
An effective vaccine against human immunodeficiency virus (HIV) should protect against mucosal transmission of genetically divergent isolates. As a safe alternative to live attenuated vaccines, the immunogenicity and protective efficacy of a DNA vaccine containing simian immunodeficiency virus (SIV) strain 17E-Fr (SIV/17E-Fr) gag-pol-env was analyzed in rhesus macaques. Significant levels of cytotoxic T lymphocytes (CTL), but low to undetectable serum antibody responses, were observed following multiple immunizations. SIV-specific mucosal antibodies and CTL were also detected in rectal washes and gut-associated lymphoid tissues, respectively. Vaccinated and naive control monkeys were challenged intrarectally with SIV strain DeltaB670 (SIV/DeltaB670), a primary isolate whose env is 15% dissimilar to that of the vaccine strain. Four of seven vaccinees were protected from infection as determined by the inability to identify viral RNA or DNA sequences in the peripheral blood and the absence of anamnestic antibody responses postchallenge. This is the first report of mucosal protection against a primary pathogenic, heterologous isolate of SIV by using a commercially viable vaccine approach. These results support further development of a DNA vaccine for protection against HIV.


We recently described the derivation of a dengue serotype 2 virus (DEN2mutF) that exhibited a host range-restricted phenotype; it was severely impaired for replication in cultured mosquito cells (C6/36 cells). DEN2mutF virus had selected mutations in genomic sequences predicted to form a 3' stem-loop structure (3'-SL) that is conserved among all flavivirus species. The 3'-SL constitutes the downstream terminal [-]95 nucleotides of the 3' noncoding region in flavivirus RNA. Here we report the introduction of these same mutational changes into the analogous region of an infectious DNA derived from the genome of a human-virulent dengue serotype 1 virus (DEN1), strain Western Pacific (DEN1WP). The resulting DEN1 mutant (DEN1mutF) exhibited a host range-restricted phenotype similar to that of DEN2mutF virus. DEN1mutF virus was attenuated in a monkey model for dengue infection in which viremia is taken as a correlate of human virulence. In spite of the markedly reduced levels of viremia that it induced in monkeys compared to DEN1WP, DEN1mutF was highly immunogenic. In addition, DEN1mutF-immunized monkeys retained high levels of neutralizing antibodies in serum and were protected from challenge with
high doses of the DEN1WP parent for as long as 17 months after the single immunizing dose. Phenotypic revertants of DEN1mutF and DEN2mutF were each detected after a total of 24 days in C6/36 cell cultures. Complete nucleotide sequence analysis of DEN1mutF RNA and that of a revertant virus, DEN1mutFRev, revealed that (i) the DEN1mutF genome contained no additional mutations upstream from the 3'-SL compared to the DEN1WP parent genome and (ii) the DEN1mutFRev genome contained de novo mutations, consistent with our previous hypothesis that the defect in DEN2mutF replication in C6/36 cells was at the level of RNA replication. A strategy for the development of a tetravalent dengue vaccine is discussed.


http://jvi.asm.org/cgi/content/abstract/76/7/3232

Postweaning multisystemic wasting syndrome (PMWS) is a disease of nursery and fattening pigs characterized by growth retardation, paleness of the skin, dyspnea, and increased mortality rates. Porcine circovirus 2 (PCV2) has been demonstrated to be the cause of PMWS. However, other factors are needed for full development of the syndrome, and porcine reproductive and respiratory syndrome virus (PRRSV) infection has been suggested to be one of them. Twenty-four conventional 5-week-old pigs were distributed in four groups: control (n = 5), PRRSV inoculated (n = 5), PCV2 inoculated (n = 7), and PRRSV and PCV2 inoculated (n = 7). The two groups inoculated with PRRSV showed growth retardation. Pigs inoculated with both PRRSV and PCV2 had increased rectal temperature. One of these pigs developed wasting, had severe respiratory distress, and died. The most important microscopic lesion in pigs inoculated with PCV2 was lymphocyte depletion with histiocytic infiltration of the lymphoid organs, more severe and in a wider range of tissues in doubly inoculated pigs. Interstitial pneumonia was observed in the three inoculated groups. PCV2 nucleic acid was found by in situ hybridization in larger amounts and in a wider range of lymphoid tissues in PRRSV- and PCV2-inoculated than in PCV2-inoculated pigs. TaqMan PCR was performed to quantify the PCV2 loads in serum during the experiment. PCV2 loads were higher in doubly inoculated pigs than in pigs inoculated with PCV2 alone. These findings indicate that severe disease can be reproduced in conventional 5-week-old pigs by inoculation of PRRSV and PCV2. Moreover, these results support the hypothesis that PRRSV infection enhances PCV2 replication.


http://jvi.asm.org/cgi/content/abstract/78/7/3777

Feline immunodeficiency virus (FIV) is a lentivirus that causes AIDS-like immunodeficiency disease in domestic cats. Free-ranging lions, Panthera leo, carry a chronic species-specific strain of FIV, FIV-Ple, which so far has not been convincingly connected with immune pathology or mortality. FIV-Ple, harboring the three distinct strains A, B, and C defined by pol gene sequence divergences, is endemic in the large outbred population of lions in the Serengeti ecosystem in Tanzania. Here we describe the pattern of variation in the three FIV genes gag, pol-RT, and pol-RNase among lions within 13 prides to assess the occurrence of FIV infection and coinfection. Genome diversity within and among FIV-Ple strains is shown to be large, with strain divergence for each gene approaching genetic distances observed for FIV between different species of cats. Multiple infections with two or three strains were found in 43% of the FIV-positive individuals based on pol-RT sequence analysis, which may suggest that antiviral immunity or interference
evoked by one strain is not consistently protective against infection by a second. This comprehensive study of FIV-Ple in a free-ranging population of lions reveals a dynamic transmission of virus in a social species that has historically adapted to render the virus benign.


