested. However, the pattern of the monoclonal reactivity differed in that small dots of staining were noted in the nucleus. These two monoclonal preparations had previously been shown to specifically react with an HSV-encoded alkaline nuclease. The nuclear reactivity detected with these monoclonal preparations also was noted in tumor tissues derived from the cervix and vulva.

1314 MODIFICATION OF HERPES SIMPLEX VIRUS (HSV) PATHOGENICITY IN MICE BY INJECTION OF ANTI-IDIOTYPE ANTIBODIES, Ronald C. Kennedy and Gordon R. Dreesman, Baylor College of Medicine, Houston, TX 77030

The in vivo administration of anti-idiotype antibodies followed by exposure to antigen has led to suppression of the idiotype (ID) associated immune response in some systems, while an enhanced response has been reported for others. We have previously prepared rabbit anti-ID to a number of IgM monoclonal antibodies to HSV type 2. BALB/c mice were injected with a pool of alum-precipitated affinity-purified anti-ID antibodies prepared to three individual IgM anti-HSV2 monoclonals. Control groups were inoculated with one of the following: pre-inoculation rabbit IgG, anti-ID to anti-hepatitis B surface antigen (anti-HBs), or saline. Fourteen days later all mice were challenged with an LD_{50} of HSV2 by the intraperitoneal route. Mice that had been exposed to the anti-ID reagent specific for anti-HSV had a significantly shorter mean survival time (8.8 days) than that of the control groups (17.4-18.1 days, $p < 0.005$). This decrease in survival time was dose-dependent in that the effect was not noted by administration of less than 5 μg of anti-ID per mouse. These observations indicate that immune mechanisms to a lethal challenge of HSV2 in mice can be altered via an ID–anti-ID network, thereby increasing the apparent pathogenicity of HSV infection.

1315 HUMAN CYTOMEGALOVIRUS LATENCY: AN IN VITRO MODEL, Linda J. Bucher, Brian Wigdahl, and Fred Rapp, The Penn. State Univ. Col. of Med., Hershey, PA 17033

The interaction of human cytomegalovirus (HCMV) with permissive human embryo lung (HEL-F) cells results in the sequentially ordered expression of the virus genome; production of immediate-early, early, and late virus-specific polypeptides; generation of infectious progeny virus; and ultimately, in cell death. As a consequence of more limited expression of the virus genome, determined, in part, by the nature of the host cell, HCMV infection may also result in transformation of the host cell, virus persistence, or virus latency. As one approach to delineate the mechanism of HCMV latency in vivo, we initially designed an in vitro model in which HCMV was maintained in a noninfectious form in HEL-F cells for 8 days by increasing the incubation temperature from 37 to 40.5°C after a 14-day treatment with human leukocyte interferon (IFN-α) and acyclovir (ACV). We now report a longer combined inhibitor treatment that extends the latency interval thereby facilitating the analysis of the latent state. HEL-F cells pretreated with IFN-α (200 IU/ml) and ACV (300 μM) were infected with HCMV (0.1 PFU/cell) and treated for 23 days at 37°C with the same inhibitor combination. Infectious HCMV was undetectable after removal of inhibitors and during an 80-day incubation at 40.5°C; no HCMV antigens were detectable during this period. As determined by infectious center analyses, 0.3 to 0.5% of the cells after inhibitors were removed contained a virus genome that could be reactivated; this value declined to 0.001% after 50 days at 40.5°C. The extension of the latency interval will permit the characterization of the HCMV genome during latency and examination of its role in transformation

1316 IDENTIFICATION BY IN SITU CYTOHYBRIDIZATION OF HUMAN CELLS INFECTED WITH HCMV, J.R. McCarrey, M.A. Churchill, M.W. Welsing, J.A. Zaia, and A.D. Riggs, Beckman Research Institute and Medical Center of the City of Hope, Duarte, CA 91010.

Human cells infected with Towne strain human cytomegalovirus (HCMV) were identified by in situ cytohybridization with a ^{32}P-labelled HCMV genomic clone. This clone contains a portion of the coding sequence for an HCMV-specific glycoprotein (HCMV-gp64) (Pande, et al., 1983) which is expressed in high abundance during the late stages of HCMV-infections monitored in vitro. Initially the effectiveness of the in situ cytohybridization technique was demonstrated on human foreskin fibroblast cells maintained in culture and infected with HCMV in vitro. A comparison of results is presented which demonstrates the specificity of the HCMV-gp64 probe to such in vitro infected cells versus similar uninfected control cells. Subsequently this technique was shown to be effective on sections of lung tissue taken at autopsy from bone marrow transplant patients who had died from HCMV pneumonia. Results are presented to show that the HCMVgp-64 probe hybridizes specifically to HCMV-infected lungs. The availability of a probe to a gene for which both the time of expression and the function of the product are known provides an extremely valuable tool. These results will be compared with those obtained from probes of known early genes to further delineate the process of HCMV infection. In addition monoclonal antibodies to HCMV-gp64 are being used to analyse expression of this gene at the protein level, thus affording a comparison of regulation mechanisms operating at the transcriptional versus post-transcriptional levels. As demonstrated here, the use of in situ cytohybridization facilitates these analyses in vivo, and allows a simultaneous analysis of and correlation with the histopathology of the infected tissue

1317 REDUCED CYTOMEGALOVIRUS (CMV) RESPONDER T CELL FREQUENCY IN CONGENITAL CMV INFECTION. Anthony Hayward and Myron Levin, University of Colorado, Denver, CO 80262

Infants with congenital CMV excrete the virus for long periods but their lymphocytes show little in vitro proliferative response to CMV antigen. We investigated the basis for this lack of response using limiting dilution cultures to enumerate CMV-specific responder T cells in blood from adults and from infants with congenital/neonatal CMV infection. The CMV antigen was prepared from infected HELF cells and stimulated a DR-restricted proliferative response by $T4^+$ cells. Immune (antibody positive) adults had 1:10,000 - 1:28,000 CMV-responsive T cells while the frequency in antibody negative adults was $<$1:300,000. The infected infants all had anti-CMV IgM antibody but their responder T cell frequency was $<$ 1:60,000. Normal responses to PHA and to alloantigen by lymphocytes from the infected infants excluded global immunosuppression as an explanation for this defect. Defective handling of CMV antigen by the infants' monocytes was also excluded by showing that these cells could present CMV antigen to maternal CMV-specific T cell blasts. One infant had a severely depleted $T4^+$ subset as a possible factor contributing to the low responder cell frequency but in the remainder the $T4^+$ subset was not reduced though the $T8^+$ cells were increased. Our results suggest that, following congenital/neonatal CMV infection, the virus specific $T4^+$ response either fails centrally or it is suppressed peripherally.

1318 DISSOCIATION BETWEEN IN-VITRO ANTIBODY PRODUCTION AND T-CELL PROLIFERATION TO HERPES SIMPLEX VIRUS IN IMMUNOSUPPRESSED PATIENTS, Patrick Tellez, Lorrie Odom and Anthony Hayward, National Jewish Hospital, The Children's Hospital and University of Colorado Medical Center, Denver, CO 80262

Children receiving anti-leukemic therapy often become susceptible to severe herpes virus infection. To investigate lymphocyte function in this setting, we studied lymphocytes from ALL patients in first remission, and receiving maintenance chemotherapy, for their HSV-specific responder cell frequency (RCF) by limiting dilution assay and their in-vitro anti-HSV antibody production using a cell culture and ELISA technique developed for this purpose. Concurrently, we determined their serum anti-HSV antibody titers and their lymphocyte response to PHA. In contrast with normal seropositive people tested, patients were found to lack a correlation between RCF (range 1:7800- 1:60,000) and in-vitro antibody production. Both normals and patient donors seronegative for anti-HSV antibody had low RCFs and no in-vitro antibody synthesis. Among the seropositive patients, all immune parameters measured varied independently of one another. This suggests that the T-cell help involved in antigen-specific proliferative responses can be impaired independently of T-cell help for specific antibody production and that diminished PHA responses need not influence either. The previously observed paradox of preserved humoral immunity in the face of impaired cellular immune function may be due, in part, to a differential sensitivity between those T-cells which may proliferate in culture and those which may provide help for B-cells, when subjected to the chemotherapy or radiation in treatment of ALL.

1319 A PROSPECTIVE STUDY OF ASSOCIATION OF HERPES SIMPLEX VIRUS INFECTION WITH CERVICAL NEOPLASIA IN WOMEN EXPOSED TO DIETHYLSTILBESTROL (DES) IN UTERO. Ervin Adam, Karen Adler-Storthz, Raymond H. Kaufman, Joseph L. Melnick and Gordon R. Dreesman, Baylor College of Medicine, Houston, TX 77030.

Nine hundred fifty nine women of middle or upper socioeconomic background, exposed to DES in utero, were periodically examined for development of cervical neoplasia during a period of five to eight years. The documentation included, in addition to data pertinent to the DES study, history of apparent oral and genital herpes before enrollment into the study and between each subsequent visit. A blood sample was drawn at the time of entry and periodically for subsequent serological studies. Twenty-eight women developed biopsy-proven cervical neoplasia of different grades during the observation period. Each of these women was matched by another DES exposed woman by method of enrollment, age and time of entry into the study. The serum samples from the case-control groups were tested for antibodies to HSV1 and HSV2 by the microneutralization test as well as by the MICRO-SPIRA test using (1) two major glycoproteins, VP123 for HSV1 and VP119 for HSV2, and (2) whole virus as coating antigens, respectively. There were no significant differences observed between women with cervical neoplasia and women in the control group, either in the initial or in subsequent sera for development of antibodies to HSV2 structural proteins. This observation is not totally unexpected since 1) we have demonstrated HSV non-structural proteins in genital tumors with absence of circulating HSV antibodies and 2) we have previously detected serum antibody to HSV non-structural proteins in women with cervical cancer.

1320 CYTOMEGALOVIREMIA IN HEALTHY ASYMPTOMATIC PREGNANT ADOLESCENTS, D. J. Lang, J.F. Kummer, S. Zweig and M.J. Lang, City of Hope Medical Center, Duarte, California 91010

Based upon clinical observations it has been deduced that human cytomegalovirus (HCMV) may be transmitted with blood. It has been estimated that approximately 5% of healthy individuals are asymptomatic carriers of HCMV in blood. Although isolation of this virus from blood is relatively easily accomplished in immunosuppressed individuals or from those with symptomatic HCMV-associated clinical conditions, efforts to recover virus from the blood of asymptomatic healthy carriers have been largely and repeatedly unsuccessful. Only one report has documented viremia in healthy individuals (in 1969 Diosi and associates reported recovery of HCMV from blood in 2 of 35 blood bank donors).

In the course of studies of healthy pregnant teenagers in North Carolina and in Maryland, HCMV was recovered from the blood in 5 of 96 and 2 of 41 subjects respectively, or 5% in each population. There was no demonstrable association with prenatal transmission of HCMV (which occurred frequently in the North Carolina study) with subject well-being or with any recognizable aspect of the course and outcome of pregnancy.

The risk of HCMV reactivation in young pregnant women may relate to gestational and endocrine factors. In addition it is possible that, as in the case of Herpesvirus hominis, reactivation of HCMV occurs most frequently soon after primary infection and that in general the risk of reactivation and transmission of this virus may be inversely related to the age of the donor-host at the time of virus acquisition. These observations may assist in defining risk factors for transfusion-related transmission as well as for reactivation and prenatal acquisition of HCMV.

1321 ANTIBODY TO HERPES SIMPLEX VIRUS TYPE-1 AND SURVIVAL OF PATIENTS WITH ORAL CANCER E.J.Shillitoe, D.Greenspan[1], J.S.Greenspan[1] and S.Silverman Jr[1]. Dept. of Microbiology, U. of Texas Dental Branch, Houston and [1]Dept. of Oral Medicine, U. of California Dental School, San Francisco.

Earlier studies showed that patients with untreated squamous cell carcinoma of the mouth have higher levels of antibody to Herpes simplex virus type-1 (HSV-1), as measured by an ELISA, than matched controls. The 70 patients in those studies were entered into a long term survival study and survivors have now completed four to five years. Patients with higher than the median level of IgG antibody to HSV-1 had a five-year actuarial survival of 76% while those with IgG antibody below the median had a five-year survival of 44%. The reverse was seen with IgM antibody to HSV-1 in that patients with levels above the median had actuarial survivals of of 56% while those with antibody below the median had actuarial survivals of 81%. No relationship was seen between high or low levels of IgA antibody (actuarial survivals of 68% and 67% respectively) or with antibody to Cytomegalovirus. When survivals were compared with pre-treatment tumor size, those with small (T1) tumors had actuarial survivals of 75% while those with large (T4) tumors had survivals of 57%. Pre-treatment assessment of antibody to HSV-1 therefore distinguished the prognosis as accurately as did tumor staging. The results suggest that some oral carcinomas are associated with HSV-1 and that these are the more aggressive tumors.

Supported by DE R01-05330 and DE K04-00088

1322 HERPESVIRUS ENCEPHALITIS IN ADULT HOMOSEXUAL MEN, Richard D. Dix, Tarvez Tucker, David Waitzman, James W. Schmidley, Richard L. Davis, Carolyn Katzen, and John Mills, University of California, San Francisco, CA 94143

HSV-1 has been shown to be responsible for a majority of adult cases of herpesvirus encephalitis. In contrast, invasion of adult central nervous system (CNS) tissue by HSV-2 or CMV is exceedingly rare. We report on three cases of atypical herpesvirus encephalitis in adult homosexual men suffering from persistent lymphadenopathy or acquired immune deficiency syndrome (AIDS). The first patient with lymphadenopathy presented with clinical signs and symptoms consistent with herpes simplex encephalitis. Biopsy of the temporal lobe revealed the presence of HSV-2. The second patient with AIDS developed a diffuse inflammatory encephalitis and died. HSV-1 was recovered at time of autopsy from spinal cord, cerebellum, temporal lobes, and frontal lobes. The third patient with AIDS complicated by perianal HSV-2 infection developed an ascending myelitis and died. HSV-2 was recovered from CNS tissue including spinal cord, brain stem, and cerebellum but not temporal lobes. Additionally, CMV was recovered simultaneously from lungs, cerebrospinal fluid, spinal cord, and brain tissue. These results suggest that AIDS and similar disturbances of cell mediated immunity in adult homosexual men may (i) increase the frequency of HSV-2 and CMV encephalitis in adults and (ii) in some cases alter the normal pathogenesis of herpes simplex encephalitis.

1323 INVESTIGATION OF THE GENES RESPONSIBLE FOR TROPISM AND VIRULENCE OF HSV, G.Darai and A.Rösen, Institute of Medical Virology, University of Heidelberg, F.R.Germany

Intertypic recombinants of HSV-1 and 2 which were generated from parental strains of HSV-1/HFEM and HSV-2/3345 (kindly provided by Dr.Halliburton, Dept.of Microbiology, Univ. Leeds, UK) were screened for their pathogenicity in tree shrew which is highly susceptible for wild-type HSV-1 and 2. It was found that HSV recombinant $RB5_0$ is pathogenic for tree shrew. In contrast no clinical pictures were observed when the animals were inoculated with HSV recombinants: $RS1_3$, $RB2_3$, $RB2_8$, $RB2_9$, $RB2_{10}$, $RB5_2$, $RB7_1$, $RB7_3$ and $RB7_7$.

The pathogenicity of parental strains of these recombinants were studied in the same system, too. These studies revealed that HSV-2/3345 is pathogenic for tree shrew similar to other wild type HSV. But in the case of HSV-1/HFEM which has a deletion of about $2x10^6$ dalton at 0.7-0.8 map units of the genome it was found firstly that this strain is apathogenic in tree shrew when the animals were inoculated i.v., i.p., and/or s.c. Secondly, that this strain is pathogenic for tree shrew when the animals were inoculated intracerebrally. Similar results were found using the BALB/c-mice system.

The state of viral latency in those animals which were infected with HSV-1/HFEM (i.v., i.p., and s.c.) was investigated. These studies revealed that this strain is unable to colonize the ganglia of tree shrew; in contrast the latent infectious virus was recovered from the spleen of latently infected animals.

This observation offers new opportunities for investigating the gene functions of HSV using the replacement of the suspected gene regions of apathogenic HSV by replacing them with the corresponding intact DNA fragments of a pathogenic HSV strain.