http://jvi.asm.org/cgi/content/abstract/79/7/3938

Prior studies, which have relied upon the use of pseudovirions generated in heterologous cell types, have led to sometimes conflicting conclusions regarding the role of the minor capsid protein of papillomaviruses, L2, in the viral life cycle. In this study we carry out analyses with true virus particles assembled in the natural host cell to assess L2's role in the viral infectious life cycle. For these studies we used the organotypic (raft) culture system to recapitulate the full viral life cycle of the high-risk human papillomavirus HPV31, which was either wild type or mutant for L2. After transfection, the L2 mutant HPV31 genome was able to establish itself as a nuclear plasmid in proliferating populations of poorly differentiated (basal-like) human keratinocytes and to amplify its genome to high copy number, support late viral gene expression, and cause formation of virus particles in human keratinocytes that had been induced to undergo terminal differentiation. These results indicate that aspects of both the nonproductive and productive phases of the viral life cycle occur normally in the absence of functional L2. However, upon the analysis of the virus particles generated, we found an approximate 10-fold reduction in the amount of viral DNA encapsidated into L2-deficient virions. Furthermore, there was an over-100-fold reduction in the infectivity of L2-deficient virus. Because the latter deficiency cannot be accounted for solely by the 10-fold decrease in encapsidation, we conclude that L2 contributes to at least two steps in the production of infectious virus.


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The number, chromosomal distribution, and insertional polymorphisms of endogenous feline leukemia viruses (enFeLVs) were determined in four domestic cats (Burmese, Egyptian Mau, Persian, and nonbreed) using fluorescent in situ hybridization and radiation hybrid mapping. Twenty-nine distinct enFeLV loci were detected across 12 of the 18 autosomes. Each cat carried enFeLV at only 9 to 16 of the loci, and many loci were heterozygous for presence of the provirus. Thus, an average of 19 autosomal copies of enFeLV were present per cat diploid genome. Only five of the autosomal enFeLV sites were present in all four cats, and at only one autosomal locus, B4q15, was enFeLV present in both homologues of all four cats. A single enFeLV occurred in the X chromosome of the Burmese cat, while three to five enFeLV proviruses occurred in each Y chromosome. The X chromosome and nine autosomal enFeLV loci were telomeric, suggesting that ectopic recombination between nonhomologous subtelomeres may contribute to enFeLV distribution. Since endogenous FeLVs may affect the infectiousness or pathogenicity of exogenous FeLVs, genomic variation in enFeLVs represents a candidate for genetic influences on FeLV leukemogenesis in cats.

Silvestri, G., A. Fedanov, et al. (2005). "Divergent Host Responses during Primary Simian Immunodeficiency Virus SIVsm Infection of Natural Sooty Mangabey and Nonnatural Rhesus
To understand how natural sooty mangabey hosts avoid AIDS despite high levels of simian immunodeficiency virus (SIV) SIVsm replication, we inoculated mangabeys and nonnatural rhesus macaque hosts with an identical inoculum of uncloned SIVsm. The unpassaged virus established infection with high-level viral replication in both macaques and mangabeys. A species-specific, divergent immune response to SIV was evident from the first days of infection and maintained in the chronic phase, with macaques showing immediate and persistent T-cell proliferation, whereas mangabeys displayed little T-cell proliferation, suggesting subdued cellular immune responses to SIV. Importantly, only macaques developed CD4+-T-cell depletion and AIDS, thus indicating that in mangabeys limited immune activation is a key mechanism to avoid immunodeficiency despite high levels of SIVsm replication. These studies demonstrate that it is the host response to infection, rather than properties inherent to the virus itself, that causes immunodeficiency in SIV-infected nonhuman primates.

Feline infectious peritonitis virus (FIPV), a coronavirus, is the causative agent of an invariably lethal infection in cats. Like other coronaviruses, FIPV contains an extremely large positive-strand RNA genome of ca. 30 kb. We describe here the development and use of a reverse genetics strategy for FIPV based on targeted RNA recombination that is analogous to what has been described for the mouse hepatitis virus (MHV) (L. Kuo et al., J. Virol. 74:1393-1406, 2000). In this two-step process, we first constructed by targeted recombination a mutant of FIPV, designated mFIPV, in which the ectodomain of the spike glycoprotein was replaced by that of MHV. This switch allowed for the selection of the recombinant virus in murine cells: mFIPV grows to high titers in these cells but has lost the ability to grow in feline cells. In a second, reverse process, mFIPV was used as the recipient, and the reintroduction of the FIPV spike now allowed for selection of candidate recombinants by their regained ability to grow in feline cells. In this fashion, we reconstructed a wild-type recombinant virus (r-wtFIPV) and generated a directed mutant FIPV in which the initiation codon of the nonstructural gene 7b had been disrupted (FIPV(Delta)7b). The r-wtFIPV was indistinguishable from its parental virus FIPV 79-1146 not only for its growth characteristics in tissue culture but also in cats, exhibiting a highly lethal phenotype. FIPV(Delta)7b had lost the expression of its 7b gene but grew unimpaired in cell culture, confirming that the 7b glycoprotein is not required in vitro. We establish the second targeted RNA recombination system for coronaviruses and provide a powerful tool for the genetic engineering of the FIPV genome.

The importance of the small envelope (E) protein in the assembly of coronaviruses has been demonstrated in several studies. While its precise function is not clearly defined, E is a pivotal player in the morphogenesis of the virion envelope. Expression of the E protein alone results in its incorporation into vesicles that are released from cells, and the coexpression of the E protein with
the membrane protein M leads to the assembly of coronavirus-like particles. We have previously generated E gene mutants of mouse hepatitis virus (MHV) that had marked defects in viral growth and produced virions that were aberrantly assembled in comparison to wild-type virions. We have now been able to obtain a viable MHV mutant in which the entire E gene, as well as the nonessential upstream genes 4 and 5a, has been deleted. This mutant (ΔE) was obtained by a targeted RNA recombination method that makes use of a powerful host range-based selection system. The ΔE mutant produces tiny plaques with an unusual morphology compared to plaques formed by wild-type MHV. Despite its low growth rate and low infectious titer, the ΔE mutant is genetically stable, showing no detectable phenotypic changes after several passages. The properties of this mutant provide further support for the importance of E protein in MHV replication, but surprisingly, they also show that E protein is not essential.


http://jvi.asm.org/cgi/content/abstract/77/8/4848

Bovine herpesvirus 1 (BHV-1) is an important pathogen of cattle and infection is usually initiated via the ocular or nasal cavity. After acute infection, the primary site for BHV-1 latency is sensory neurons in the trigeminal ganglia (TG). Reactivation from latency occurs sporadically, resulting in virus shedding and transmission to uninfected cattle. The only abundant viral transcript expressed during latency is the latency-related (LR) RNA. An LR mutant was constructed by inserting three stop codons near the beginning of the LR RNA. This mutant grows to wild-type (wt) efficiency in bovine kidney cells and in the nasal cavity of acutely infected calves. However, shedding of infectious virus from the eye and TG was dramatically reduced in calves infected with the LR mutant. Calves latently infected with the LR mutant do not reactivate after dexamethasone treatment. In contrast, all calves latently infected with wt BHV-1 or the LR rescued mutant reactivate from latency after dexamethasone treatment. In the present study, we compared the frequency of apoptosis in calves infected with the LR mutant to calves infected with wt BHV-1 because LR gene products inhibit apoptosis in transiently transfected cells. A sensitive TUNEL (terminal deoxynucleotidyltransferase-mediated dUTP-biotin nick end labeling) assay and an antibody that detects cleaved caspase-3 were used to identify apoptotic cells in TG. Both assays demonstrated that calves infected with the LR mutant for 14 days had higher levels of apoptosis in TG compared to calves infected with wt BHV-1 or to mock-infected calves. Viral gene expression, except for the LR gene, is extinguished by 14 days after infection, and thus this time frame is operationally defined as the establishment of latency. Real-time PCR analysis indicated that lower levels of viral DNA were present in the TG of calves infected with the LR mutant throughout acute infection. Taken together, these results suggest that the antiapoptotic properties of the LR gene play an important role during the establishment of latency.


http://jvi.asm.org/cgi/content/abstract/77/8/4489

An open reading frame (ORF), US28, with homology to mammalian chemokine receptors has been identified in the genome of human cytomegalovirus (HCMV). Its protein product, pUS28, has been shown to bind several human CC chemokines, including RANTES, MCP-1, and MIP-1α, and the CX3C chemokine fractalkine with high affinity. Addition of CC chemokines to cells expressing pUS28 was reported to cause a pertussis toxin-sensitive increase in the
concentration of cytosolic free Ca2+. Recently, pUS28 was shown to mediate constitutive, ligand-independent, and pertussis toxin-insensitive activation of phospholipase C via Gq/11-dependent signaling pathways in transiently transfected COS-7 cells. Since these findings are not easily reconciled with the former observations, we analyzed the role of pUS28 in mediating CC chemokine activation of pertussis toxin-sensitive G proteins in cell membranes and phospholipase C in intact cells. The transmembrane signaling functions of pUS28 were studied in HCMV-infected cells rather than in cDNA-transfected cells. Since DNA sequence analysis of ORF US28 of different laboratory and clinical strains had revealed amino acid sequence differences in the amino-terminal portion of pUS28, we compared two laboratory HCMV strains, AD169 and Toledo, and one clinical strain, TB40/E. The results showed that infection of human fibroblasts with all three HCMV strains led to a vigorous, constitutively enhanced formation of inositol phosphates which was insensitive to pertussis toxin. This effect was critically dependent on the presence of the US28 ORF in the HCMV genome but was independent of the amino acid sequence divergence of the three HCMV strains investigated. The constitutive activity of pUS28 is not explained by expression of pUS28 at high density in HCMV-infected cells. The pUS28 ligands RANTES and MCP-1 failed to stimulate binding of guanosine 5'-O-(3-[35S]thiotriphosphate to membranes of HCMV-infected cells and did not enhance constitutive activation of phospholipase C in intact HCMV-infected cells. These findings raise the possibility that the effects of CC chemokines and pertussis toxin on G protein-mediated transmembrane signaling previously observed in HCMV-infected cells are either independent of or not directly mediated by the protein product of ORF US28.


Understanding the early cytokine response to lentiviral infections may be critical to the design of prevention and treatment strategies. By using the feline immunodeficiency virus (FIV) model, we have documented an interleukin 10 (IL10)-dominated response in lymphoid tissue CD4+ and CD8+ T lymphocytes within the first 4 weeks after mucosal FIV infection. This profile coincided with the period of high tissue viral replication. By 10 weeks postinfection, tissue viral levels decreased significantly, and gamma interferon (IFN(gamma)) production in CD8+ T cells had increased to restore the IL10/IFN(gamma) ratio to control levels. Concurrently, increased production of IL6 and viral RNA was detected in macrophages. These temporal associations of viral replication with cytokine balance in tissues suggest roles for IL10 in the permissive stage of infection and IFN(gamma) in the subsequent down modulation of lentiviral infection.


Feline infectious peritonitis (FIP) is a fatal immunity-mediated disease caused by mutants of a ubiquitous coronavirus. Since previous attempts to protect cats under laboratory and field conditions have been largely unsuccessful, we used our recently developed system of reverse genetics (B. J. Haijema, H. Volders, and P. J. M. Rottier, J. Virol. 77:4528-4538, 2003) for the development of a modified live FIP vaccine. With this objective, we deleted the group-specific gene cluster open reading frame 3abc or 7ab and obtained deletion mutant viruses that not only multiplied well in cell culture but also showed an attenuated phenotype in the cat. At doses at
which the wild-type virus would be fatal, the mutants with gene deletions did not cause any clinical symptoms. They still induced an immune response, however, as judged from the high levels of virus-neutralizing antibodies. The FIP virus (FIPV) mutant lacking the 3abc cluster and, to a lesser extent, the mutant missing the 7ab cluster, protected cats against a lethal homologous challenge; no protection was obtained with the mutant devoid of both gene clusters. Our studies show that the deletion of group-specific genes from the coronavirus genome results in live attenuated candidate vaccines against FIPV. More generally, our approach may allow the development of vaccines against infections with other pathogenic coronaviruses, including that causing severe acute respiratory syndrome in humans.