1324 EFFECT OF CYCLOPHOSPHAMIDE ON HSV LATENCY FOLLOWING GENITAL INFECTION IN THE GUINEA PIG, Marie L. Landry, Zhi-ming Zheng, Donald R. Mayo and G. D. Hsiung, Virology Laboratory, VA Medical Center, West Haven, CT. 06516 and Yale University School of Medicine, New Haven, CT. 06510

Guinea pigs have been used successfully as an animal model for genital herpes infection and have been especially useful in evaluating the efficacy of newly developed antiviral agents. However, it is not known what effect immunosuppression would have on HSV infected, drug treated animals. In the present study, guinea pigs were inoculated with HSV-2 strain 1868 intravaginally. Infected animals were divided into drug-treated and non-treated groups. Five drugs, acyclovir (ACV), phosphonoformate (PFA), fluoro-bdoaracytosine (FIAC), fluoro-methyl-arauracil (FMAU) and fluoro-iodoarauracil (FIAU) were used for comparison. All drugs were administered i. p. for 3 days starting on day 1 post-infection. FMAU completely inhibited the appearance of genital lesions in treated animals while untreated animals developed extensive genital lesions. In order to detect HSV latency in the FMAU treated animals, cyclophosphamide (Cy) was administered i. p., 50 mg/kg/day for 7 days, beginning 50-150 days after primary infection. With Cy treatment, 92% of FMAU treated guinea pigs had recurrent genital lesions, whereas 60% of infected, untreated guinea pigs showed recurrence. Thus, our data show that: (1) Cy increased the incidence of recurrent lesions in latently infected guinea pigs; (2) FMAU blocked the development of primary genital lesions, but it did not prevent HSV latency.

1325 CYTOSKELETAL RELATED RESPONSES TO HUMAN CYTOMEGALOVIRUS (CMV) INFECTION. T. Albrecht, J.L.H. Li, R.L. Ball, M. Nokta, W. Thompson, and D.H. Carney, The University of Texas Medical Branch, Galveston, TX 77550

Three major cytoskeletal elements have been recognized in eukaryotic cells, microtubules (MTs), intermediate filaments (IFs), and microfilaments (MFs). We have previously provided data suggesting that CMV induces a Ca^{++} influx by 6 h after virus infection (PI). Since Ca^{++} fluxes have been shown to effect the various elements of the cytoskeleton, we have examined these elements at various times after CMV infection using indirect immunofluorescence microscopy. Taxol, which stabilizes MTs against depolymerization, inhibited CMV-induced stimulation of cell DNA synthesis by up to 100%. Addition of taxol at various times PI indicated that the taxol sensitive event(s) occurred before 8 h PI, soon after the Ca^{++} influx had begun. By 24 h PI IFs were not detectable in most cells with IFA-1 antibody (which specifically decorates IFs) although after 48 h IFs were again detected. During this interval, nuclear structures indistinguishable from nuclear inclusions (NIs) were also decorated by IFA-1 and by 17BG3 (anti-vimentin monoclonal antibody). Fluorescence was absent from the nucleus of uninfected cells stained with IFA-1 or 17BG3. DNAseI has been shown to specifically decorate actin filaments. In CMV-infected cells, stress fibers were altered and NIs found to be brightly stained using this technique. When considered together, these results suggest that cytoskeletal modifications may be important cellular responses in the replication of CMV.

1326 NONSPECIFIC IMMUNE FUNCTION IN PERSISTANT EPSTEIN-BARR VIRUS (EBV) INFECTION. James F. Jones, Ruthann Kibler, Mary J. Hicks, National Jewish Hospital, Denver, CO 80206 and University of Arizona, Tucson 85724.

Immune effector cell number and nonspecific cell function were analyzed in patients with persistant/recurrent EBV infections (Clin Res 31:122A, 1983). Absolute numbers and percentages of monocytes, lymphocytes and lymphoid cell subsets were normal when examined for surface markers, morphology and enzyme content. Patient and control natural killer (NK) cell activity in whole mononuclear and enriched NK cell preparations were assayed at 10, 5, and 2.5:1 effector to K562 target ratios. Control values (n=27) were 33 $\pm$12, 23 $\pm$10, and 14 $\pm$7 % cytotoxicity, while patient values (n=11) were 18 $\pm$8, 11 $\pm$6, 6 $\pm$4 at respective ratios. The values were different at $p<0.005$, $p<0.005$, and $p<0.01$ respectively. Control values using NK enriched cells were 61 $\pm$ 13, 51 $\pm$ 17 and 42 $\pm$ 21, while patient values were 54 $\pm$ 16, 41 $\pm$ 16, and 29 $\pm$ 14, respectively; these values were not statistically different by three methods of analysis. Patient and control mononuclear preparations were stimulated with CON A/PMA and supernatants assayed for IL-2 and IFN-γ. Mean $\pm$ SD values for patient and control IL-2 were 1.17 $\pm$ 0.69 (n=16) and 2.58 $\pm$ 1.28 (n=19) Units, respectively ($p<0.001$). Patient and control IFN values were $3^{2.77}$ (n=13) and $3^{5.75}$ (n=4) Units, respectively (analysis pending larger control n). Low IFN-γ production after PHA stimulation was previously observed (patient - $3^{2.25}$ (n=8) and control $3^{4.4}$ (n=15) ($p<0.05$). Correlation of patient IL-2 and IFN-γ values yielded an R value of 0.82990, and an R^2 of 0.69973 ($p<0.005$). Whether this overall suppression is related to exaggerated control mechanisms normally seen in EBV infections or a primary defect remains to be elucidated.

1327 Cellular and humoral immune response to the late 64K glycoprotein of HCMV after natural infection in humans. J.A. Zaia, S.J. Forman, B.R. Clark, M.J. Gallagher, Y.P. Ting, E. Vanderwal-Urbina, C.L. Wright, and B.C. Racklin. City of Hope Medical Center, Duarte, CA 91010.

The late 64K structural glycoprotein of HCMV (HCMVgp64) was purified from virions plus dense body preparations by reverse phase high performance liquid chromatography (cf B.R. Clark, et al. J Virol 49: in press, 1984). HCMVgp64 was then used to evaluate the human immune response to this single polypeptide after natural HCMV infection using an enzyme-linked immunoassay (EIA) and a lymphocyte blast transformation (LBT) assay. Results were compared to assays using whole virion antigens.

The EIA utilized 20 ng HCMVgp64 coated onto 96-well microtiter plates. Antibody was detected using protein A-horse radish peroxidase conjugate and o-phenylene diamine substrate and quantitative and qualitative determinations of antibody were correlated with standard immunofluorescence, EIA and immunoblot assays for HCMV antibody. Antibody to HCMVgp64 was detectable in sera which were positive by standard anti-HCMV assays. There were no false positive reactions, and antibody was detectable late after natural infection

In the polypeptide specific LBT assay, HCMVgp64 was added to peripheral blood lymphocyte cultures from normal donors with and without prior HCMV infection. HCMVgp64 (240ng/ml) stimulated ^{3}H-thymidine incorporation 20-43 times above background and this response was as high and occasionally higher than the response to whole CMV virus antigen.

Both humoral and cellular immunity to HCMVgp64 persists late after natural infection suggesting that this protein could be important in immunity to HCMV.

1328 AN EXPERIMENTAL OBSERVATION ON ONCOGENIC POTENTIAL OF ANULTRAVIOLET INACTIVED HSV-2(W) IN MICE. Sun Yu, Chen Minhui, Chang Youxin, Chang Weiyin and Chin-min Hsiang* Laboratories of Cell Biology and of Virology, Virus Research Institute, Hubei Medical College, Wuchang, Hubei, People's Republic of China

The potential oncogenic activity of an ultraviolet inactived HSV-2(W) strain in mice was studied. The same strain of living HSV-2(W) was set in a contral group. Under the same conditions of the experiment, the oncogenic activity was of no difference between two groups within 6 months of observation. But after 6 months to one year, 12 of 48 animals (25%) in the ultraviolet inactivated HSV-2 (W) group developedcarcinomas at cervix and/or vagina, whereas in the control group, only 3 out of 45 animals (6.7%) developed carcinomas at the same areas. There was a significant difference with the rate of tumor formation between the two groups. The data suggest that the ultraviolet inactivated HSV-2 (W) could increase the potential oncogenic activity. Its mechanism was briefly discussed.

* present address: 517 Grant Place, Frederick, Md. 21701 or
Bldg 376, Ft. Detrick. Frederick, Md. 21701

1329 A ROLE FOR T LYMPHOCYTES IN HERPES SIMPLEX VIRUS-INDUCED OCULAR DISEASE, Judith A. Whittum and Robert A. Prendergast, Johns Hopkins University School of Medicine, Baltimore, MD 21205

Herpes simplex virus type 1 (HSV-1) inoculated into the anterior chamber of eyes of BALB/c mice results in a differential pattern of ocular disease in the injected and uninjected eyes: virus-injected eyes develop primarily anterior segment disease (cornea, lens, anterior chamber and iris/ciliary body) while the retinas remain essentially normal. In sharp contrast, an intense inflammatory reaction results in total retinal destruction in the opposite uninjected eyes. We have evidence that preservation of the retina in virus-injected eyes is a T cell-dependent process. Three methods of obtaining T cell-depleted mice have been utilized: 1) athymic BALB/c nu/nu mice, 2) cyclophosphamide (CY) treatment 2 days before virus presentation, and 3) sublethal irradiation (450 r) 1 day prior to virus challenge. In all three groups of experiments, T cell depletion resulted in ablation of retinal preservation in virus-inoculated eyes. Within 14 days, the retinas of both eyes were severely damaged by an inflammatory reaction. These results strongly suggest a role for T lymphocytes in retinal protection. CY-treated mice retain the characteristic of anterior chamber associated immune deviation (ACAID) in that anti-HSV delayed type hypersensitivity (DTH) responses are suppressed. These data suggest that a CY-sensitive T cell is responsible for retinal preservation, and differs from T lymphocytes which suppress DTH immune responsiveness. (Supported in part by a grant from the National Society to Prevent Blindness and a fellowship from Fight for Sight, Inc., New York City, NY)

1330 PATHOGENESIS OF MURINE CYTOMEGALOVIRUS IN NATURAL KILLER CELL-DEPLETED MICE, Jack F. Bukowski, Bruce A. Woda, and Raymond M. Welsh, UMass Medical Center, Worcester, MA

Selective depletion of NK cells by treatment with antibody to asialo GM1 (anti-AGM1) enhances virus synthesis and virus-induced hepatitis during acute murine cytomegalo (MCMV), mouse hepatitis, and vaccinia virus infections (J. Immunol. 131:1531). We report here that NK cell depletion enhanced MCMV pathogenesis in i.v. or i.p. but not intranasally inoculated mice. NK cell depletion early (0-2 days) but not late (6-8 days) after i.p. inoculation resulted in more severe and longer-lasting spleen and liver necrosis, correlating with higher (up to 1000-fold) virus titers and delayed clearance of virus. When compared to control mice, NK cell-depleted mice had significant increases in MCMV-induced suppression of the Con A response (up to 13-fold greater), spleen leukopenia, morbidity, and mortality. NK cell depletion of mice persistently infected with MCMV resulted in 6-8-fold increases in salivary gland virus titers. Athymic nude mice treated with anti-AGM1 were more susceptible to infection. Adoptive transfer of anti-thy 1.2 and C'-treated or untreated adult spleen cells protected NK cell-deficient suckling mice from lethal infection; thus, T cells do not protect. Spleen cells from anti-AGM1-treated mice did not protect. NK cell-depleted mice reconstituted with cloned NK cells (obtained from Drs. G. Dennert and J. Warner) were more resistant to MCMV infection. While MCMV infection rendered fibroblasts less sensitive to lysis by MCMV-activated NK cells, pretreatment of targets with interferon protected uninfected cells, but left infected cells susceptible to NK cell-mediated lysis. These data support the hypothesis that NK cells acting early in infection can limit the severity, extent, and duration of acute MCMV infection, and that they may also be involved in controlling persistent infection.

1331 HYPERACTIVITY RESULTING FROM A HOST RANGE MUTANT HSV-1 VIRUS. L. S. Crnic, L. J. Yamamoto,* and L. I. Pizer*. Univ. of Colo. School of Med., Denver, CO 80262

Balb/c mice were injected subcutaneously with 10^6 plaque forming units of a host range mutant herpes simplex type 1 virus in the first day of life. The mutant had been selected to grow on primate but not mouse cells in vitro and so produced a mild infection in the neonatal mice. Control groups were either injected with the vehicle (media and cell fragments) or not manipulated.

Virus was evident in the brains by 3 days, reached peak titers of 2.9×10^3 plaque forming units per brain by 21 days, and viable virus was not detectable at 60 days in most brains. Small brain and body weight deficits were apparent throughout development in the virus-treated mice but were gone by adulthood. Hyperactivity in the virus-treated mice consisted of high activity levels throughout development. At 60 days of age, the infected mice crossed an average of 144 ± 34 squares while the vehicle group crossed 76 ± 26 and the controls 67 ± 37 squares in the open field. Prior studies have shown the hyperactivity to persist into adulthood in the absence of infectious virus. The adult mice also showed deficits in passive avoidance learning (7.25 ± 2.49 trials to criterion for infected versus 4 ± 0.35 for control), although their performance on a radial arm maze was not impaired, indicating that the passive avoidance deficit was probably not due to a learning deficit but to difficulty in inhibiting behavior.

This sort of virus infection is a potential cause of the specific behavioral disorders of childhood. In addition, the selective destruction of brain areas by mild virus infections could be a useful tool for the study of brain function.

Molecular Genetics

1332 Detection and typing of Herpes Simplex virus by hybridization with synthetic oligonucleotides, Robert N. Bryan and Jerry Ruth, Molecular Biosystems, Inc. San Diego, CA 92121.

We have synthesized short (20-25 deoxyribonucleotides) DNA hybridization probes which are complimentary to either the Herpes Simplex virus Type 1 thymidine kinase gene (HSV-1 TK gene), the HSV-2 TK gene, or both. Some of these probes were synthesized with modified nucleosides which allows the subsequent addition of non-radioactive reporter groups such as biotin, fluorescein, or dinitrobenzene. Sensitivity and specificity of both radioactive and non-radioactive probes have been determined for hybridization against a variety of targets including pBR322 carrying the HSV-1 TK gene (pHSV106), DNA from HeLa S3 cells infected with HSV-1 (strain F) or HSV-2 (strain 333), and DNA from clinical samples.

1333 EPSTEIN-BARR VIRUS GENE EXPRESSION, Paul J. Farrell[1], Mark Biggin[2], Toby Gibson[2], Alan Bankier[2], Richard Baer[2], Graham Hudson[2], Graham Hatfull[2] & Bart Barrell[2]. (1) Ludwig Institute for Cancer Research, MRC Centre, Cambridge, England. (2) Laboratory of Molecular Biology, MRC Centre, Cambridge, England.

We have been characterising RNA polymerase II promoters and transcription units in the B95-8 strain of Epstein-Barr Virus. About twenty-five RNA pol II promoters have been mapped on to the viral DNA sequence. The abundance of mRNA derived from each promoter has been determined in the latent and productive (early/late) virus cycles. We have localised DNA sequences encoding a viral DNA polymerase and also identified two genes which are homologous to the Herpes simplex virus ribonucleotide reductase gene region. mRNAs encoding the surface glycoproteins gp350/220, including a spliced mRNA have been mapped. Also a spliced latent mRNA produced from the EcoR1-Dhet region has been characterised.

1334 THE STRUCTURE OF MAREK'S DISEASE VIRUS GENOME AND IDENTIFICATION OF POSSIBLE TUMOR INDUCING GENE OF THE VIRUS, Akiko Tanaka, Kunihiko Fukuchi, Miyuki Suto, Janet Donovan, Lynne Eklund, John Jessip, and Meihan Nonoyama, Showa University Research Institute for Biomedicine in Florida, 10900 Roosevelt Blvd. N., St. Petersburg, FL 33702

Twenty-seven BamHI fragments of MDV DNA were cloned into bacterial plasmids and restriction maps for BamHI, BglI, and SmaI endonucleases were constructed. On the map, as found in HSV-1 and -2, BMV, and HCMV, the inverted repeat regions were identified. SmaI digestion gave evidence of a direct repeat SmaI-M (0.9 Kb) in BamHI-F. A majority of the population had two copies of SmaI-M, some containing up to 16 copies. Hirai, et al. (Virology 115, 385-389, 1981), reported that MDV avirulent strains and attenuated strains did not contain either BamHI-D or -H, but did contain new larger fragments D1 and D2. BamHI-D and -H hybridized each other and are located at the junction between unique and inverted repeat regions. In our preparation of MDV GA strains, three types of virus with different structures of MDV DNA have been obtained: normal DNA structure with BamHI-H and -D, DNA with BamHI-H and -D1 and -D2 in place of -D, and DNA with BamHI-D1 and -D2 in place of BamHI-H and -D and two new fragments. We are examining these altered DNA structures and thus far have shown that D1, but not D2, is a fragment derived from the inverted repeat region of BamHI-D. We are in the process of testing these virus strains, all cloned from passages of the MDV GA strain, for degree of tumor induction in chicken. Since BamHI-D and -H contain the same inverted sequences, we could expect that the MDV strain with a deletion of BamHI-D may be less oncogenic and the strain with a deletion of both BamHI-D and -H may be non-oncogenic. The most tumorgenic may be the wild-type strain containing BamHI-D and -H.