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Human T-cell lymphotropic virus type 1 (HTLV-1) causes adult T-cell leukemia/lymphoma and exhibits high genetic stability in vivo. HTLV-1 contains four open reading frames (ORFs) in its pX region. ORF II encodes two proteins, p30II and p13II, both of which are incompletely characterized. p30II localizes to the nucleus or nucleolus and has distant homology to the transcription factors Oct-1, Pit-1, and POU-M1. In vitro studies have demonstrated that at low concentrations, p30II differentially regulates cellular and viral promoters through an interaction with CREB binding protein/p300. To determine the in vivo significance of p30II, we inoculated rabbits with cell lines expressing either a wild-type clone of HTLV-1 (ACH.1) or a clone containing a mutation in ORF II, which eliminated wild-type p30II expression (ACH.30.1). ACH.1-inoculated rabbits maintained higher HTLV-1-specific antibody titers than ACH.30.1-inoculated rabbits, and all ACH.1-inoculated rabbits were seropositive for HTLV-1, whereas only two of six ACH.30.1-inoculated rabbits were seropositive. Provirus could be consistently PCR amplified from peripheral blood mononuclear cell (PBMC) DNA in all ACH.1-inoculated rabbits but in only three of six ACH.30.1-inoculated rabbits. Quantitative competitive PCR indicated higher PBMC proviral loads in ACH.1-inoculated rabbits. Interestingly, sequencing of ORF II from PBMC of provirus-positive ACH.30.1-inoculated rabbits revealed a reversion to wild-type sequence with evidence of early coexistence of mutant and wild-type sequence. Our data provide evidence that HTLV-1 must maintain its key accessory genes to survive in vivo and that in vivo pressures select for maintenance of wild-type ORF II gene products during the early course of infection.


http://jvi.asm.org/cgi/content/abstract/78/8/4330

The largest outbreak on record of Ebola hemorrhagic fever (EHF) occurred in Uganda from August 2000 to January 2001. The outbreak was centered in the Gulu district of northern Uganda, with secondary transmission to other districts. After the initial diagnosis of Sudan ebolavirus by the National Institute for Virology in Johannesburg, South Africa, a temporary diagnostic laboratory was established within the Gulu district at St. Mary's Lacor Hospital. The laboratory used antigen capture and reverse transcription-PCR (RT-PCR) to diagnose Sudan ebolavirus infection in suspect patients. The RT-PCR and antigen-capture diagnostic assays proved very effective for detecting ebolavirus in patient serum, plasma, and whole blood. In samples collected very early in the course of infection, the RT-PCR assay could detect ebolavirus 24 to 48 h prior to detection by antigen capture. More than 1,000 blood samples were collected, with multiple
samples obtained from many patients throughout the course of infection. Real-time quantitative RT-PCR was used to determine the viral load in multiple samples from patients with fatal and nonfatal cases, and these data were correlated with the disease outcome. RNA copy levels in patients who died averaged 2 log10 higher than those in patients who survived. Using clinical material from multiple EHF patients, we sequenced the variable region of the glycoprotein. This Sudan ebolavirus strain was not derived from either the earlier Boniface (1976) or Maleo (1979) strain, but it shares a common ancestor with both. Furthermore, both sequence and epidemiologic data are consistent with the outbreak having originated from a single introduction into the human population.


Subgroup D adenovirus (Ad) types 8, 19, and 37 (Ad8, -19, and -37, respectively) are causative agents of epidemic keratoconjunctivitis and genital tract infections. Previous studies showed that Ad37 binds to a 50-kDa membrane glycoprotein expressed on human ocular (conjunctival) cells. To identify and characterize the role of the 50-kDa glycoprotein in Ad37 infection, we partially purified this molecule from solubilized Chang C conjunctival cell membranes by using lentil lectin chromatography and preparative sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Liquid chromatography coupled to nano-electrospray ionization-tandem mass spectrometry was subsequently used to identify four Ad37 receptor candidates: CD46, CD87, CD98, and CD147. Immunodepletion analyses demonstrated that the 50-kDa protein is identical to CD46 (also known as membrane cofactor protein). The Ad37, but not Ad5, fiber knob bound to the extracellular domain of CD46, demonstrating a direct interaction of an Ad37 capsid protein with CD46. An antibody specific for the N-terminal 19 amino acids of CD46 also blocked Ad37 infection of human cervical carcinoma and conjunctival cells, indicating a requirement for CD46 in infection. Finally, expression of a 50-kDa isoform of human CD46 in a CD46-null cell line increased cell binding by wild-type Ad37 and gene delivery by an Ad vector pseudotyped with the Ad37 fiber, but not by a vector bearing the Ad5 fiber. Together, these studies demonstrate that CD46 serves as an attachment receptor for Ad37 and shed further light on the cell entry pathway of subgroup D Ads.


Noroviruses are single-stranded RNA viruses with high genomic variability. They have emerged in the last decade as a major cause of acute gastroenteritis. It remains so far unclear whether norovirus evolution is driven by sequence mutation and/or recombination. In this study, we have assessed the occurrence of recombination in the norovirus capsid gene. For this purpose, 69 complete capsid sequences of norovirus strains accessible in GenBank as well as 25 complete capsid sequences generated from norovirus-positive clinical samples were examined. Unreported recombination was detected in about 8% of norovirus strains belonging to genetic clusters I/1 (n = 1), II/1 (n = 1), II/3 (n = 1), II/4 (n = 3), and II/5 (n = 1). Recombination breakpoints were mainly located at the interface of the putative P1-1 and P2 domains of the capsid protein and/or within the P2 domain. The recombination region displayed features such as length, sequence composition (upstream and downstream GC- and AU-rich sequences, respectively), and predicted RNA secondary structure that are characteristic of homologous recombination activators. Our results suggest that recombination in the norovirus capsid gene may naturally
occur, involving capsid domains presumably exposed to immunological pressure.


Tamarins (Saguinus species) infected by GB virus B (GBV-B) have recently been proposed as an acceptable surrogate model for hepatitis C virus (HCV) infection. The availability of infectious genomic molecular clones of both viruses will permit chimeric constructs to be tested for viability in animals. Studies in cells with parental and chimeric constructs would also be very useful for both basic research and drug discovery. For this purpose, a convenient host cell type supporting replication of in vitro-transcribed GBV-B RNA should be identified. We constructed a GBV-B subgenomic selectable replicon based on the sequence of a genomic molecular clone proved to sustain infection in tamarins. The corresponding in vitro-transcribed RNA was used to transfect the Huh7 human hepatoma cell line, and intracellular replication of transfected RNA was shown to occur, even though in a small percentage of transfected cells, giving rise to antibiotic-resistant clones. Sequence analysis of GBV-B RNA from some of those clones showed no adaptive mutations with respect to the input sequence, whereas the host cells sustained higher GBV-B RNA replication than the original Huh7 cells. The enhancement of replication depending on host cell was shown to be a feature common to the majority of clones selected. The replication of GBV-B subgenomic RNA was susceptible to inhibition by known inhibitors of HCV to a level similar to that of HCV subgenomic RNA.


Boid inclusion body disease (BIBD) is a fatal disorder of boid snakes that is suspected to be caused by a retrovirus. In order to identify this agent, leukocyte cultures (established from Python molurus specimens with symptoms of BIBD or kept together with such diseased animals) were assessed for reverse transcriptase (RT) activity. Virus from cultures exhibiting high RT activity was banded on sucrose density gradients, and the RT peak fraction was subjected to highly efficient procedures for the identification of unknown particle-associated retroviral RNA. A 7-kb full retroviral sequence was identified, cloned, and sequenced. This virus contained intact open reading frames (ORFs) for gag, pro, pol, and env, as well as another ORF of unknown function within pol. Phylogenetic analysis showed that the virus is distantly related to viruses from both the B and D types and the mammalian C type but cannot be classified. It is present as a highly expressed endogenous retrovirus in all P. molurus individuals; a closely related, but much less expressed virus was found in all tested Python curtus individuals. All other boid snakes tested, including Python regius, Python reticulatus, Boa constrictor, Eunectes notaeus, and Morelia spilota, were virus negative, independent of whether they had BIBD or not. Virus isolated from P. molurus could not be transmitted to the peripheral blood mononuclear cells of B. constrictor or P. regius. Thus, there is no indication that this novel virus, which we propose to name python endogenous retrovirus (PyERV), is causally linked with BIBD.
Herpesvirus gene expression is divided into immediate-early (IE) or \{alpha\} genes, early (E) or \{beta\} genes, and late (L) or \{gamma\} genes on the basis of temporal expression and dependency on other gene products. By using real-time PCR, we have investigated the expression of 35 human herpesvirus 6B (HHV-6B) genes in T cells infected by strain PL-1. Kinetic analysis and dependency on de novo protein synthesis and viral DNA polymerase activity suggest that the HHV-6B genes segregate into six separate kinetic groups. The genes expressed early (groups I and II) and late (groups V and VI) corresponded well with IE and L genes, whereas the intermediate groups III and IV contained E and L genes. Although HHV-6B has characteristics similar to those of other roseoloviruses in its overall gene regulation, we detected three B-variant-specific IE genes. Moreover, genes that were independent of de novo protein synthesis clustered in an area of the viral genome that has the lowest identity to the HHV-6A variant. The organization of IE genes in an area of the genome that differs from that of HHV-6A underscores the distinct differences between HHV-6B and HHV-6A and may provide a basis for further molecular and immunological analyses to elucidate their different biological behaviors.

The papain-like protease p29, derived from the N-terminal portion of the hypovirus CHV1-EP713-encoded open reading frame (ORF) A polyprotein, p69, was previously shown to contribute to reduced pigmentation and sporulation by the infected host, the chestnut blight fungus Cryphonectria parasitica, while being dispensable for virus replication and attenuation of fungal virulence (hypovirulence). We now report that deletion of the C-terminal portion of p69, which encodes the highly basic protein p40, resulted in replication-competent mutant viruses that were, however, significantly reduced in RNA accumulation. While the \(\Delta\)p40 mutants retained the ability to confer hypovirulence, \(\Delta\)p40-infected fungal strains produced more asexual spores than strains infected with either wild-type CHV1-EP713 or a \(\Delta\)p29 mutant virus. As observed for \(\Delta\)p29-infected colonies, pigment production was significantly increased in \(\Delta\)p40-infected fungal strains relative to that in CHV1-EP713-infected strains. Virus-mediated suppression of laccase production was not affected by p40 deletion. A gain-of-function analysis was employed to map the p40 symptom determinant to the N-terminal domain, encompassing p69 amino acid residues Thr(288) to Arg(312). Evidence that the gain of function was due to the encoded protein rather than the corresponding RNA sequence element was provided by introducing frameshift mutations on either side of the activity determinant domain. Moreover, restoration of symptoms correlated with increased accumulation of viral RNA. These results suggest that p40 indirectly contributes to virus-mediated suppression of fungal pigmentation and conidiation by providing an accessory function in hypovirus RNA amplification. A possible role for p40 in facilitating ORF B expression and the relationship between hypovirus RNA accumulation and symptom expression are discussed.
Human herpesvirus 6 (HHV-6) is a potentially immunosuppressive agent that has been suggested to act as a cofactor in the progression of human immunodeficiency virus disease. However, the lack of suitable experimental models has hampered the elucidation of the mechanisms of HHV-6-mediated immune suppression. Here, we used ex vivo lymphoid tissue to investigate the cellular tropism and pathogenic mechanisms of HHV-6. Viral strains belonging to both HHV-6 subgroups (A and B) were able to productively infect human tonsil tissue fragments in the absence of exogenous stimulation. The majority of viral antigen-expressing cells were CD4+ T lymphocytes expressing a nonnaive phenotype, while CD8+ T cells were efficiently infected only with HHV-6A. Accordingly, HHV-6A infection resulted in the depletion of both CD4+ and CD8+ T cells, whereas in HHV-6B-infected tissue CD4+ T cells were predominantly depleted.