1335 NUCLEOTIDE SEQUENCE OF MOUSE GENOMIC DNA RELATED TO THE TERMINAL REPETITIONS OF THE HSV-1 GENOME. Jaime Gomez-Marquez, Alvaro Puga and Abner Louis Notkins. Laboratory of Oral Medicine, National Institute of Dental Research, National Institutes of Health. Bethesda, MD 20205.

We and others have identified regions of the genome of several herpesviruses related to an evolutionarily conserved family of mammalian DNA. Several recombinant DNA clones were identified and isolated from a mouse genomic library by hybridization with cloned HSV-1 DNA fragments derived from the TR_S component. One of the homologous regions was located within a 1.1 kb SmaI fragment proximal to the domain of the a sequence. The related region in one of the mouse genomic clones was located within a 1100 bp EcoRI-HindIII fragment, which was subcloned in the M13 derivatives mp8 and mp9 in order to determine its nucleotide sequence. The fragment consists of a 210-nucleotide core of interspersed tandem repetitions of the four monomeric units GGGCAT, GGGTTA, CTGGG and TCTGGGG flanked by non-repeated sequences. The repetitive core shows a high degree of nucleotide homology with repetitive sequences within the HSV-1 SmaI fragment in a region downstream from the polyadenylation site of the gene coding for Vmw175 (ICP4). Comparison of the DNA sequence of the mouse fragment with sequences in the Los Alamos Nucleic Acid Databank gave significant scores (8-13 standard deviations above mean) with sequence elements dispersed in the immunoglobulin switch regions, likely to be involved in class-switch recombination. The presence of these sequences in the HSV genome suggests the possibility that quasi-homologous recombination events may occur between viral and host genomes.

1336 TRANS-ACTING FUNCTIONS ENCODED BY HSV RECOGNIZE CIS CLEAVAGE AND PACKAGING SIGNALS PRESENT ON HCMV DNA, Richard R. Spaete and Edward S. Mocarski, Jr., Stanford University, Stanford, CA 94305

We have constructed a chimeric plasmid containing an HSV origin of replication (class II) and a putative cleavage and packaging site from the HCMV (Towne) genome. This construct, designated pON 302, was cotransfected with standard HSV DNA as helper virus and serially passaged on BSC or HEp-2 cells. DNA was prepared from these cells, electrophoresed in agarose gels following digestion by restriction enzymes, transferred to nitrocellulose filters and probed with ^{32}P-labeled plasmid vector (pUC9) DNA. The results of these analyses show that a subset of input chimeric plasmids were authentically replicated and packaged in virions contained within HSV virus stocks in the manner previously described for defective HSV virus DNA. As such, input chimeric plasmid monomers generated concatemeric viral length molecules consisting of head-to-tail iterations of the input repeat unit. These replicated and packaged chimeric defective viral DNA molecules were cleaved to monomeric repeat units with Hind III and rescued in bacteria. A majority of the recovered plasmids were identical to the input plasmid and thus confirmed the Southern blot analysis. This study demonstrates that cis cleavage and packaging sites present on HCMV are recognized by trans-acting functions presumably encoded within the HSV genome. This is a less efficient process than when HSV recognizes its own packaging function. The study shows a functional relatedness in packaging signals which have been preserved over evolutionary time in biologically diverse herpesviruses. Hybridization analysis suggests that the corresponding cleavage/packaging sites from HSV and HCMV genomes share little or no homology.

1337 AN INSERTION MUTANT OF HERPES SIMPLEX VIRUS THAT PRODUCES A SECRETED AND MODIFIED FORM OF gD-1, Marylou G. Gibson and Patricia G. Spear, University of Chicago, Chicago, IL 60637

Sequences at the carboxy terminal end of the gene coding for glycoprotein D of herpes simplex virus type 1 (gD-1) were deleted and this modified form of the gene was inserted into the TK gene (tk) of a recombinant virus containing a type 2 glycoprotein D (gD-2). The resultant insertion mutant expressed a modified, secreted form of gD-1 as well as the normal membrane-bound form of gD-2. The plasmid used to generate the recombinant virus had the carboxy terminal end of the gD-1 gene joined at a NarI site to sequences upstream of and on the noncoding strand of the tk gene. Based on published nucleotide sequences of the gD-1 and tk genes, the fusion of the truncated gD-1 gene and upstream noncoding tk sequences should produce a protein which has lost 82 amino acids due to the deletion but gained 47 amino acids as a result of the fusion. In fact, the molecular weight of the secreted form of gD-1 is 56,500 d, in agreement with its predicted size; its partially processed form (MW 53,500 d) can be detected intracellularly. The secreted form of the protein can be found in the cell medium within 2.5 h after labeling. Secreted gD-1 is glycosylated and the secretion and complete processing of gD-1 are dependent upon processes blocked by monensin treatment. We conclude that the carboxy terminal amino acids of gD-1, including a stretch of hydrophobic amino acids, are required for anchoring of the protein into the cell membrane. Soluble forms of relatively pure gD-1 can be obtained from cell medium and are being tested in a variety of functional assays.

1338 TRANSCRIPTION OF A HUMAN CYTOMEGALOVIRUS-DNA REGION, WHICH IS CAPABLE OF TRANSFORMING RODENT CELLS. Gerhard Jahn[1], Jay A. Nelson[2], James K. McDougall[2], and Bernhard Fleckenstein[1]. [1]Institut für Klinische Virologie der Universität Erlangen-Nürnberg, W.-Germany; [2]Fred Hutchinson Cancer Research Center, Seattle, WA, USA

A cloned DNA fragment of human cytomegalovirus (HCMV) is capable of transforming NIH 3T3 and primary rat embryo cells. DNA-sequence analysis demonstrated that the viral insert was punctuated by multiple stops in all six reading frames. This sequence is located within a region that is transcribed into a 5.0 kb RNA during the immediate early (IE) phase of virus replication in the lytic system. In contrast to the other IE-transcripts, a part of the 5.0 kb RNA appeared polyadenylated, while the majority of the same transcript was found in the poly(A) pool. Also, different from the other IE-genes, the DNA coding for the 5.0 kb IE-RNA was transcribed in high quantities during the late phase of virus replication. These results suggest that transcription of the segment, which includes the transforming sequence, is exempt from the temporal regulation of HCMV transcription which is characteristic for other IE-genes.

1339 LOCALIZATION OF PSEUDORABIES VIRUS GENES USING E. COLI EXPRESSION PLASMID LIBRARIES, Alan K. Robbins, John H. Weis, Lynn W. Enquist and Roger J. Watson, Molecular Genetics, Inc., Minnetonka, MN 55343

We describe a general system to construct libraries of E. coli plasmids expressing foreign antigens. We demonstrate the utility of the expression library for localization of specific genes within the Pseudorabies virus genome. Antibodies directed against Triton X-100 disrupted PRV virions were used to make antibody affinity columns for the purification of several viral proteins. Proteins were further purified by SDS/polyacrylamide gel electrophoresis and gel slices containing these proteins used to inoculate rabbits to produce antibodies. Genomic PRV DNA was fragmented and inserted into an expression vector that expressed PRV open reading frames as proteins fused to β-galactosidase. A number of expression plasmids that specified fusion proteins that reacted in a Western blot analysis with rabbit antibodies directed against PRV proteins, were isolated. PRV DNA sequences represented in these expression plasmids were mapped on the PRV genome. PRV β-galactosidase fusion proteins produced by the expression plasmids were used to inoculate rabbits. Antibodies produced against these fusion proteins recognized PRV specific proteins. This protocol should have general application in localizing genes within large DNA virus genomes.

1340 icr ts149: AN HSV-1 MUTANT DEFECTIVE IN A LATE STAGE OF GLYCOPROTEIN PROCESSING. Bette A. Pancake, Richard A. Respess and Priscilla A. Schaffer, Dana-Farber Cancer Institute and Harvard Medical School, Boston, MA 02115. *Present address: Department of Pharmacology, UMDNJ, Rutgers Medical School, Piscataway, NJ 08854.

icr ts149 was selected for its ability to render cells resistant to immune cytolysis mediated by complement and rabbit antiserum to glycoprotein gB at 39.6°C, but not at 34°C. In addition, mutant-infected cells are resistant to cytolysis mediated by hyperimmune antiserum to KOS-infected cells - antiserum which contains antibodies to all the major viral glycoproteins. Based on its DNA$^+$ phenotype, wild-type polypeptide profile and ability to produce enveloped virions at the nonpermissive temperature, icr ts149 is a late mutant. Furthermore, although wild-type amounts of all ^{125}I-labelled glycoproteins are found at the infected-cell surface, decreased amounts of ^{14}C-glucosamine-labelled gB and gC were detected in metabolically labelled cells, suggesting that aberrantly glycosylated glycoproteins are synthesized and inserted into the surface plasma membrane. Preliminary studies of the ability of a series of lectins to bind to mutant and wild-type virus-infected cells at 39.6°C support the hypothesis that one or more viral glycoproteins is/are altered with respect to glycosylation. The ts mutation in icr ts149 has been mapped physically by marker rescue to the 1.4Kb Eco RI Q fragment, which is not known to encode viral glycoprotein genes. ts$^+$ revertants and rescuants of icr ts149 exhibit wild-type phenotypes with respect to cytolysis sensitivity and labelling with ^{14}C-glucosamine. Our results indicate that icr ts149 possesses a single ts mutation in a late viral gene affecting glycoprotein processing and consequently presentation of glycoproteins at the cell surface.

1341 VZV ENVELOPE GLYCOPROTEIN GP63: BIOSYNTHESIS AND CROSS-REACTIVITY WITH HSV, Clark M. Edson[1], Betsy A. Hosler[2], Richard Respess[3], Robert Schooley[4], David J. Waters[2], and David A. Thorley-Lawson[1]. [1]Tufts Univ. Sch. of Med., [2]State Biologic Labs., [3]Dana-Farber Cancer Inst., [4]Mass. Gen. Hospital, Boston, MA 02111.

A 63,000 dalton Varicella-Zoster virus[VZV] envelope glycoprotein[gp63] was identified in immunoprecipitation studies with mouse monoclonal antibodies to VZV. Pulse-chase labeling experiments revealed that gp63 was synthesized by proteolytic cleavage of a 125,000 dalton precursor glycoprotein[gp125]. This proteolysis was partly inhibited by growth in the presence of monensin, suggesting that the cleavage takes place during intracellular transport of the glycoprotein at the level of the Golgi. Viral material obtained from the vesicle fluid of a zoster patient contained only gp63 and no detectable gp125, indicating that the proteolysis is not merely a tissue-culture artifact.

Rabbit antisera to HSV-1 and HSV-2 both immunoprecipitated gp63 and gp125, establishing a biochemical basis for the reported serologic cross-reactivity between HSV and VZV. Comparison of the known biochemical properties of the HSV envelope glycoproteins with those of gp63, suggests that HSV gA/gB may be responsible for eliciting the cross-reactive antibodies. Results of immunoprecipitation experiments with monospecific antisera to individual HSV glycoproteins will be presented and possible roles for gp63 in VZV infection will be discussed.

1342 SEQUENCES IN HCMV WHICH SHOW HOMOLOGY TO HUMAN DNA. Richard D. Rasmussen, Silvija I. Staprans, Sydney B. Shaw, and Deborah H. Spector. University of California, San Diego, La Jolla, CA 92093.

In an initial characterization of our set of cloned EcoRI fragments from human cytomegalovirus (HCMV), strain AD169, we found that several regions in the HCMV genome hybridized to normal cell DNA. Included in this set of fragments were 7 EcoRI fragments (C,F,H,I,P,R,b) which shared limited homology with a portion of v-myc, the oncogene of the avian retrovirus MC-29. Recently we have been involved in studies to define more precisely the nature of the cell-related sequences in HCMV. In Southern hybridization experiments we have found that all of these HCMV v-myc-related sequences are more closely related to the avian retrovirus v-myc oncogene than to the human c-myc gene, an observation which is supported by DNA sequence analysis of the v-myc-related segment of EcoRI fragment R. This region of the HCMV genome is GC rich and contains a 50 bp stretch sharing 80% homology with two separate GC rich regions in the 5' half of v-myc. However, these homologous sequences in v-myc are found to be in regions of divergence between the v-myc and human c-myc genes. We have additionally found that two of the regions of HCMV which contain v-myc-related sequences (EcoRI fragments R and b) as well as at least one other HCMV fragment (EcoRI fragment d) share homology with a small region of the human DNA coding for 28s ribosomal RMA. At present we do not know if the observed homology is of any functional significance or merely a fortuitous consequence of the high GC content of these sequences.

1343 EXPRESSION OF AN IMMEDIATE EARLY HSV GENE FROM A LATE PROMOTER, Kent Wilcox and Steve Faber, Medical College of Wisconsin, Milwaukee, WI 53226.

The immediate early viral polypeptide ICP4 (or Vmw175) is synthesized within two hours after infection of animal cells by herpes simplex virus (HSV). ICP4 functions as a transcriptional regulatory protein to induce the synthesis of delayed early proteins. The net synthesis of ICP4 in a cell infected by wild-type HSV is relatively low due to a limited production of ICP4-specific mRNA. Apparently, transcription of the ICP4 gene is both positively and negatively controlled by regulatory elements located upstream from the coding sequences. We have constructed three related amplicons in which a transcription initiation site and the sequences encoding Type I ICP4 are located immediately downstram from either (1) the immediate early promoter for ICP4, (2) the late promoter for ICP5 (Vmw154), or (3) nonpromoting sequences in an adjacent procaryotic vector. Each of these amplicons has been transfected into Vero cells along with HSV-2 helper virus and serially passaged to generate defective virions containing tandem repeats of the recombinant ICP4 genes. Analyses of the synthesis of Type 1 ICP4 in cells infected with each of the three defective stocks revealed barely-detectable amounts of Type 1 ICP4 in cells infected with defective virions having either nonpromoter or immediate early promoter sequences adjacent to the Type I ICP4 gene. In contrast, a much greater amount of ICP4 was detected in cells infected with defective virions having a late promoter adjacent to the Type I ICP4 gene. Experiments to establish the kinetics of ICP4 synthesis in cells infected by these defective stocks are in progress.

1344 MAPPING OF THE RNA TRANSCRIPTS ENCODED OR PARTIALLY ENCODED BY THE BglII-N FRAGMENT OF HERPES SIMPLEX VIRUS TYPE 2 DNA, Mary K. Howett, Frank J. Jenkins, and Fred Rapp, The Pennsylvania State University College of Medicine, Hershey, PA 17033

The region of herpes simplex virus type 2 (HSV-2) DNA mapping from 0.58 to 0.62 map units (the BglII-N fragment) has been demonstrated by a number of investigators to morphologically transform cells in culture. This property has generated interest in the coding capacity of this fragment. We previously reported detection of several RNA species encoded by this DNA fragment and have now completed careful mapping of eight RNA transcripts that are fully or partially encoded by HSV-2 BglII-N DNA sequences during lytic infection of monkey cells. Cloned subfragments of this DNA region were generated and utilized for approximate mapping studies in RNA blot hybridization experiments. Subsequently, ^{32}P labeling of either the 5' or 3' end of selected DNA subfragments followed by hybridization to RNA, S1 nuclease digestion, and agarose gel electrophoresis were used to precisely locate the transcripts and to determine the direction of transcription. The similarities of the coding capacity of this region of HSV-2 DNA with published maps from the corresponding area of herpes simplex virus type 1, and a detailed map of eight transcripts ranging in size from 1.5 to 7 kilobases will be presented.

1345 A DELAYED SHUT-OFF STRAIN OF HERPES SIMPLEX VIRUS TYPE 1 (HSV-1 KOS) CAN COMPETITIVELY INHIBIT THE RAPID SHUT-OFF OF CELLULAR PROTEIN SYNTHESIS INDUCED BY THE HSV-2 186 STRAIN IN MIXED INFECTIONS, Thomas M. Hill and John R. Sadler, Department of Biochemistry, Biophysics & Genetics, University of Colorado Health Sciences Center, Denver, CO 80262

The herpes simplex viruses induce dramatic changes in cellular protein synthesis following viral infection. Certain strains of HSV characteristically inhibit the synthesis of cellular proteins very quickly (rapid shut-off) whereas other strains demonstrate a relatively slow inhibition of host protein synthesis (delayed shut-off). We have demonstrated that the rapid shut-off strain HSV-2 186 uses a virion component to induce cellular shut-off and that the delayed shut-off strain HSV-1 KOS requires viral protein synthesis to initiate cellular shut-off. In mixed infections of Friend erythroleukemia cells with HSV-2 186 and HSV-1 KOS, the HSV-1 strain antagonizes the HSV-2-induced rapid shut-off of host protein synthesis. Exclusion of the HSV-2 strain by HSV-1 is not the cause of the observed interference: Southern blot analysis of HSV-1 x HSV-2 infected cell nuclear DNA preparations shows that the amount of HSV-2 DNA reaching the cellular nucleus at 1.5 hours post-infection is identical in either mixed or single infections. UV-irradiated HSV-1 KOS is also capable of preventing the rapid shut-off of cellular protein synthesis by HSV-2 186, suggesting that a virion component of the HSV-1 strain is responsible for the observed viral interference. We postulate that HSV-1 KOS possesses a virion component which competes for the same target site within the cell as the HSV-2 186 rapid shut-off component, but that the HSV-1 component is not capable of rapidly inhibiting cellular protein synthesis under our conditions of infection.