The expression of different cellular antigens was dramatically altered in HHV-6-infected tissues: whereas CD4 was upregulated, both CD46, which serves as a cellular receptor for HHV-6, and CD3 were downmodulated. However, CD3 downmodulation was restricted to infected cells, while the loss of CD46 expression was generalized. Moreover, HHV-6 infection markedly enhanced the production of the CC chemokine RANTES, whereas other cytokines and chemokines were only marginally affected. These results provide the first evidence, in a physiologically relevant study model, that HHV-6 can severely affect the physiology of secondary lymphoid organs through direct infection of T lymphocytes and modulation of key membrane receptors and chemokines.


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A family of cellular nucleic acid binding proteins (CNBPs) contains seven Zn2+ fingers that have many of the structural characteristics found in retroviral nucleocapsid (NC) Zn2+ fingers. The sequence of the NH2-terminal NC Zn2+ finger of the pNL4-3 clone of human immunodeficiency virus type 1 (HIV-1) was replaced individually with sequences from each of the seven fingers from human CNBP. Six of the mutants were normal with respect to protein composition and processing, full-length genomic RNA content, and infectivity. One of the mutants, containing the fifth CNBP Zn2+ finger (CNBP-5) packaged reduced levels of genomic RNA and was defective in infectivity. There appear to be defects in reverse transcription in the CNBP-5 infections. Models of Zn2+ fingers were constructed by using computational methods based on available structural data, and atom-atom interactions were determined by the hydrophobic orthogonal dynamic analysis of the protein method. Defects in the CNBP-5 mutant could possibly be explained, in part, by restrictions of a set of required atom-atom interactions in the CNBP-5 Zn2+ finger compared to mutant and wild-type Zn2+ fingers in NC that support replication. The present study shows that six of seven of the Zn2+ fingers from the CNBP protein can be used as substitutes for the Zn2+ finger in the NH2-terminal position of HIV-1 NC. This has obvious implications in antiviral therapeutics and DNA vaccines employing NC Zn2+ finger mutants.


http://jvi.asm.org/cgi/content/abstract/78/15/8349

By analysis of a single, variable, and short DNA sequence of 447 bp located within open reading frame 22 (ORF22), we discriminated three major varicella-zoster virus (VZV) genotypes. VZV isolates from all six inhabited continents that showed nearly complete homology to ORF22 of the European reference strain Dumas were assigned to the European (E) genotype. All Japanese isolates, defined as the Japanese (J) genotype, were identical in the respective genomic region
and proved the most divergent from the E strains, carrying four distinct variations. The remaining isolates carried a combination of E- and J-specific variations in the target sequence and thus were collectively termed the mosaic (M) genotype. Three hundred twenty-six isolates collected in 27 countries were genotyped. A distinctive longitudinal distribution of VZV genotypes supports this approach. Among 111 isolates collected from European patients, 96.4% were genotype E. Consistent with this observation, approximately 80% of the VZV strains from the United States were also genotype E. Similarly, genotype E viruses were dominant in the Asian part of Russia and in eastern Australia. M genotype viruses were strongly dominant in tropical regions of Africa, Indochina, and Central America, and they were common in western Australia. However, genotype M viruses were also identified as a minority in several countries worldwide. Two major intertypic variations of genotype M strains were identified, suggesting that the M genotype can be further differentiated into subgenotypes. These data highlight the direction for future VZV genotyping efforts. This approach provides the first simple genotyping method for VZV strains in clinical samples.


http://jvi.asm.org/cgi/content/abstract/76/16/8298

In the present study, we describe a new simian immunodeficiency virus (SIV), designated SIVgsn, naturally infecting greater spot-nosed monkeys (Cercopithecus nictitans) in Cameroon. Together with SIVsyk, SIVgsn represents the second virus isolated from a monkey belonging to the Cercopithecus mitis group of the Cercopithecus genus. Full-length genome sequence analysis of two SIVgsn strains, SIVgsn-99CM71 and SIVgsn-99CM166, revealed that despite the close phylogenetic relationship of their hosts, SIVgsn was highly divergent from SIVsyk. First of all, they differ in their genomic organization. SIVgsn codes for a vpu homologue, so far a unique feature of the members of the SIVcpz/human immunodeficiency virus type 1 (HIV-1) lineage, and detailed phylogenetic analyses of various regions of the viral genome indicated that SIVgsn might be a mosaic of sequences with different evolutionary histories. SIVgsn was related to SIVsyk in Gag and part of Pol and related to SIVcpz in Env, and the middle part of the genome did not cluster significantly with any of the known SIV lineages. When comparing the two SIVgsn Env sequences with that of SIVcpz, a remarkable conservation was seen in the V3 loop, indicating a possible common origin for the envelopes of these two viruses. The habitats of the two subspecies of chimpanzees infected by SIVcpz overlap the geographic ranges of greater spot-nosed monkeys and other monkey species, allowing cross-species transmission and recombination between coinfecting viruses. The complex genomic structure of SIVgsn, the presence of a vpu gene, and its relatedness to SIVcpz in the envelope suggest a link between SIVgsn and SIVcpz and provide new insights about the origin of SIVcpz in chimpanzees.