1346 REGULATED HIGH-LEVEL EXPRESSION OF THE HERPES SIMPLEX TYPE 1 THYMIDINE KINASE GENE IN E. COLI, Joan L. Betz, David L. Hare and John R. Sadler, University of Colorado Health Sciences Center, Denver, CO 80262

A plasmid cloned Herpes Simplex Virus type 1 (HSV-1) thymidine kinase gene (TK) was expressed in E. coli by inserting a 203 bp lac UV5/L8 promoter-operator segment, in frame, 53 nucleotides 5' to the native TK translational start codon. The hybrid gene resulting from this construction encodes a polypeptide which has 25 additional amino acids on the amino terminus of the HSV TK protein. The fusion polypeptide phenotypically complements a tdk- mutation of E. coli. We have demonstrated that the fusion polypeptide contains the HSV-1 TK sequences by maxicell, immunoprecipitation and native gel electrophoretic techniques. Activity of the fusion TK product is completely inhibited by anti-HSV-1 antiserum, whereas the activity of E. coli TK is unaffected by the antiserum. When the recombinant plasmid is in a strain containing a $lacI^q$ gene (overproduction of lac repressor), the inducer galactoside IPTG causes a greater than 1000-fold coordinate induction of the plasmid-encoded TK and the chromosomal lacZ (β-galactosidase) activities. The fusion TK polypeptide was shown to be as stable as β-galactosidase by pulse-chase induction experiments. HSV TK-specific RNA isolated from the bacterial strain has a short half-life, similar to many bacterial messages.

1347 MOLECULAR MECHANISM OF PROVIRAL MuLV AMPLIFICATION IN HUMAN LYMPHOMA LINES BY EPSTEIN-BARR VIRUS, Frederic A. Troy and John Menke, Univ. of Calif., Davis, CA 95616

To examine the molecular mechanism whereby EBV-conversion amplifies proviral MuLV antigens in human lymphomas (Lasky, R.D. and Troy, F.A. (1984) PNAS, In Press), DNA restriction mapping analyses of Ramos lymphoma lines and its EBV-converted sublines have been initiated. The MuLV copy number, methylation-demethylation events and insertional activation of MuLV by EBV-integration into the lymphocyte genome have been examined. Using EBV DNA fragments BamH1A, BamH1C and EcoRI DJ_{het} cloned in pBR322 and MuLV clone 4070A in pBR322 to map the location of the two viruses within the chromosomal DNA of Ramos converted lines, we have determined that: 1)The EBV clones only hybridized to the EBV-converted lines of Ramos and BJAB and not to their EBV-negative parental lines; 2)Increased copy number of the MuLV provirus does not appear to be the mechanism of MuLV amplification since Ramos and AW-Ramos both contain ca. 8 proviruses; 3)Use of methylation sensitive restriction enzymes indicated that methylation of lymphoid DNA does not appear to be the cause of amplification; 4)Chromosomal DNA fragments have been located that contain both EBV DJ_{het} probe and the MuLV sequences. AW-Ramos contained coincident bands with both the EBV DJ_{het} probe and the MuLV whole virus clone. Coincident bands for AW-Ramos, EHRA-Ramos and Ramos-B95-8 were also seen using restriction endonuclease digestion. We interpret the large number of coincident bands to indicate the likelihood that EBV integrates close to a MuLV provirus. Further studies will be required to confirm that the two viruses are on the same fragment and to more accurately determine the distance between EBV and MuLV.

1348 VIRUS SPECIFICITY OF THE GENE FRAGMENT CODING FOR THE 64,000 DALTON GLYCOPROTEIN OF HUMAN CYTOMEGALOVIRUS (HCMV). Margaret A. Churchill, Hema Pande, Steven W. Baak, and John A. Zaia. Beckman Research Institute of the City of Hope, Duarte, CA 91010.

A 64-66K dalton glycoprotein (HCMV-gp64) is present in relative abundance during the late stages of infection with HCMV (Towne strain). A cloned 800 base pair fragment, which codes for a portion of this protein, was used as a probe for HCMV-specific DNA sequences in infected cells. This fragment maps from 0.5 to 0.51 map units in the HCMV (Towne) genome. This portion of the genome has not been reported to be homologous to either cellular or to onc genes. We investigated whether the cloned fragment would hybridize to extracts from various herpesvirus-infected or uninfected cells. The 800 base pair fragment was ^{32}P-labeled and used as a probe in a dot blot assay. Hybridization was detected with extracts from cells infected with three laboratory strains of HCMV (Towne, AD169, and Davis) and with all clinical isolates of HCMV tested to date. The cloned fragment did not hybridize to extracts from cells infected with herpes simplex virus types 1 and 2, varicella zoster virus, Epstein-Barr virus, several simian cytomegaloviruses, and uninfected cells. Since the fragment coding for HCMV-gp64 hybridizes specifically with nucleotide sequences found in HCMV-infected cells, this probe can be used to differentiate HCMV from other herpesviruses.

1349 DIFFERENT PATTERNS OF EBV-DETERMINED PROTEINS SYNTHETIZED DURING REPLICATIVE INFECTION IN NORMAL CELLS AND AFTER INDUCTION OF RAJI CELLS, F. Sinangil and D.J. Volsky, UNMC, Dept. of Pathology and Eppley Inst. for Cancer Res. Omaha, NE. 68105.

Lytic infection of rodent lymphocytes by EBV, following implantation of viral receptors, permits studying viral replicative cycle in normal cells. Mouse or rat splenocytes were isolated, implanted with EBV receptors and infected by EBV as previously described. 5-10% of cells expressed the early (EA) and virus capsid (VCA) antigens 48 hrs after the infection. The cells released infectious viral particles but were not immortalized. EBV-determined proteins were identified by SDS-PAGE of immunoprecipitates obtained from extracts of (^{35}S)-methionine-labelled EBV-infected cells. 26 EBV-specific proteins ranging from 15 to 168 kd were detected 24 h after infection. 27, 44, 63, 72 and 87 kd proteins were dominant. Similar analysis of chemically-induced or superinfected Raji cells yielded a completely different polypeptide pattern, including 19 EBV-specific proteins ranging from 19.5 to 355 kd, with dominant bands at 37, 49.5, 58, 70, 90, 125, 152 and 170 kd. Most notable difference was the predominance of a 44 kd band in mouse and rat cell extracts, and lack of polypeptides over 168 kd. Since at least two proteins present in Raji cell extracts, 260 and 355 kd, are components of the viral capsid, our results indicate that the assembly of new EBV virions in normal cells was not completed 24 h after primary infection. The patterns of EBV-specific proteins in mouse and rat lymphocytes, but not in Raji cells, were identical, indicating that we were detecting the actual translational products of the infecting viral genome. These findings are being confirmed by electron microscopy, pulse-chase experiments, and use of metabolic inhibitors and DNA synthesis blockers. Our approach permits analysis of the transcriptional and translational products of different EBV strains in normal cells and kinetic study of the viral reproductive cycle.

1350 EXPRESSION OF EBV DNA AND CLONED DNA FRAGMENTS IN FRESH HUMAN B LYMPHOCYTES: EBV ANTIGENS INDUCTION AND EFFECT ON CELL TRANSFORMATION. D.J. Volsky, T. Gross, R. Bartzatt, C. Kuszynski, B. Volsky and F. Sinangil, UNMC, Dept. of Pathol. and Eppley Inst. for Cancer Res., Omaha, NE. 68105.

Identification of Epstein-Barr virus (EBV) genes responsible for inducing lymphocyte immortalization and contributing to the process of lymphomagenesis has been hampered by the lack of viral mutants. In an alternative approach, we study the expression of purified EBV DNA and cloned DNA fragments in normal human lymphocytes (HL). DNA is introduced into HL by our recently developed gene transfer method based on reconstituted Sendai virus envelopes (RSVE). We report here that the RSVE-tranferred purified EBV DNA (B95-8 strain) induced EBV-nuclear antigen (EBNA) in 0.2-1% of HL 48 h after transfer. The viral DNA also induced 5-15-fold increase in cellular DNA synthesis, however, cell immortalization was not achieved. Cloned, RSVE-transfserred SalIF-1 (9 kbp) and a smaller BamHI K (5.2 kbp) fragment of B95-8 EBV DNA also induced EBNA in up to 4% of cells. However, no stimulation of cellular DNA synthesis or promotion of cell immortalization was detected with these fragments, suggesting that the EBNA-coding gene(s) is not an autonomous transforming gene of EBV. In contrast, 9 kbp BamHI D-1 (AG876 virus), or combination of BamHI X and H (B95-8 virus, 2 and 7 kbp, respectively) fragments of EBV DNA significantly stimulated HL DNA synthesis. No EBNA-positive HL were detected, and immortalization was not achieved. The antigens associated with the lytic cycle of the virus, EA and VCA, were not observed after DNA or any fragment transfer. These results suggest that cell transformation by EBV requires collaboration between several viral and perhaps cellular genes. Our approach will be useful for analysing these interactions.

1351 STIMULATION OF DNA SYNTHESIS IN MOUSE LYMPHOCYTES AFTER INITIATION WITH CHEMICALS AND SUBSEQUENT INFECTION WITH EBV. C. Kuszynski and D. J. Volsky, Dept. of Pathol. & Lab. Med. and the Eppley Inst. for Cancer Res., UNMC, Omaha, NE. 68105.

Cell transformation by EBV is restricted to mature human B lymphocytes. This host cell restriction, and the lack of viral mutants and permissive system for virus replication, have made study of EBV difficult. We have approached this problem by attempting chemical/viral transformation in mouse lymphocytes (ML). The cells were implanted with functional viral receptors and infected by intact EBV as previously reported. Full viral lytic cycle rather than cell transformation was observed, suggesting lack of mouse cell control over the EBV life cycle. To investigate whether this mode of EBV-host cell interaction can be altered, we pretreated ML in vitro with known chemical initiators, dimethylbenzanthracene (DMBA), methylnitrosourea (MNU) and N-methyl-N'-nitrosoguanidine (MNNG). The pretreated cells were receptor-implanted and infected by EBV. Cell proliferation was tested by (^{3}H)-thymidine incorporation into total cellular DNA. Cell growth was monitored for 4-6 weeks after the chemical/viral treatment. Our results show that chemical pretreatment increased the proliferation of EBV-infected ML 10-15 times above the control. Addition of the virus at various times after initiation also had a stimulatory effect on proliferation, with 24-48 hours post chemical yielding maximum increase in ML DNA synthesis. Addition of P3HR-1-substrain of EBV prior to B95-8 substrain further increased the stimulation. The life span of cells in culture was prolonged. The effect of these manipulations on EBV lytic cycle in mouse lymphocytes is now under investigation. Since increased cellular DNA synthesis is required for cell tranformation by the virus, the observed stimulation, if maintainable, may yield EBV-transformed mouse cells.

1352 INTRODUCTION OF EBV DNA INTO LYMPHOCYTES USING RECONSTITUTED SENDAI VIRUS ENVELOPES (RSVE): QUANTITATIVE AND QUALITATIVE DEFINITION OF THIS GENE TRANSFER SYSTEM. R.L. Bartzatt, F. Sinangil and D.J. Volsky, Dept. of Pathology & Eppley Inst., UNMC, Omaha, NE. 68105.

The known DNA-transfer techniques cannot efficiently introduce isolated EBV DNA and subgenomic fragments into lymphocytes. We have recently developed gene transfer method based on reconstituted Sendai virus envelopes (RSVE). DNA is trapped inside RSVE during envelope reconstitution and transferred into cell interior by vesicle-cell fusion. Because of the increasing importance of DNA transfer for functional analysis of eucaryotic genomes, and for gene therapy in the future, we have undertaken analysis of the efficiency of the RSVE-mediated EBV DNA transfer compared to other DNA transfer methods. Using a calcein fluorescence quenching procedure we determined the volume of the internal aqueous compartment of RSVE. The size of the compartment, or trapping volume of RSVE, depended on the concentration of Triton X-100-solubilized SV envelope proteins, reaching about 4% of the total volume of the system with 2 mg SVE protein/ml. Further increase in the trapping volume, to 17% and more, could be achieved by concentrating the RSVE 4-5-fold, followed by solubilization and reconstitution. The proportion of DNA trapped within RSVE depended on the size of the genome. While about 3% of the (^{3}H)-labelled cloned EcoRI B fragment of EBV DNA (33 kbp) added to the reconstitution mixture (50μ g DNA per 2 mg SVE protein/ml) was unaccessible to DNAse after reconstitution, indicating entrappment within RSVE, only 1.5% of intact EBV DNA (approx. 170 kbp) was trapped under the same conditions. The efficiency of the transfer varied, as judged by the expression of intact EBV DNA and EBNA-coding fragment, between 0.2% and 4%. These studies will permit optimization of the RSVE-mediated EBV DNA transfer into lymphocytes.

1353 TRANSFORMATION OF NORMAL HUMAN LYMPHOCYTES BY COINFECTION WITH UV-INACTIVATED EPSTEIN-BARR VIRUS (EBV) AND PURIFIED EBV DNA. T. Gross, R. L. Bartzatt, and D. J. Volsky, UNMC, Dept. of Pathology & Eppley Inst. for Cancer Res. Omaha, NE. 68105.

EBV has the capability of efficiently transforming human B-lymphocytes in vitro. The transforming activity and its relation to the genesis of Burkitt's lymphoma, are poorly understood. In an attempt to evaluate the interaction between EBV and the natural host cell genome, we have applied Sendai virus (RSVE)-mediated gene transfer to introduce purified EBV-DNA and cloned DNA fragments into human lymphocytes (HL). We found that EBV DNA, isolated from B95-8 cells and RSVE-transferred into HL, induced EBNA in 0.2-1% of cells and stimulated cellular DNA synthesis, as measured by (^{3}H)-thymidine incorporation. However, these effects were transient and cells were not immortalized, indicating that purified EBV DNA lacked the capability to transform HL. Conceivably, certain component(s) of the virion that is normally carried in by intact virus during infection might be required for cell transformation. To evaluate this possibility, we UV-irradiated the B95-8 virus to inactivate viral DNA, and used it for coinfection with RSVE-transferred purified B95-8 virus DNA. Cells expressed EBNA, increased cellular DNA synthesis, and transformation was achieved. One of the established cell lines, NEB-1, has been kept continuously in culture for more than 3 months, and is submitted to detailed molecular analysis. Similar coinfection experiments were also performed using UV-inactivated nontransforming P3HR-1 EBV substrain. Although blastogenesis, increased cellular DNA synthesis, and cell clumping were observed, no immortalized cell lines were obtained in these experiments. Using the coinfection with UV-inactivated tranforming virus, cloned fragments of EBV DNA and DNAs of different EBV strains are now studied to understand the requirements for cellular tranformation by EBV.

1354 GENETIC ANALYSES OF HSV-1 dDNA LAMBDA AND PLASMID CHIMERIC MOLECULES PROPAGATED IN EUKARYOTIC CELLS, Susan E. Bear[1], Anamaris M. Colberg-Poley[2], Barrie Carter[2] and Lynn W. Enquist[3], [1]National Cancer Institute, FCRC, Frederick, Md. 21701; [2] National Institute of Arthritis, Diabetes and Digestive and Kidney Diseases, NIH, Bethesda, Md. 20205; [3]Molecular Genetics Inc., Minnetonka, Minn. 55343.

Defective interfering particles(dDNA) of HSV-1 have been recognized as possible eukaryotic cloning vectors for large fragments of DNA. Inclusion of a prokaryotic replication origin into dDNA creates a chimeric DNA molecule with propagation potential in prokaryotic and eukaryotic cells. We examined two chimeric molecules, each containing the same 9.5 kb class I dDNA fragment(HSV 12-7) but a different prokaryotic replicon: 1)a recombinant DNA molecule of dDNA and the lambda vector λgtWES·λB, and 2)a smaller hybrid molecule of dDNA and the plasmid vector pBR325. Transfection of circularized monomer-chimeric molecules into Vero cells and infection with HSV-1 helper virus resulted in the amplification and packaging of the chimeric molecules into infectious HSV particles. However, restriction endonuclease analysis of this virion DNA from early passages showed extensive novel rearrangements in the λ DNA. Some novel rearrangements also appeared in the propagated pBR325::12-7 chimeric molecule. The amplified pBR325::12-7 DNA could transform E. coli to ampicillin and tetracycline resistance. However, the λgtWES::12-7 DNA isolated from HSV virions could not be successfully recovered in bacteriophage particles by in vitro packaging of HSV virion DNA.

1355 CHARACTERIZATION OF THE HSV 1 GLYCOPROTEIN gB GENE, Rae Lyn Burke, Carol Pachl, Richard Najarian, Chiron Corporation, 4560 Horton Street, Emeryville, CA 94608

We have cloned and sequenced the gene for glycoprotein gB from HSV 1 (strain Patton) The gB coding region contains those elements characteristic of a membrane glycoprotein including an NH_2-terminal signal sequence, N-linked glycosylation sites, and a hydrophobic anchor region near the COOH-terminus. The identity of the gB sequence has been verified by hybrid selection and translation of the mRNA followed by immunoprecipitation with a gB-specific monoclonal antibody.

Glycoprotein B has been purified from HSV 1-infected cells by immunoaffinity chromatography followed by preparative gel electrophoresis. Sequence analysis of this protein using automated Edman degradation has been performed to determine the NH_2-terminus of the mature protein and to verify the nucleic acid sequence analysis. Experiments to express this glycoprotein in yeast and mammalian expression systems will be presented.

1356 TEMPERATURE-SENSITIVE MUTANTS OF HSV-1 DEFECTIVE IN A LATE REGULATORY FUNCTION, Neal A. DeLuca, Moira A. Courtney and Priscilla A. Schaffer, Dana-Farber Cancer Institute and Harvard Medical School, Boston, Massachusetts 02115

A large number of temperature-sensitive (ts) mutants of HSV-1 in the gene encoding the immediate early transcriptional regulatory protein, ICP4, have been isolated and characterized with respect to the expression of the immediate early (IE), early (E), and late (L) viral gene products. The hallmark of these mutants is the overproduction of IE, and the underproduction of E and L gene products. The present study involves the preliminary genetic and molecular characterization of two unique regulatory mutants of HSV-1, ts48 and ts303. Both mutants exhibit inefficient complementation with 8 ts mutants in complementation group 1-2 which defines the gene for ICP4. Marker rescue experiments place the mutation in ts48 in the 3' portion of the coding sequence for ICP4. SDS-PAGE analysis of ts48- and ts303-infected cell polypeptides synthesized at the nonpermissive temperature demonstrates that IE polypeptides ICP4 and ICP27 are overproduced with the simultaneous production of E polypeptides ICP6, ICP8, gB, ICP25, ICP29 and others. Late gene products, however, are either absent or underrepresented under similar conditions. 20-60% of wild-type levels of viral DNA are synthesized at the nonpermissive temperature in ts48- and ts303-infected cells indicating that IE and E gene functions are intact (or nearly so) and that the block in ts48 and ts303 is in a regulatory event subsequent to that exhibited by other mutants in group 1-2 which are DNA^-. Thus, the phenotypic properties of ts48 and ts303 are distinctly different from those of other mutants in ICP4 and suggest a multifunctional role for this important regulatory protein.

1357 STUDIES OF CIS-ACTING REPLICATION FUNCTIONS OF HERPES SIMPLEX VIRUS DNA. Niza Frenkel, Louis P. Deiss and Richard R. Spaete. University of Chicago, Ill. 60637.

We have used the herpes simplex virus (HSV) amplicon system to study cis-acting signals in the replication of HSV DNA. By assaying the ability of deleted versions of seed amplicons to propagate into defective genomes in the presence of helper virus DNA we have mapped three replication origins within the standard HSV genome. The firsttwo origins (ori-1 and ori-1') have been localized within the c inverted repeat sequences, whereas the third origin (ori-2) has been mapped within a 750 bp segment around coordinate 0.40. Ori-2 sequences are deleted during cloning in bacteria but are restored during transfections with helper virus DNA by an efficient recombinational event within the 300 bp sequences flanking the deletion. We have mapped the cleavage/packaging signal of HSV DNA and the signal for a amplification to within 250 bp sequences of the a sequence. Cotransfection of cells with amplicons containing duplicated sequences in direct or inverted orientations results in homologous recombinations leading to deletions and inversions. These homologous recombinations occurred regardless of the type of sequences involved in as much as inversions and deletions were observed when the duplicated sequences were derived from the S-L junctions, portions of the tk gene or pBR-322 DNA. The relevance of these recombinations to the inversions of the S and L components in standard virus DNA is currently under investigation.

1358 DRUG-RESISTANT HERPESVIRUS MUTANTS: ISOLATION AND UTILITY, Donald M. Coen, Department of Pharmacology, Harvard Medical School, Boston, MA 02115

Drug-resistant virus mutants are valuable for several reasons including 1) assessment of the likelihood of resistance and cross-resistance in the clinic, 2) analysis of the mechanism of action of antiviral drugs, 3)identification and functional subdivision of viral genes, and 4) facilitation of the isolation and characterization of mutants affecting viral gene expression. Each of these rationales can be illustrated by the results of studies of herpes simplex virus (HSV) mutants resistant to acyclovir (ACG), arabinosyladenine (araA), and/or phosphonoacetic acid (PAA): 1) These mutants can be isolated in a single step by plaque isolation under drug-containing overlays. (Repeated plaque purification has proven essential to avoid mixed populations and to recognize mutants exhibiting less common phenotypes such as ACG-resistance due to polymerase (pol) mutations.) This suggests possible clinical problems while the mutants' properties suggest possible clinical solutions. 2) Pol gene mutations can confer resistance to any of these three drugs (in the case of araA, in the presence or absence of adenosine deaminase inhibitors) implying that polymerase is a target for the drugs. 3) The pol gene was initially identified with the aid of PAA-resistant mutants. Pol mutations conferring varying sensitivities to drugs which mimic polymerase substrates have been mapped to distinct sites in the pol gene, providing a beginning for dissecting functional sites on the polymerase molecule. 4) A cloned mutant thymidine kinase (TK) gene conferring conditional resistance to ACG has been used to incorporate altered TK promoters into HSV without selecting for their effect on TK expression. The relative effect of such altered promoters can be assayed by examining the relative ACG-sensitivity of mutant isolates.

1359 EARLY GLYCOPROTEINS SPECIFIED BY HERPES VIRUSES, Vikram Misra and Christine Baugh, Department of Vet. Microbiology, W.C.V.M., University of Saskatchewan, Canada S7N 0W0

Herpes viruses temporally control the expression of their genes such that viral proteins can be categorized as 'immediate early', 'early' or 'late'. We have demonstrated that gVPII, a glycoprotein of the Bovine herpes virus-1 is an 'Early' protein and that at least one glycoprotein specified by field isolates of herpes simplex viruses may fall into this category. These glycoproteins are of interest since they are made early in infection and are the only immunologicaly important viral markers expressed on the surface of virus infected cells in the absence of viral DNA replication. Studies on the characterization of these proteins and their interaction with the immune system will be presented.

1360 A CIS-ACTING ELEMENT OF THE EBV GENOME THAT PERMITS PLASMID REPLICATION, J. Yates, N. Warren, D. Reisman, and B. Sugden, University of Wisconsin, Madison, WI 53706.

DNA fragments representing 99% of the EBV genome were cloned onto a plasmid vector pKan2 which expresses resistance to the antibiotic G418 when introduced into mammalian cells. Using $CaHPO_4$ precipitation, we introduced the recombinant plasmids into EBV genome-positive and -negative cells. pBamC, pKan2 containing the Bam C fragment of EBV, yielded 10-100 times more G418-resistant colonies than did any other plasmid when tested in the EBV genome-positive cell lines D98/Raji or D98/P3HR1. With the EBV genome-negative cells 143 or Balb3T3 neither pBamC nor any other recombinant plasmid gave markedly more G418-resistant colonies than did the vector, pKan2.

Clones of D98/Raji transfected with pBamC contained 5-20 copies of pBamC plasmid per cell, as determined by Southern blot analysis of Hirt extracts. Cutting with methyl-sensitive endonucleases showed that the plasmid had been replicated. Unaltered pBamC plasmid could be recovered in E. coli transformed with the Hirt supernatants.

Deletion analysis of pBamC revealed that 1790 bp to the left of EcoRI J are sufficient for stable plasmid replication. An 800 bp region composed of a family of 30 bp direct repeats and a 250 bp region containing elements of dyad symmetry about 1 kbp away are both required for function in our assays.

We refer to the identified cis-acting element(s) as oriP, for origin of plasmid replication. Our experiments suggest that EBV expresses trans-acting products that act on oriP to control replication and/or maintenance of EBV plasmids in transformed cells.

1361 INSERTIONAL MUTAGENESIS OF THE HSV-1 GENE ENCODING THE dUTPASE. Valerie G. Preston and Fiona Fisher, Inst. of Virology, Church St. Glasgow,UK.

A novel approach was used to identify the gene encoding the HSV-1 dUTPase. The entire immediate-early promoter or the far upstream regulatory sequence of the Vmw175 (ICP4) gene was inserted in front of genes located between map units 0.67 and 0.68 and the plasmids screened for the ability to induce dUTPase activity in a transient expression assay. One clone, containing several genes, induced high levels of enzyme activity. The specific gene was identified by linker insertion within its coding sequences.

1362 ALTERED EXPRESSION OF MHC GENES IN HSV AND OTHER TRANSFORMED CELLS. Robin A. Robinson, Mary L. Hedley. Sharon Mc Millan, and Jim Forman. Univ. Texas Health Sci. Ctr. Dallas, Dallas, TX 75235.

One means by which viral tumor genes may mediate the transforming phenotype is regulation of the expression of specific cellular genes. Several murine strains of primary and 3T3 cells transformed morphologically by herpes simplex virus types 1 & 2 (HSV-1, -2), adenovirus (Ad) types 5 & 12, or SV40 & polyoma were examined for the expression of major histocompatibility gene (MHC) transcripts and polypeptides with respect to the parent nontransformed cells. Enhanced levels (> 10 fold increase) in transcipt abaundancies under steady state conditions of several MHC class I genes were noted in HSV-, SV40-, Ad 12, and Py transformed cells as compared to the nontransformed cell transcript levels. HSV-1 and Ad 12 transformed, as well as infected, cells exhibited reductions in the steady state levels of several other MHC genes. A model for the role of regulation of MHC expression by viral tumor genes during transformation and other viral genes during productive infection will be presented.

Defenses Against Viruses

1363 FINE MAPPING OF HSV GLYCOPROTEIN D EPITOPES USING MONOCLONAL ANTIBODIES. Roselyn J. Eisenberg, Michael Nobel, Manuel Ponce de Leon, Deborah Long, Valerie Rinaldt and Gary H. Cohen, University of Pennsylvania, Philadelphia, Pennsylvania 19104.

Previously, we used a panel of 17 monoclonal antibodies to define eight antigenic determinants of gD, based on differences in neutralization, immunofluorescence and immunoprecipitation assays. Recently, we localized and synthesized one antigenic site of gD (Group VII), corresponding to residues 8-23 of the mature glycoprotein. A second site (Group V) has been localized to within the last 30 residues of the protein. Thus, two of the eight groups of monoclonal antibodies have been fine-mapped with respect to the structure of gD. A number of laboratories have developed additional monoclonal antibodies to gD-1 and gD-2. The following laboratories have agreed to co-operate in this joint effort: A. Minson, H. Marsden, P. Spear, S. Marlin, W. Rawls, S. Jeansson, H. Friedman, M. Zweig and L. Pereira. The object of this investigation was to relate these antibodies to the original grouping, and to determine whether there are additional antigenic determinants on the glycoprotein. A dot-blot assay was used for characterization. A series of antigens representing native and denatured gD-1 and gD-2, as well as fragments of the molecule, were spotted onto nitrocellulose and reacted with antibody and then iodinated protein A to determine their reactivity. Thus far, we have used this technique to identify additional monoclonal antibodies that fit within our original grouping, but we have not yet identified additional groups. One antibody (from H.F.),is virtually identical in the dot-blot assay to antibodies in Group VII.

1364 PROTECTIVE ROLE OF MATERNAL CYTOMEGALOVIRUS SPECIFIC ANTIBODY FOR NEONATAL GUINEA PIGS, Brigitte P. Griffith, Jacquelyn T. Lavallee and G.D. Hsiung, Yale University School of Medicine, New Haven, CT and VA Medical Center, West Haven, CT. 06516

The capacity of cytomegalovirus (CMV) specific antibody acquired transplacentally, through breast milk feeding or by passive transfer, to protect newborn guinea pigs against severe disease due to CMV was assessed. Seropositive mothers with significant levels of CMV antibody prior to pregnancy were selected for study. Neonates born to seropositive and seronegative mothers were cross-fostered for 10 days and then challenged with virulent CMV. Ten days after virus challenge, babies in both groups were evaluated for the extent of viral infection, hematological changes, spleen size and neutralizing antibodies. Higher levels of CMV specific antibody were detected in babies born to seropositive mothers. Neonates (N=10) born to seropositive mothers and nursing on seronegative mothers developed a mild subclinical infection, and no deaths occurred. In contrast, 3/10 neonates born to seronegative mothers and nursing on seropositive mothers died. The remaining animals in that group had severe CMV infection, anemia, lymphopenia and enlarged spleens. In separate experiments, anti guinea pig CMV serum, normal guinea pig serum or PBS was administered to newborns from seronegative mothers, once a day for 5 days. When challenged with CMV, deaths and generalized CMV infection occurred only in the babies not treated with CMV specific antiserum. These results suggest that acquisition of CMV specific antibody either from mothers or by passive transfer confers on neonatal guinea pigs protection against death and severe disease due to CMV infection.

1365 Monoclonal antibodies against varicella-zoster virus(VZV). K.Yamanishi,T.Okuno, H. Campo-Vera, J.Namazue, K.Shiraki, and M.Takahashi. Osaka University, Osaka, Japan

VZV contains four major glycoproteins designed as gp 1(115K),gp 2(106-80K),gp 3(64K) and gp 5 (55K). Thirty-eight hybridomas secrecting monoclonal antibodies against VZV proteins have been established. Nineteen clones of these thirty eight hybridomas produced antibodies to glycosylated polypeptides of VZV, which could be classified into three groups on the basis of the electrophoretic patterns. The first group reacted specially with polypeptides with apparent molecular weights of 94K and 83K(both presumed to correspond to gp 2)in infected cells, on the surface membrane of infected cells, and on the virion. The second group precipitated polypeptides with molecular weight of 94K,83K and 55K(corresponding to gp 5)in infected cells, and 94K 83K 55K,45K on the surface membrane of infected cells and 94K,83K and 55K on the virions. Finally the third group reacted with polypeptides with molecular weights of 116K,106K and 64K(corresponding to gp 3)in infected cells, 64K on the surface membrane of infected cells, and on the virion. By pulse-chase experiments, antibodies from former two groups precipitated new polypeptides with molecular weights of 75K and 49K, respectively which were presumed to be precursor proteins. It was also found that a glycoprotein of 45K was detected in culture fluid of infected cells. Tunicamycin and endoglycosidaseH were used for oligosaccharide analysis. The fully processed forms of gp 2 had complex type N-linked, and O-linked oligosaccharides. The mature form of gp 3 contained both complex and high-mannose type N-linked oligosaccharides and that of gp 5 had only complex type N-linked oligosaccharides. Monoclonal antibodies of all groups had complement dependent cytolytic activity, and only antibodies against gp 2 could neutralize infectivity in the presence of rabbit complement.

1366 SUPPRESSION OF NK ACTIVITY AND T CELL PROLIFERATION INDUCED BY FRESH ISOLATES OF CYTOMEGALOVIRUS. Rachel D. Schrier, George P.A. Rice and Michael B.A. Oldstone, Scripps Clinic and Research Foundation, La Jolla, California 92037

Human cytomegalovirus (CMV) infections are commonly associated with immunosuppression. However, much of the in vitro work has failed to show direct effects of this virus on lymphocyte functions. Rather than working exclusively with cell free CMV strain AD 169, a common lab strain, we have been investigating the effects of low passage fresh CMV isolates (cell free and cell associated) on NK activity and T cell proliferation. Our results indicate that cell associated low passage CMV isolates (15 have been tested) markedly depressed NK activity. Cell associated AD 169 also induced suppression but to a lesser extent. Suppression of NK activity was clearly manifested only after 7 days of culture and could not be attributed to virus titer, viability, or overabundance of interferon. In contrast, cell free supernatant of either AD 169 or fresh isolates, while containing virus, did not depress NK activity.

The effects of cell free and cell associated AD 169 and fresh isolates on T cell proliferation differed in several respects. Cell free AD 169 did not suppress the proliferative response to either antigen or mitogen. Cell associated AD 169 depressed both types of proliferation from 0-50% while both cell free and cell associated low passage isolates completely abrogated both antigen and mitogen responses (>90%). This effect was apparent following only 3 days of culture with virus. From these and other data we suggest that CMV can cause direct suppression of lymphocyte functions. The mechanisms involved in these differential effects of various virus preparation are currently under study.