http://jvi.asm.org/cgi/content/abstract/78/16/8771

Wild aquatic birds are the primary reservoir of influenza A viruses, but little is known about the viruses' gene pool in wild birds. Therefore, we investigated the ecology and emergence of influenza viruses by conducting phylogenetic analysis of 70 matrix (M) genes of influenza viruses isolated from shorebirds and gulls in the Delaware Bay region and from ducks in Alberta, Canada, during >18 years of surveillance. In our analysis, we included 61 published M genes of isolates from various hosts. We showed that M genes of Canadian duck viruses and those of shorebird
and gull viruses in the Delaware Bay shared ancestors with the M genes of North American poultry viruses. We found that North American and Eurasian avian-like lineages are divided into sublineages, indicating that multiple branches of virus evolution may be maintained in wild aquatic birds. The presence of non-H13 gull viruses in the gull-like lineage and of H13 gull viruses in other avian lineages suggested that gulls' M genes do not preferentially associate with the H13 subtype or segregate into a distinct lineage. Some North American avian influenza viruses contained M genes closely related to those of Eurasian avian viruses. Therefore, there may be interregional mixing of the two clades. Reassortment of shorebird M and HA genes was evident, but there was no correlation among the HA or NA subtype, M gene sequence, and isolation time. Overall, these results support the hypothesis that influenza viruses in wild waterfowl contain distinguishable lineages of M genes.


http://jvi.asm.org/cgi/content/abstract/76/23/12087

Human immunodeficiency virus type 1 (HIV-1) can infect nondividing cells productively because the nuclear import of viral nucleic acids occurs in the absence of cell division. A number of viral factors that are present in HIV-1 preintegration complexes (PICs) have been assigned functions in nuclear import, including an essential valine at position 165 in integrase (IN-V165) and the central polypurine tract (cPPT). In this article, we report a comparison of the replication and infection characteristics of viruses with disruptions in the cPPT and IN-V165. We found that viruses with cPPT mutations still replicated productively in both dividing and nondividing cells, while viruses with a mutation at IN-V165 did not. Direct observation of the subcellular localization of HIV-1 cDNAs by fluorescence in situ hybridization revealed that cDNAs synthesized by both mutant viruses were readily detected in the nucleus. Thus, neither the cPPT nor the valine residue at position 165 of integrase is essential for the nuclear import of HIV-1 PICs.


http://jvi.asm.org/cgi/content/abstract/76/23/11793

The predominant rotavirus electropherotypes (e-types) during 17 epidemic seasons (1980 through 1997) in Finland were established, and representative virus isolates were studied by nucleotide sequencing and phylogenetic analysis. The virus isolates were either P[8]G1 or P[8]G4 types. The G1 and G4 strains formed one G1 lineage (VP7-G1-1) and one G4 lineage, respectively. Otherwise, they belonged to two P[8] lineages (VP4-P[8]-1 and -2) unrelated to their G types. Phylogenetic analysis of partial sequences of all 11 RNA segments obtained from the strains also revealed genetic diversity among gene segments other than those defining P and G types. With the exception of segments 1, 3, and 10, the sequences of the other segments could be assigned to 2 to 4 different genetic clusters. The results of this study suggest that, in addition to the RNA segments encoding VP4 and VP7, the other RNA segments may segregate independently as well. In total, the 9 predominant e-types represented 7 different RNA segment combinations when the phylogenetic clusters of their 11 genes were determined. The extensive genetic diversity and number of e-types among rotaviruses are best explained by frequent genetic reassortment.

http://jvi.asm.org/cgi/content/abstract/77/23/12523

During a large serosurvey of wild-caught primates from Cameroon, we found 2 mona monkeys (Cercopithecus mona) out of 8 and 47 mustached monkeys (Cercopithecus cephus) out of 302 with human immunodeficiency virus (HIV)-simian immunodeficiency virus (SIV) cross-reactive antibodies. In this report, we describe the full-length genome sequences of two novel SIVs, designated SIVmon-99CMCML1 and SIVmus-01CM1085, isolated from one mona (CML1) and one mustached (1085) monkey, respectively. Interestingly, these viruses displayed the same genetic organization (i.e., presence of a vpu homologue) as members of the SIVcpz-HIV type 1 lineage and SIVgsn isolated from greater spot-nosed monkeys (Cercopithecus nictitans). Phylogenetic analyses of SIVmon and SIVmus revealed that these viruses were genetically distinct from other known primate lentiviruses but were more closely related to SIVgsn all across their genomes, thus forming a monophyletic lineage within the primate lentivirus family, which we designated the SIVgsn lineage. Interestingly, mona, mustached, and greater spot-nosed monkeys are phylogenetically related species belonging to three different groups of the genus Cercopithecus, the C. mona, C. cephus, and Cercopithecus mitis groups, respectively. The presence of new viruses closely related to SIVgsn in two other species reinforces the hypothesis that a recombination event between ancestral SIVs from the family Cercopithecinae is the origin of the present SIVcpz that is widespread among the chimpanzee population.


http://jvi.asm.org/cgi/content/abstract/77/23/12430

Control of viremia in natural human immunodeficiency virus type 1 (HIV-1) infection in humans is associated with a virus-specific T-cell response. However, still much is unknown with regard to the extent of CD8+ cytotoxic T-lymphocyte (CTL) responses required to successfully control HIV-1 infection and to what extent CTL epitope escape can account for rises in viral load and ultimate progression to disease. In this study, we chose to monitor through full-length genome sequence of replication-competent biological clones the modifications that occurred within predicted CTL epitopes and to identify whether the alterations resulted in epitope escape from CTL recognition. From an extensive analysis of 59 biological HIV-1 clones generated over a period of 4 years from a single individual in whom the viral load was observed to rise, we identified the locations in the genome of five CD8+ CTL epitopes. Fixed mutations were identified within the p17, gp120, gp41, Nef, and reverse transcriptase genes. Using a gamma interferon ELISpot assay, we identified for four of the five epitopes with fixed mutations a complete loss of T-cell reactivity against the wild-type epitope and a partial loss of reactivity against the mutant epitope. These results demonstrate that sequential accumulation of CTL escape in a patient during disease progression, indicating that multiple combinations of T-cell epitopes are required to control viremia.