1367 STRUCTURAL ANALYSES OF THE VARICELLA VIRUS GLYCOPROTEINS. Charles Grose, Eduardo A. Montalvo, and William E. Friedrichs, University of Texas Health Science Center, San Antonio, Texas 78284

We have produced panels of murine monoclonal antibodies against the major glycoproteins of varicella-zoster virus (VZV). Based on the results of radioimmune precipitation analyses, the glycoproteins have been divided into three groups: gp118; gp140 / gp66; and gp98 / gp88 / gp62 / gp45. The latter group, which also has been designated VGA (varicella glycoprotein antigen) complex is a major component of the infected cell membrane. High-mannose glycoproteins have been distinguished by their susceptibility to digestion with endo-beta-N-acetylglucosaminidase H, and sialated forms by the use of neuraminidase. As with other herpesviruses, the VZV glycoproteins contain mainly asparagine-linked sugar moieties with terminal sialic acid residues. Structural intermediates in VZV-infected cells have been identified by use of the glycosylation inhibitors tunicamycin and monensin. From these studies, we have established that glycoprotein gp98 is a mature product which is synthesized in a series of steps from a 70,000 molecular weight nonglycosylated precursor; gp88 is the penultimate form. The glycoprotein gp66 is the major cleaved product of a disulfide linked 140,000 dalton glycoprotein. The glycoprotein gp118, which possesses an epitope inducing complement-independent neutralization, also appears to be a mature form.

1368 PURIFICATION AND IMMUNOLOGICAL ACTIVITY OF CYTOMEGALOVIRUS GLYCOPROTEINS, Lenore Pereira, Marjorie Hoffman, Cynthia Chan and Dale Dondero, Viral and Rickettsial Disease Laboratory, California Department of Health Services, 2151 Berkeley Way, Berkeley, California 94704.

In published studies, we reported the production of monoclonal antibodies to cytomegalovirus (CMV) and identified three glycoproteins which comigrated with polypeptides immune precipitated by sera from children with congenital and neonatal CMV infections (Pereira et. al., Infect. Immun. 36:924, 1982; Pereira et. al., Infect. Immun. 39:100, 1983). Analyses of CMV glycoproteins using monoclonal antibodies as site specific immunological probes have shown that CMV infected cells contain at least six antigenically distinct glycoproteins. We have designated these glycoprotein gA, gB, gC, gD, gE, and gF. Immunoaffinity columns constructed from monoclonal antibodies have been used to purify gA, gC, and gD. Results of studies on purified CMV glycoproteins showed the following: i) Purified glycoproteins retained their immunological activity by immune precipitation with monoclonal antibodies. ii) Comparison of the electrophoretic profiles of glycoproteins eluted from immunoaffinity columns with those immune precipitated from extracts of CMV infected cells showed that all electrophoretically distinct forms of gA and gC were purified whereas only the fastest migrating form of gD was purified. iii) Immune reactions of patient sera with purified glycoproteins, denatured in sodium dodecyl sulfate, electrophoretically separated, and immobilized on nitrocellulose paper showed that purified glycoproteins would be suitable antigens for quantitation of antibody in ~~serological tests.~~

1369 CHARACTERISATION of PSEUDORABIES ANTIGENS, Eric Saman and Raymond Thys, Janssen Pharmaceutica Research Laboratories, Belgium.

This study aims at the identification of pseudorabies viral antigens that are important in the host immune response upon infection with the virus. Using in vitro translation of mRNA from infected cells, we were able to identify one major antigen, by immunoprecipitation of the in vitro translation products with antiserum to pseudorabies virus. This antigen has an apparent molecular mass of 140 kd on denaturing and reducing gels. The coding region for this antigen was localized in the viral genome and this information was cloned for further study Another approach to identify viral antigens, important for the immunological response was taken. This consists of shotgun cloning of viral genomic fragments in a bacterial expression vector, and asking for expression of the corresponding proteins. The bacterial colonies are subsequently screened for the presence of viral antigens with an antiserum to the virus. Colonies that are identified as expressing some viral antigenic determinants should have obtained a recombinant plasmid containing information coding for this antigen or part of it. These data will allow then the localization of the relevant genetic information in the viral genome as well as the screening of the antigens produced by the bacteria, for eliciting neutralizing antibodies upon injection in mice. In this way we hope to identify several important viral antigens. Production of these antigens in bacteria would allow the composition of synthetic vaccines.

1370 HSV-1 VIROSOMES BIND TO AND FUSE WITH CELL SURFACES, David C. Johnson,* Michele Wittels, and Patricia G. Spear, The University of Chicago, Chicago, IL 60637

The envelope glycoproteins and at least one non-glycosylated protein were extracted from herpes simplex virus type 1 virions with the detergent octyl glucoside and were mixed with egg phosphatidyl choline. The proteins were incorporated into liposomes after removal of the detergent by dialysis as evidenced by their cosedimentation with labelled lipid on sucrose gradients. The glycoprotein-containing liposomes or virosomes bound to HEp-2 or Vero cells and inhibited binding of HSV-1 virions. The cell-bound material was enriched for gB and VP16 indicating variability in virosome composition and suggesting a requirement for one or more of these proteins in the binding reaction. Electron microscopic examination of cells that had been exposed to virosomes at 4°C and then incubated at 37°C provided evidence of fusion of virosomes with the plasma membrane. We are presently modifying the protein and lipid content of the virosomes to investigate the roles of various glycoproteins and lipids in the binding and fusion processes.
*Present address: Department of Pathology, MacMaster University, Hamilton, Ontario, Canada.

1371 MONOCLONAL ANTIBODIES SPECIFIC TO AND CROSS-REACTIVE WITH MAREK'S DISEASE VIRUS(MDV) AND HERPESVIRUS OF TURKEYS(HVT), Kazuyoshi Ikuta, Kazuhiro Nakajima, Kumiko Maotani, Shigeharu Ueda, Shiro Kato and Kanji Hirai*, Research Institute for Microbial Diseases, Osaka University, Osaka 565, and *Tokai University School of Medicine, Isehara 259-11, Japan.

To study on the modes of the immunity of HVT to Marek's disease(MD), we have isolated the mouse hybridoma cells secreting monoclonal antibodies cross-reactive with MDV and HVT glycoproteins. One group of monoclonal antibodies reacted with the secreted glycoproteins(gA) and also with the surface of cells infected with MDV and HVT. The analysis on two-dimensional gels revealed that the processing steps of the precursor form of MDV-gA to the processed form (54K to 70K) had changed during serial passage of oncogenic MDV strains. The other group of monoclonal antibodies reacted with three glycoproteins(gB), MDV-gp115/110, gp63 and gp50; HVT-gp115, gp62 and gp52, which were related to virus neutralization. Chickens or rabbits immunized with MDV- or HVT-gB purified by affinity chromatography coupled with monoclonal antibodies produced neutralizing antibodies reactive with both MDV and HVT. Moreover, immunization of chickens with HVT-gB resulted in partial protection against MD. These results suggest the possible role of HVT-gB in humoral or cell-mediated immunity to MD.

No viral antigen specific to MD lymphoblastoid cell lines (MDLCL) has not been reported. We attempted to isolate monoclonal antibodies against the MDV antigen purified from extracts of MDLCL, MDCC-MSB1, by affinity chromatography coupled with MDV-infected chicken serum IgG. Monoclonal antibodies obtained were found to react with three phosphorylated polypeptides in the MW ranges of 40K to 20K in the MDV-infected cells. The antigen was detected by immunofluorescence test when MDCC-MSB1 or MKT1 cells were cultured at 33°C, but not at 41°C.

1372 A TYPE SPECIFIC MONOCLONAL ANTIBODY IDENTIFYING A NOVEL GLYCOPROTEIN OF HSV-1. A Minson, A. Buckmaster, and U. Gompels, Department of Pathology, University of Cambridge, U.K.

A monoclonal antibody, LP11, efficiently neutralises HSV-1 in the absence of complement. The antibody is directed against a highly conserved, type 1 specific, epitope since it neutralises all but one of 65 HSV-1 isolates and none of 35 HSV-2 isolates. The antibody precipitates two polypeptide species of approximate molecular weights 115,000 and 120,000 from extracts of infected cells labelled with ^{35}S-methionine or ^{14}C-glucosamine. The electrophoretic behaviour of the polypeptides, and of the precursors seen in pulse and pulse-chase experiments, distinguish them from all previously described HSV-1 glycoproteins with the possible exception of the 110K species described by Showalter et al. (Infection and Immunity 34, 684, 1981).

The reactivity of antibody LP11 with intertypic recombinants of HSV-1 and HSV-2 maps the target antigen close to gB, within map co-ordinates 0.29 - 0.33. HSV-1 mutants resistant to LP11 have been isolated and are being used in marker rescue experiments to define more precisely the location of the gene for this glycoprotein.

1373 IgG SUBCLASS ANTIBODIES RELATED TO CLINICAL MANIFESTATIONS OF HERPES SIMPLEX VIRUS (HSV), R. Marie Coleman, Susan C. Williams, Donald J. Phillips, Charlotte M. Black, Charles B. Reimer, and Andre J. Nahmias. Emory University School of Medicine, Atlanta, GA. 30303 and Centers for Disease Control, Atlanta, GA 30333

Monoclonal antibodies specific for human IgG1, IgG2, IgG3 and IgG4 were used in an enzyme immunosorbent assay to determine subclass specific antibodies to Triton X-100 extracted antigens of HSV-1 and HSV-2 infected HEp-2 cells. The sera tested were from 151 patients with various clinical herpetic manifestations and from 12 seropositive adult and newborn controls. IgG1 and IgG2 antibodies appeared first after infection and were detected in 180/186 sera. IgG3 and IgG4 antibodies were not detected in sera from most patients with primary or initial genital infections, neonatal herpes, or seropositive adult and newborn controls. In addition to IgG1 and IgG2, IgG3 and IgG4 antibodies were found predominantly in sera from patients with recurrent genital and orolabial infections or non-primary herpes encephalitis. IgG4 antibodies occurred significantly ($p<0.005$) more frequently in sera from males than in those from females with recurrent genital infections.

These data suggest that the study of IgG subclasses to HSV may provide important markers of immune responsiveness by different hosts to the various forms of herpetic infections. Investigations underway involving subclass allotypes as well as subclass antibody responses to purified HSV proteins should provide more detailed markers of the immune response to HSV infections.

1374 NEUTRALIZING ANTIBODIES REACT WITH A COMPLEX OF CYTOMEGALOVIRUS ENVELOPE GLYCOPROTEINS William J. Britt, University of Alabama in Birmingham, Birmingham, Alabama 35294.

The presence of neutralizing antibodies is thought to be important for the control of human cytomegalovirus infection (CMV). Although recovery from CMV is usually associated with the production of neutralizing antibodies, the protein specificity of these antibodies remains poorly defined. To simplify the characterization of CMV neutralizing antibodies found in human sera, we generated CMV-specific murine monoclonal antibodies and compared the specificity of these monoclonal antibodies with human sera. Six monoclonal antibodies were found to bind intact virions and four of these antibodies neutralized infectious virus in-vitro. All of the monoclonal antibodies as well as a number of human immune sera precipitated three virion glycoproteins of estimated molecular weights of 160 kilodaltons (kd), 116kd and 55kd. These envelope glycoproteins were subsequently shown to be covalently linked by disulfide bonding within mature virions. This covalent linkage of envelope proteins provided an explanation of why monospecific reagents such as monoclonal antibodies immune precipitated three proteins of differing molecular weight. Additional supporting evidence for this hypothesis was obtained by generating proteolytic peptide maps of the 116kd and 55kd proteins. Using a variety of proteolytic enzymes, our results indicated these two proteins shared little sequence homology, thus making it unlikely that the monoclonal antibodies were reacting with shared antigenic determinants expressed by these proteins. These findings suggested that additional, conformationally dependent determinant(s) may be present on envelope glycoprotein complexes and that these determinant(s) may be recognized by neutralizing antibodies.

1375 ANTISERUM TO A SYNTHETIC PEPTIDE THAT REACTS WITH HSV-1 GLYCOPROTEIN C, Martin Zweig, Stephen D. Showalter and Berge Hampar, National Cancer Institute/Frederick Cancer Research Facility, Frederick, Maryland 21701

The nucleotide sequence of DNA encoding HSV-1 glycoprotein C(gC) was recently determined by Frink and co-workers. We immunized a rabbit with a conjugate of bovine serum albumin and a hydrophilic synthetic peptide of 12 amino acids predicted by a portion of this sequence. The antiserum obtained reacted with antigen in cells infected with HSV-1 and HSV-2 in immunofluorescence tests. Enzyme-linked immunosorbent assays demonstrated that this antiserum reacted with immunoaffinity purified HSV-1 gC and HSV-2 gC (formerly designated HSV-2 gF). Although the antiserum did not detectably immunoprecipitate radiolabeled proteins from infected cell extracts, it reacted in immunoblot assays with purified HSV-1 gC and HSV-2 gC preparations that were electrophoretically transferred to nitrocellulose membranes. The antiserum preferentially reacted with a 65-70K MW component of HSV-1 gC and its reactivity was enhanced after the glycoprotein was treated with glycosidases. These findings confirm that HSV-1 and HSV-2 gC are immunologically related and also define a specific conserved peptide region of HSV-1 gC.

1376 VIRUS-SPECIFIC IgG SUBCLASSES IN HERPES SIMPLEX AND VARICELLA-ZOSTER INFECTIONS, Vivi-Anne Sundqvist, Annika Linde and Britta Wahren, National Bacteriological Laboratory, S-105 21 Stockholm, Sweden.

Using monoclonal antibodies to different subclasses of human IgG, an enzyme-linked immunosorbent assay (ELISA) has been used to study the subclass pattern in serum samples from patients with primary or reactivated infections of HSV and VZV. IgG1 and IgG3 with anti HSV activity were seen in patients with primary and reactivated disease, as well as in healthy seropositive subjects and immunoglobulins. In occasional patients IgG4 was seen alone or together with IgG1 and IgG3. In varicella, IgG3 subclass specific antiviral antibodies were predominant while IgG1 was the dominant subclass in zoster. IgG3 appeared to be the first subclass to react with HSV and VZV in primary infections.

1377 MURINE MONOCLONAL ANTIBODY TO A SINGLE PROTEIN NEUTRALIZES THE INFECTIVITY OF HUMAN CYTOMEGALOVIRUS, Lucy Rasmussen and Thomas C. Merigan, Stanford University School of Medicine, Stanford, California 94305

Murine monoclonal antibodies to human cytomegalovirus (CMV) strain AD169 were selected which neutralized virus infectivity. One antibody producing hybridoma, 1G6, was used to produce ascites fluid from which immunoglobulin was isolated. This antibody efficiently neutralized CMV AD169, other CMV laboratory strains (Towne, Davis) and clinical isolates of CMV in early (less than 10) tissue culture passage in the absence of complement. The antibody immunoprecipitated a single 86,000 dalton protein from both laboratory and clinical CMV strains. This viral protein was demonstrated by indirect immunofluorescence to be localized in the cytoplasm of CMV infected cells.

1378 MAPPING THE ANTIGENIC REGIONS OF EPSTEIN-BARR NUCLEAR ANTIGEN USING SYNTHETIC PEPTIDES, Gary Rhodes, Richard A. Houghten, Dennis A. Carson and John H. Vaughan, Scripps Institute and Research Foundation, La Jolla, CA 92037

The viral DNA encoding for the Epstein-Barr Nuclear Antigen (EBNA) contains a repeating sequence which is expressed as a run of over 200 amino acids containing only glycine and alanine (Hennessy and Kieff, PNAS 80, 5665 (1983)). We have synthesized peptides from the middle, ends and outside this repeating region of the protein and raised rabbit antibodies against them. We find that antibodies to the peptides containing the repeating gly-ala sequence recognize the EBNA protein from two different transformed cell lines and this recognition is blocked by the peptides to which the antibodies were raised.

Reciprocally, we find that human sera containing antibodies to EBNA will recognize and react either with the peptides alone or coupled to proteins. Sera with no antibodies to EBNA or to the viral capsid antigen do not recognize the peptides. Futhermore, antibody titers to the peptides in the sera of patients with acute and convalesent mononucleosis rise in conjuction with those directed against EBNA. Sera with high titers to EBNA recognized some of the peptide sequences better than others and did not recognize at all the peptide outside the repeating unit. This implies that human antibodies to EBNA are directed at selected portions of the protein and further studies of peptides should afford a method of mapping these antigenic determinants.

1379 PRODUCTION AND CHARACTERIZATION OF HUMAN MONOCLONAL ANTIBODIES AGAINST VARICELLA-ZOSTER VIRUS, Steven K.H. Foung, Susan Perkins, Celine Korapchak, Edgar G. Engleman, F. Carl Grumet and Ann Arvin, Stanford University, Stanford, CA 94305

A human-mouse cell line, SBC-H20, that fuses efficiently to human B lymphocytes and produces stable human Ig secreting hybridomas has been developed. This line was derived from a fusion between a mouse myeloma line, SP2/08A2, and human peripheral B lymphocytes isolated from a normal donor. Fusion between SBC-H20 and B lymphocytes isolated from a patient recovering from an acute varicella (VZV) infection, yielded multiple hybridomas secreting human monoclonal anti-VZV antibodies. When tested by solid phase RIA against commercial VZV antigens, spent supernatants from 2 different hybrid clones (diluted 1:256) showed significant reactivity, mean cpm of two values. 2-1D5-1246 (control=80); 1-A2=854 (control=112). By a standard assay, both human monoclonal antibodies neutralized VZV at approximately 5ug/ml of protein. 2-1D5 is an IgG1 (kappa) and 1-A2 is an IgG1 (lambda). Both antibodies bound to VZV infected cells by indirect immunofluorescence. By Western blot gel electrophoresis, 1-A2 identified a viral protein of 92,000 molecular weight. In one patient tested by immunofluorescent binding, 1-A2 identified cell surface viral antigens on monocytes isolated during acute VZV infection.

1380 ANTIHERPETIC ACTIVITY OF 9-(3,4-DIHYDROXYBUTYL)GUANINE (DHBG), A. Larsson, A.-C. Ericson, C.-E. Hagberg, N.-G. Johansson, B. Lindborg, K. Stenberg and B. Öberg, Department of Antiviral Chemotherapy, Research and Development Laboratories, Astra Läkemedel AB, 151 85 Södertälje, Sweden.

The effect on herpes simplex virus (HSV) multiplication in vitro and in vivo as well as the mechanism of action of 9-(3,4-dihydroxybutyl)guanine (DHBG) was investigated. The compound was shown to be a good substrate for the HSV (HSV-1 and HSV-2) induced thymidine kinase (TK) with a high affinity and high phosphorylation rate for the enzyme. No affinity was observed for the corresponding cellular TK. The monophosphate of DHBG was converted to the diphosphate form by the cellular guanosine monophosphate kinase. DHBG was shown to inhibit different HSV-1 and HSV-2 strains at concentrations where no effect on the normal cellular metabolism was observed. The (R)-enantiomer was more inhibitory than the (S)-enantiomer of DHBG. In animal models, a good therapeutic effect was observed for the (R)-enantiomer of DHBG on cutaneous HSV-1 infection in guinea pigs, in a systemic HSV-2 infection in mice and on herpes keratitis in rabbits.

1381 INHIBITION OF HERPESVIRUS REPLICATION BY MACROPHAGE PEPTIDES, John F. Weaver, Gloria Lee, Abla A. Creasey, Laura V. Doyle, Charles V. Herst, Edward C. O'Rourke, and Thomas J. White, Cetus Corporation, Emeryville, CA 94608; Michael E. Selsted and Robert I. Lehrer, Dept. of Medicine, Univ. of California, Los Angeles, CA 90024.

Rabbit alveolar activated macrophages produce two unusual peptides, MCP-1 and MCP-2 which have potent antiviral activity on human cells in culture. At micromolar concentrations, these peptides inhibit the replication of Herpes simplex viruses I and II, Cytomegalovirus, and Vesicular stomatitis virus. Antiviral activity was measured by quantitative virus yield reduction techniques and assessment of the inhibition of viral cytopathic effects. Pretreatment of cells with peptide prior to virus challenge is sufficient to inhibit virus replication, but maximal activity is observed when the peptides remain in contact with the cells throughout the assay period. We have investigated the effects of these peptides on host cell metabolism and compared them to interferon by studying their mechanism of action. We examined cellular processes known to be affected by interferon as well as interferon induction. Although the peptides exhibit antiviral activity, they do not appear to share a common mechanism of action with interferon, or exhibit the antiproliferative and immunomodulatory activities associated with interferon.

1382 FAILING IN PREVENTION OF HSV-2 LATENCY IN SENSORY GANGLIA OF MICE WITH AN INACTIVATED SUBUNIT VACCINE Ac NFU$_1$ (S^-) BHK PREPARED FROM HSV-1,

M.H. Chen, J.G. Wu[1]. G.R.B. Skinner, and C.E. Hartley[2] 1.Department of Cell Biology, Virus Research Institute, Hubei Medical College, Wuchang, China. 2. Department of Medical Microbiology, The Medical School, University of Birmingham, U.K.

We have previously reported that the vaccine afforded significant protection to experimental HSV-2 infection in mice (G.R.B. Skinner et al,1982) and reduced the frequency of HSV-2 induced cervical cancer in mice (M.H. Chen et al, 1983). In the present study, we carried out a research into the efficacy of the vaccine to protect HSV-2 latency of sensory ganglia of mice. Female hybrid mice received two vaccinations intraperitionally at an interval of two weeks. Then, mice were intravaginally inoculated with strain 333 or Wu of HSV-2 once a week for 25 times. Lumbosacral spinal ganglia and trigeminal ganglia were removed from animals which survived over one year for recovery of latent HSV-2. The percentages of animals harboring latent virus in their lumbosacral spinal ganglia and trigeminal ganglia in both immunized (40%) and non-immunized (30%) groups were not significantly different. The lumbosacral spinal ganglia were also examined histo-pathologically. The foci of neurons with cell fusion, atrophy, degeneration, necrosis or fibrosis could be found in both immunized and non-immunized animals.

1383 MODE OF INHIBITION OF N^4-SUBSTITUTED 2-ACETYLPYRIDINE THIOSEMICARBAZONES, John C. Drach, Steven R. Turk and Charles Shipman, Jr., University of Michigan, Ann Arbor, MI.

Over 60 substituted derivatives of 2-acetylpyridine thiosemicarbazone synthesized by D. L. Klayman and coworkers at Walter Reed Army Institute of Research have been evaluated as potential antiviral agents. The effects of these drugs on the replication of herpes simplex viruses (HSV) types 1 and 2 and their capacity to cause cellular cytotoxicity were studied in vitro. The drugs also were evaluated in a cutaneous HSV-infected Guinea pig model for efficacy in vivo. These studies have identified five N^4-substituted compounds which warrant further investigation as potential antiherpes drugs.

Mode-of-action studies in uninfected KB cells with the parent compound, 2-acetylpyridine thiosemicarbazone, have shown that DNA synthesis was reversibly inhibited within 30 minutes of exposure. Incubation of cells with [^{14}C]uridine or [^{32}P]orthophosphate in the presence of drug resulted in reduction in the amount of labeling of DNA but not RNA. Formation of thymidine nucleotides from [^{3}H]deoxyuridine was not inhibited in the same dosage range suggesting thymidylate synthetase was not inhibited. Preliminary experiments with crude ribonucleoside diphosphate reductase indicated this enzyme is a major target of drug inhibition. Studies in HSV-infected cells have shown potent inhibition of viral DNA synthesis. Isolated HSV DNA polymerase and mammalian DNA polymerases α and β were not inhibited. Furthermore, DNA synthesis was not inhibited in nuclei isolated from HSV-2-infected cells indicating that the drug did not affect DNA polymerases or the DNA template. Recent results on the inhibition of HSV-1-induced ribonucleoside diphosphate reductase also will be presented.

1384 ANTIVIRAL ACTIVITY OF 5-ETHYL-2'-DEOXYURIDINE (EDU) ON HERPES SIMPLEX VIRUSES (HSV) IN CELL CULTURE, MICE AND GUINEA PIGS, Raymond F. Schinazi, R. Taylor Scott, Jeanne Peters and Andre J. Nahmias, Emory University and VA Medical Center, Atlanta, GA 30303.

EDU is a nucleoside antiviral currently licensed in several European countries for the treatment of HSV keratitis. Clinical trials for several herpetic conditions, including genital herpes, are ongoing in the US and Canada. It was, therefore, important to determine its effectiveness in animal models. We initially determined the susceptibility of clinical isolates of HSV-1 (14) and HSV-2 (13) to EDU in plaque reduction assays in Vero cells. The mean ED_{50}s ($\pm$1 SD) were 8.62 $\pm$ 1.17 μM and 7.79 $\pm$ 1.25 μM, respectively. This difference was not statistically significant. No apparent toxicity to rapidly growing Vero cells at 1 mM was noted. Oral or intraperitoneal (ip) treatments of EDU were ineffective in Swiss mice (6 weeks old) infected intracerebrally with different doses of HSV-1 (KOS) or HSV-2 (G), even when treated with 1 g/Kg/day (bid x 4d, Rx 5 h after virus inoculation). However, a significant antiviral effect was observed when EDU was given ip in 15% DMSO, suggesting that DMSO can effectively open the blood-brain barrier to EDU. The drug was not toxic to mice even at 1.6 g/Kg/day. Topical 3% EDU cream was evaluated in a blind fashion in the guinea pig model of genital infection with HSV-2 (MS). The drug, given bid x 7d, was compared with placebo and other antivirals. EDU treatment was more effective in reducing lesion severity than placebo, whether begun 3 or 24 h after infection (ascertained serologically and virologically). These results indicate that EDU is effective in the guinea pig genital model. Its lack of toxicity at very high doses, as well as its poor transport to the CNS suggests that EDU may be safer than other antiviral drugs.

1385 INTRACELLULAR CONCENTRATIONS OF DEOXYTHYMIDINE MAY INFLUENCE THE ANTIVIRAL ACTIVITY OF ANTIHERPES NUCLEOSIDE ANALOGUES, Johan Harmenberg and Gunnar Abele, Department of Virology, National Bacteriological Laboratory, S-105 21 Stockholm, Sweden.

Many antiherpes nucleoside analogues utilize deoxythymidine kinase (dTK) for intracellular activation of the compounds. Presence of the natural substrate of dTK which is deoxythymidine (dT) would therefore possibly decrease the activation rate of these substances. We have found high amounts of dT in green monkey kidney cells (GMK) and low amounts in human lung fibroblasts (HL). The nucleoside analogue acyclovir has been shown to exert 20-100 fold lower antiviral activity in GMK than HL cells.
Utilizing high performance liquid chromatography (HPLC) we have analysed the dT content of organs and tissues from guinea pigs, mice and humans. In guinea pig skin we have found dT concentration in the order of 30 μM while in human skin only low amounts have been found. Deoxythymidine concentrations in other tissues will be discussed.

1386 INHIBITION OF CYTOMEGALOVIRUS INDUCED CYTOMEGALY AND NUCLEAR INCLUSIONS BY CYCLIC NUCLEOTIDE MODULATORS, Mostafa Nokta, Odd Steinsland, and Thomas Albrecht, The University of Texas Medical Branch, Galveston, TX 77550.

Human embryo skin muscle (SM) cells show a progression of cytopathic effects (c.p.e.) following cytomegalovirus (CMV) infection. As early as 5 h PI rounding and "contraction" (early events) begin to occur; by 24 h "relaxation" and enlargement (late events), have begun along with the development of nuclear inclusions (NIs). We have shown that papaverine (PAP), which blocks phosphodiesterase, prevented rounding and "contraction" of SM cells. We now report the effect of PAP, isobutyl methylxanthine (IBMX), and forscolin (FN) on "relaxation", enlargement, and development of NIs. FN ($7.3x10^{-5}$M), a drug which raises cAMP levels, as well as PAP ($8.8x10^{-5}$M) and IBMX ($1.4x10^{-5}$M), which increase both cAMP and cGMP levels in many systems, inhibited enlargement when added at 5 or 12 h PI and measured at 48 or 72 h PI. Cells treated with these drugs contained NIs of diminished size. The observed effects of these drugs could be mediated through variation of intracellular Ca^{++} since: 1) Cyclic AMP has been shown associated with a decrease in intracellular Ca^{++} in several tissues, 2) We have shown a Ca^{++} influx 6 h after CMV infection. 3) Verapamil (VR, $6x10^{-5}$M), a Ca^{++} entry blocker, inhibits enlargement and development of NIs, when added at 5 or 12 h and measured at 48 or 72 h PI. 4) Calmodulin blockers such as triflouroperazine (TFP, $7.3x10^{-5}$M) blocked enlargement and inhibited development of NIs when added at 24 h and measured at 72 h PI. 5) The Ca^{++} ionophore A23187 ($3x10^{-5}$M) enlarged uninfected cells 5 minutes after its addition. These results indicate that increased levels of cAMP may prevent cytomegaly and inhibit CMV replication by decreasing intracellular Ca^{++} concentration.

1387 INHIBITION OF CELLULAR α DNA POLYMERASE AND HERPES SIMPLEX VIRUS-INDUCED DNA POLYMERASES BY THE TRIPHOSPHATE OF 9-[[2-HYDROXY-1-(HYDROXYMETHYL)ETHOXY]METHYL] GUANINE, Phillip A. Furman, Wayne H. Miller, Richard L. Miller, Catherine U. Lambe and Marty H. St. Clair, Burroughs Wellcome Co., Research Triangle Park, NC 27709.

The triphosphate form of the acyclovir analog 9-[[2-hydroxy-1-(hydroxymethyl)ethoxy]methyl]-guanine (BW 759U) inhibited the DNA polymerases from several strains of herpes simplex virus type 1. Both viral and the cellular α DNA polymerases were less sensitive to inhibition by BW 759U-triphosphate than to inhibition by acyclovir triphosphate. Interestingly, two acyclovir triphosphate resistant DNA polymerases were as sensitive to BW 759U-triphosphate as were the DNA polymerases induced by wild type viruses (K_i=0.05-0.01 μM).. The cellular α DNA polymerase was much less sensitive to inhibition than the DNA polymerases induced by the various herpes simplex virus strains investigated. Incubation of Vero cells infected by the KOS strain of herpes simplex virus type 1 with BW 759U resulted in the formation of substantial quantities of the triphosphate of this compound.

1388 INHIBITION OF THE DNA POLYMERASES OF VARICELLA ZOSTER VIRUS AND HUMAN CYTOMEGALOVIRUS BY THE NUCLEOSIDE ANALOGS 9-[(2-HYDROXYETHOXY)METHYL]GUANINE (ACV) AND 9-[(2-HYDROXY-1-(HYDROXYMETHYL)ETHOXY)METHYL]GUANINE (BW 759U), Karen K. Biron, Peter J. Stenbuck and Jann B. Sorrell, Burroughs Wellcome Co., Research Triangle Park, NC 27709.

The nucleoside analog 9-[(2-hydroxyethoxy)methyl]guanine (ACV) and its congener 9-[(2-hydroxy-1-(hydroxymethyl)ethoxy)methyl]guanine (BW 759U) exert similar antiviral activity against VZV in vitro as measured by the plaque reduction assay. The kinetics of inhibition of the VZV induced DNA polymerase by the triphosphates of these antiviral compounds were studied using assay conditions optimal for the virus induced enzyme. The triphosphate of ACV (ACVTP) behaved as a competitive inhibitor for the viral polymerase catalyzed incorporation of deoxyguanosine triphosphate (dGTP) into the synthetic template primer poly dC·oligo dG (Km dGTP=6μM, K_i ACVTP =.04μM). The triphosphate of BW 759U (759TP) was roughly 5-fold less effective as a competitive inhibitor in this system (K_i 759TP=0.2μM). Guinea pig tumor cell DNA polymerase α was 50-fold less sensitive to inhibition by the triphosphate of BW 759U as compared to ACV triphosphate using the calf thymus template (K_i ACVTP=.14μM, K_i 759TP=10.5μM).

Human CMV is relatively insensitive to ACV in vitro, while BW 759U is quite active. For example, the ED_{50} against strain AD169 for ACV is 108μM, while the ED_{50} for BW 759U is 2μM. A comparison of the relative sensitivity of the virus induced DNA polymerase of strain AD169, however, revealed that this virus induced enzyme is also roughly 5-fold less sensitive to competitive inhibition by BW 759U as compared to ACV triphosphate (K_i ACVTP=.33μM, K_i 759TP= 1.4μM).

1389 CORRELATION OF INCORPORATION OF ARABINOSYL ANALOGS INTO HERPES SIMPLEX VIRUS DNA AND MUTAGENESIS, Lowell E. Schnipper, Glenn J. Bubley, H.S. Allaudeen*, Clyde S. Crumpacker, Beth Israel Hospital and Harvard Medical School, Boston, MA 02215 and *Smith, Kline and Beckman, King of Prussia, PA.

1-β-D-arabinofuranosylcytosine (ara-C) is an effective inhibitor of Herpes Simplex Virus Type 1 (HSV-1) replication. Analysis of the wild type virus, HSV-1 strain (KOS) grown in the presence of ara-C demonstrates a strong correlation between incorporation of ara-C in internucleotide linkage and inhibition of viral DNA synthesis. An ara-C and ara-A resistant mutant, HSV 1 ($araA^r$), expresses an altered DNA polymerase activity (Ki for ara ATP = 3.4 uM) and incorporates less ara-C into viral DNA than wild type. Synergistic inhibition of HSV-1 is observed in the presence of PAA and the arabinosyl analog (araA). The observed synergy may relate to increased formation of arabinosyl DNA since HSV-1 infected cells demonstrate a 20-40% enhancement in incorporation of ^{3}H-ara-C into viral DNA at subinhibitory concentrations of PAA. In studies employing purified HSV-1 DNA polymerase, in vitro, subinhibitory concentrations of PAA (0.1 ug/ml) completely inhibits incorporation of ^{3}H-ara ATP into an activated DNA template. Viral DNA containing ara-C in internucleotide linkage demonstrates increased single strand breaks when analyzed on alkaline sucrose gradients, and fragment size is related to the extent of formation of arabinosyl DNA. When HSV-1 (KOS) is grown in $5x10^{-7}$M (the ID_{50}) and $5x10^{-6}$M (ID_{90}) ara-C, the progeny viruses express a concentration-dependent increase in the frequency of thymidine kinase (TK) deficient particles when compared to KOS, and this suggests that mutagenesis at the TK locus is related to the formation of (araC) DNA.

1390 REINFECTION WITH WILD-TYPE HERPES SIMPLEX VIRUS TYPE 2 (HSV-2) AFTER PRIMARY GENITAL INFECTION WITH A THYMIDINE KINASE DEFICIENT (TK-) HSV-2 MUTANT, L.R. Stanberry and S. Kit, Univ. Cincinnati, Children's Hospital Research Foundation, Cinti, OH, and Baylor College of Medicine, Houston, TX.

TK- HSV-2 genital infection is characterized by vaginal virus replication but little or no clinical disease. In contrast, TK+ HSV-2 induces vaginal virus replication and also severe vesiculo-ulcerative genital skin lesions, urinary retention, and hindlimb paralysis. We investigated whether the prior TK- infection protected against subsequent challenge with TK+ virus. Weanling, female guinea pigs were initially intravaginally inoculated with a TK- HSV-2 mutant of strain 333 or tissue culture medium. Three weeks after inoculation, when vaginal virus replication had ceased, both groups were challenged with wild type TK+ HSV-2.

Following TK+ challenge, the mock-infected group developed clinically severe genital infection with prolonged vaginal virus replication. In contrast, the group of animals previously infected with TK- HSV-2 exhibited no clinical disease and had significantly less vaginal virus replication. Although acute and latent ganglionic infection occurred as a consequence of primary (initial) genital infection with either TK+ or TK- strains, we could not demonstrate TK+ superinfection of neural tissues in animals previously infected with TK- HSV-2.

Initial TK- HSV-2 genital infection modifies subsequent wild type TK+ HSV-2-induced genital infection. In this model re-infection with wild type TK+ HSV-2 is clinically inapparent, vaginal HSV-2 replication is reduced, and ganglionic superinfection is prevented.

1391 IMMUNOGENICITY AND PREVENTIVE EFFICACY OF A SUBUNIT HERPES SIMPLEX VIRUS VACCINE IN CEBUS MONKEYS, George D. Wilbanks, Gordon R.B. Skinner and Mary E. Turyk, Rush University, Chicago, IL 60612.

Adult female Cebus monkeys were vaccinated with $AcNFU_1(S^-)MRC$, a subunit formaldehyde-inactivated Herpes simplex virus (HSV) preparation. After two or three vaccinations, animals developed significant levels of neutralizing antibodies to HSV-1 and to HSV-2. Following subcutaneous vulva challenge with live HSV-2, antibody levels to both HSV-1 and HSV-2 were significantly re-stimulated. In unvaccinated animals infected with HSV-2 in an identical manner, neutralizing antibodies were stimulated, but to a much lesser extent.

There was evidence of protection in vaccinated monkeys against subcutaneous challenge with 10^8 plaque-forming units of HSV-2. Three vaccinations administered within nine months of the challenge offered the best protection. Two vaccinations and exposure to live virus 16 months previous to the challenge seemed to offer good protection to an animal that had elevated antibody levels at challenge, but not to an animal without remaining antibodies. A previous genital infection did not give protection against reinfection.

1392 PREVENTION OF RECURRENT HERPES GENITALIS BY AN ANTI-PROSTAGLANDIN AGENT, Linda M. Janus, George D. Wilbanks, Lisa A. Nagel and Mary E. Turyk, Rush University, Chicago, IL 60612.

Reactivation of the latent Herpes virus may be prostaglandin mediated. A clinical trial of an anti-prostaglandin agent (Motrin in a dose of 600 mg four times daily) is being compared to a placebo medication taken in a similar manner. Thirty female patients with documented recurrent Herpes genitalis and in good general health are randomized to receive either medication. They are seen every three months as well as at the time of a reoccurence to document the number, severity, and duration of their lesions. In addition, questionnaires are administered at these times to document the level of stress the patient is experiencing. Preliminary data will be presented at the time of the meeting since the code has not yet been broken.

1393 THE FATE OF HERPES SIMPLEX VIRUS IN THE NERVOUS SYSTEM OF MICE UNDERGOING NUCLEOSIDE ANALOGUE THERAPY, H.J. Field, J.R. Anderson and S. Efstathiou, Department of Pathology University of Cambridge, U.K.

Herpes encephalitis was established in mice by means of intranasal inoculation. From the third day after inoculation acyclovir or dihydroxypropoxymethylguanine therapy was used to inhibit virus replication. The presence of virus DNA in the brainstem and trigeminal ganglia was studied by means of the Southern blotting technique. The quantity and form of virus sequences in the neural tissue were correlated with amount and distribution of infectious virus and virus antigen in these neural tissues from the acute phase until the time when infectious virus was no longer detectable. The results will be discussed in relation to human encephalitis and possible latency of virus in the CNS.

1394 NUCLEIC ACID HYBRIDIZATION, A METHOD FOR STUDYING THE EFFECT OF ANTIVIRAL COMPOUNDS, Håkan Gadler, Alf Larsson[X] and Ellen Sølver, National Bacteriological Laboratory, S-105 21 Stockholm and [X]Astra Läkemedel AB, S-151 85 Södertälje, Sweden.

The inhibition of viral replication by an antiviral compound can be studied by various techniques. The effect on viral DNA synthesis can be determined after addition of radioactive thymidine to infected cell cultures. Separation of labelled viral and cellular DNA is then carried out on a CsCl gradient. This technique, however, is very cumbersome and time-consuming, especially when examining numerous samples. A simple, rapid technique utilizing nucleic acid hybridization and with recombinant virus DNA clones as probes, has therefore been developed. The method has been applied to studies on herpes simplex virus (HSV) and cytomegalovirus (CMV), but can equally well be applied to all DNA-viruses. This technique is applicable to screening different compounds with suspected antiviral activity or clinical virus isolates for determination of resistance to antivirals. The effect of several newly described nucleoside analogs (e.g. hydroxybutylguanine, dihydroxybutylguanine, dihydroxypropoxymethylguanine) on HSV and CMV DNA synthesis will be described. Since the mode of action of all of these new antiviral compounds is not known, experiments performed to elucidate the mechanism will be detailed.

1395 NATURAL KILLER CELL ACTIVITY IN INTERFERON-TREATED HERPES ZOSTER PATIENTS Rosemarie Berger, Barbara Gisin, Christian Herzog and Max Just University Children's Hospital, Basle, CH 4000 Switzerland

Otherwise healthy herpes zoster patients were treated with recombinant leucocyte A interferon not later than 5 days after the appearance of the first symptoms (pain). They received 5 daily doses of 1.5 or 3 x 10^6 Units or placebo. The diagnosis of herpes zoster was established by virus isolation from the vesicle fluid and by titration of antibody at day 1 and day 14 of the treatment. The level of endogenous interferon in the patients serum was determined before the first injection of exogenous interferon. Lymphocytes were isolated by ficoll separation from blood samples taken before the beginning of the treatment and at day 5 before the administration of the fifth dose of interferon. The lytic activity of the natural killer cells was tested with ^{51}Cr-labeled K 562 cells. In most patients the NK cell activity markedly increased during the course of the disease. This enhanced activity is probably not only due to the administration of exogenous interferon as it was also observed in the placebo treated control group.

1396 DRUG RESISTANCE AND HYPERSENSITIVITY: MUTATIONS MAPPING TO DISTINCT SITES IN THE HSV DNA POLYMERASE LOCUS, D. Coen[1], H. Chiqu[1], H. Fleming[1], J. Hall[2], and M. Retondo[1] [1]Harvard Medical School, Boston, MA 02115 and [2]University of Arizona, Tucson, AZ 85721

We can distinguish at least twelve mutants known or likely to contain mutations in the HSV DNA polymerase (pol) locus on the basis of their relative sensitivities to the antiviral drugs commonly abbreviated PAA, PFA, araA, araT, FMAU, FIAU, BVdU, ACG, and 2'NDG, as well as their relative sensitivities to aphidicolin (Aph) and temperature and their production of mutations at other loci. We presume these mutants to be mutated at different sites within the pol locus and in four cases have mapped their mutations to distinct restriction fragments of 1.1 kbp or less. Mutations in two mutants which differ slightly in terms of drug sensitivity but greatly in terms of production of mutations at other loci have been mapped to an 0.8 kbp region. Cross-resistance patterns of the mutants suggest possible treatments for resistant clinical isolates and that polymerases capable of discriminating against acyclo- and aranucleoside triphosphates cannot discriminate against the triphosphates of the closely related compounds 2'NDG and FMAU. Numerous Aph-, PAA-, FMAU-, FIAU-, BVdU-, and 2'NDG-hypersensitive (hs) mutants have been found. A $BVdU^{hs}$ mutation has been mapped to an 0.8 kbp region in the pol locus lying at least 0.5 kbp away from a previously reported BVdU-resistance mutation. $2'NDG^{hs}$ and Aph^{hs} mutations have been mapped to different regions. These results indicate that HSV polymerase is a target of these drugs and that mutants previously viewed as merely sensitive to given drugs may in fact contain mutations affecting drug sensitivity. Analysis of hypersensitivity should aid in the functional dissection of the pol locus. To this end, DNA sequence analysis of these mutations is in progress.

1397 INDUCTION OF INTERFERON HAS A ROLE IN THE AUTO-INTERFERENCE OF HERPES SIMPLEX VIRUS REPLICATION IN MONONUCLEAR PHAGOCYTES, Tapani Hovi, Kimmo Linnavuori, Ilkka Julkunen and Pekka Tiainen, Department of Virology, University of Helsinki, SF-00290 FINLAND

Freshly isolated human peripheral blood monocytes support poorly herpes simplex virus replication, as compared to cultures of macrophage-like cells derived from monocytes by one to two weeks' incubation in vitro before inoculation with the virus. However, if freshly isolated monocytes are infected with low multiplicities of herpes simplex virus (0.05 - 0.0001 p.f.u. per cell) infectious virus is produced after a couple of days' lag period. Up to 1000 i.u./ml of interferon, mainly or exclusively of type alpha, can be detected in the culture media of high multiplicity-infected monocytes while little if any interferon appears to be produced in mature macrophages under similar conditions. Anti-leukocyte interferon serum applied together with the inoculum virus shortens the lag period before the appearance of the progeny virus in low multiplicity-infected monocytes. High multiplicity infection of monocytes with herpes simplex virus inhibits morphological differentiation of the cells in a manner much like that of exogenous interferons, but the virus-induced block of differentiation cannot be prevented by anti-interferon serum. Possible correlation of the observed phenomena to the interferon-associated increase in cellular 2'-5'-oligoadenylate synthetase activity is under investigation.

1398 CONSTRUCTION OF CONTINUOUS CELL LINES EXPRESSING MEMBRANE-BOUND AND SECRETED FORMS OF GLYCOPROTEIN D FROM HERPES SIMPLEX VIRUS, Phillip Berman, Donald Dowbenko, Christian Simonsen, and Laurence Lasky, Genentech, Inc. S. San Francisco, Ca. 94080

The genes coding for glycoprotein D of Herpes Simplex Virus Types 1 and 2 (HSV-1 and HSV-2) have been cloned and sequenced. Both gD genes coded for proteins 393 amino acids long which were approximately 80% homologous. Although amino acid substitutions were found throughout the proteins, clusters of substitutions were found in the amino- terminal signal sequence and the carboxy- terminal membrane binding domain. The gene coding for HSV-1 gD was transfected into mammalian cells and stable cell lines which constitutively express a membrane- bound form of gD have been isolated. gD produced in these cells was found to be processed, glycosylated, and exported to the cell surface in a manner similar to that which occurs in virus infected cells. In other studies we have tailored HSV-1 to be secreted from mammalian cells. Removal of the gene fragment coding for the carboxy- terminal 93 amino acid residues resulted in the synthesis of a truncated protein which lacked the membrane binding domain and was secreted into the culture medium. Mice immunized with this secreted protein formed neutralizing antibodies to both HSV-1 and HSV-2 and were protected from a lethal challenge with HSV-1. These studies represent the first steps towards the development of an efficatious subunit vaccine for human HSV infections.

1399 COMPARATIVE EFFICACY OF DIFFERENT ANTIVIRAL AGENTS IN THE TOPICAL TREATMENT OF CUTANEOUS HSV-1 AND HSV-2 INFECTIONS IN HAIRLESS MICE, Erik De Clercq, Rega Institute for Medical Research, Katholieke Universiteit Leuven, B-3000 Leuven, Belgium

Intracutaneous infection of hairless (hr/hr) mice with HSV-1 or HSV-2 was used as a model for evaluating the topical efficacy of several antiherpes agents, i.e. (E)-5-(2-bromovinyl)-2'-deoxyuridine (BVDU), 1-β-D-arabinofuranosylthymine (araT), 1-β-D-arabinofuranosyl-(E)-5-(2-bromovinyl)uracil (BVaraU), 5-iodo-2'-deoxyuridine (IDU), 5-ethyl-2'-deoxyuridine (EDU) and 9-(2-hydroxyethoxymethyl)guanine (acyclovir, ACV). The compounds were applied topically 4 times a day for 5 days starting on the day of infection. They were formulated at different concentrations (1, 3 or 10 %) in either Beeler base, polyethylene glycol, Tween-glycerol, dimethylsulphoxide (DMSO), Azone 5 % in water or Azone 5 % in DMSO. Three parameters of infection were followed : development of skin lesions, paralysis (of the hind legs) and mortality.

BVDU was outstanding in its protective activity against HSV-1 infection. Regardless of the choice of the vehicle, topical BVDU completely suppressed all parameters of the infection in 80, 90 or 100 % of the mice when administered at 1, 3 or 10 %, respectively. The other drugs were markedly less active, BVaraU, araT and IDU achieving protection against HSV-1 infection in one half to two thirds of the mice only if administered at 10 % (in DMSO), and EDU being virtually inefficacious at 10 %. When applied at 10 % in DMSO, ACV completely prevented the development of skin lesions, and paralysis and mortality therewith associated, in HSV-2-infected mice. Under the same experimental conditions, BVDU, IDU and EDU proved ineffective against HSV-2 infection. These findings establish the superior activity of BVDU and ACV in the topical treatment of HSV-1 and HSV-2 infections, respectively.

1400 SEQUENTIAL STUDIES USING ACYCLOVIR AND ITS ANALOGUE IN A PROPHYLAXIS OF HERPES INFECTION IN THE IMMUNOCOMPROMISED HOST, H.Grant Prentice*, David Brigden#
*Royal Free Hospital, London, #Wellcome Research Laboratories, U.K.

Studies from our own group[1] (Study 1) and from Johns Hopkins Hospital[2] have shown that it is possible to prevent recurrence of herpes simplex (HSV) infection in the immunocompromised (IC) bone marrow transplant (BMT) recipient using intravenous (IV) Acyclovir (ACV). Because infections with herpes zoster virus (VZV) and cytomegalovirus (CMV) occur later in BMT recipients, protection against these viruses with IV ACV was not evaluable. Post trial infections in BMT recipients with herpes viruses were very common. Three subsequent studies with oral Acyclovir or its analogues have been undertaken with a view to longer term prophylaxis in the IC host. In Study 2 oral Acyclovir 400mg 6 hourly was taken for 6 weeks following the date of BMT.[3] One of 20 patients was non-compliant and developed HSV stomatitis (and fatal CMV pneumonitis on ACV). Four others developed HSV lesions. Protection with oral ACV was incomplete and study 3 was instituted, in which BMT patients received either oral ACV or were randomly allocated during one week to receive compound 134U a well absorbed ACV analogue. Pharmacokinetic profiles will be presented. In Study 4 a further ACV analogue which is near 100% absorbed, achieving peak blood levels equivalent to those seen with IV ACV will be discussed.

Refs:(1) Hann et al. Brit. Med. J. 1983, 287: 384; (2) Saral et al. N. Eng. J. Med. 1981, 305: 63; (3) Prentice et al. J. Infect. 1983, 6, (Suppl.1):17.