http://jvi.asm.org/cgi/content/abstract/77/23/12773
The wild mouse species most closely related to the common laboratory strains contain proviral env genes of the xenotropic/polytropic subgroup of mouse leukemia viruses (MLVs). To determine if the polytropic proviruses of Mus spretus contain functional genes, we inoculated neonates with Moloney MLV (MoMLV) or amphotropic MLV (A-MLV) and screened for viral recombinants with altered host ranges. Thymus and spleen cells from MoMLV-inoculated mice were plated on Mus dunni cells and mink cells, since these cells do not support the replication of MoMLV, and cells from A-MLV-inoculated mice were plated on ferret cells. All MoMLV-inoculated mice produced ecotropic viruses that resembled their MoMLV progenitor, although some isolates, unlike MoMLV, grew to high titers in M. dunni cells. All of the MoMLV-inoculated mice also produced nonecotropic virus that was infectious for mink cells. Sequencing of three MoMLV- and two A-MLV-derived nonecotropic recombinants confirmed that these viruses contained substantial substitutions that included the regions of env encoding the surface (SU) protein and the 5' end of the transmembrane (TM) protein. The 5' recombination breakpoint for one of the A-MLV recombinants was identified in RNase H. The M. spretus-derived env substitutions were nearly identical to the corresponding regions in prototypical laboratory mouse polytropic proviruses, but the wild mouse infectious viruses had a more restricted host range. The M. spretus proviruses contributing to these recombinants were also sequenced. The seven sequenced proviruses were 99% identical to one another and to the recombinants; only two of the seven had obvious fatal defects. We conclude that the M. spretus proviruses are likely to be recent germ line acquisitions and that they contain functional genes that can contribute to the production of replication-competent virus.


http://jvi.asm.org/cgi/content/abstract/78/23/12910

Passive immunization with monoclonal antibodies from humans or nonhuman primates represents an attractive alternative to vaccines for prevention of illness caused by dengue viruses (DENV) and other flaviviruses, including the West Nile virus. In a previous study, repertoire cloning to recover Fab fragments from bone marrow mRNA of chimpanzees infected with all four DENV serotypes (dengue virus serotype 1 [DENV-1] to DENV-4) was described. In that study, a humanized immunoglobulin G1 (IgG1) antibody that efficiently neutralized DENV-4 was recovered and characterized. In this study, the phage library constructed from the chimpanzees was used to recover Fab antibodies against the other three DENV serotypes. Serotype-specific neutralizing Fabs were not identified. Instead, we recovered DENV-neutralizing Fabs that specifically precipitated the envelope protein and were cross-reactive with all four DENV serotypes. Three of the Fabs competed with each other for binding to DENV-1 and DENV-2, although each of these Fabs contained a distinct complementarity determining region 3 (CDR3)-H sequence. Fabs that shared an identical or nearly identical CDR3-H sequences cross-neutralized DENV-1 and DENV-2 at a similar high 50% plaque reduction neutralization test (PRNT50) titer, ranging from 0.26 to 1.33 {micro}g/ml, and neutralized DENV-3 and DENV-4 but at a titer 10- to 20-fold lower. One of these Fabs, 1A5, also neutralized the West Nile virus most efficiently among other flaviviruses tested. Fab 1A5 was converted to a full-length antibody in combination with human sequences for production in mammalian CHO cells. Humanized IgG1 1A5 proved to be as efficient as Fab 1A5 for cross-neutralization of DENV-1 and DENV-2 at a titer of 0.48 and 0.95 {micro}g/ml, respectively. IgG1 1A5 also neutralized DENV-3, DENV-4, and the West Nile virus at a PRNT50 titer of approximately 3.2 to 4.2 {micro}g/ml. This humanized antibody represents an attractive candidate for further development of immunoprophylaxis against DENV and perhaps other flavivirus-associated diseases.
The epitope determinants of chimpanzee Fab antibody 1A5, which have been shown to be broadly reactive to flaviviruses and efficient for cross-neutralization of dengue virus type 1 and type 2 (DENV-1 and DENV-2), were studied by analysis of DENV-2 antigenic variants. Sequence analysis showed that one antigenic variant contained a Gly-to-Val substitution at position 106 within the flavivirus-conserved fusion peptide loop of the envelope protein (E), and another variant contained a His-to-Gln substitution at position 317 in E. Substitution of Gly106Val in DENV-2 E reduced the binding affinity of Fab 1A5 by approximately 80-fold, whereas substitution of His317Gln had little or no effect on antibody binding compared to the parental virus. Treatment of DENV-2 with β-mercaptoethanol abolished binding of Fab 1A5, indicating that disulfide bridges were required for the structural integrity of the Fab 1A5 epitope. Binding of Fab 1A5 to DENV-2 was competed by an oligopeptide containing the fusion peptide sequence as shown by competition enzyme-linked immunosorbent assay. Both DENV-2 antigenic variants were shown to be attenuated, or at least similar to the parental virus, when evaluated for growth in cultured cells or for neurovirulence in mice. Fab 1A5 inhibited low pH-induced membrane fusion of mosquito C6/36 cells infected with DENV-1 or DENV-2, as detected by reduced syncytium formation. Both substitutions in DENV-2 E lowered the pH threshold for membrane fusion, as measured in a fusion-from-within assay. In the three-dimensional structure of E, Gly106 in domain II and His317 in domain III of the opposite E monomer were spatially close. From the locations of these amino acids, Fab 1A5 appears to recognize a novel epitope that has not been mapped before with a flavivirus monoclonal antibody.

Epstein-Barr virus (EBV) has an accepted association with the epithelial malignancy nasopharyngeal carcinoma and has also been reported in other more controversial carcinoma settings. Evaluation of EBV association with epithelial carcinomas such as breast cancer would benefit from a better understanding of the outcome of EBV infection of these cells. Cell-free preparations of a green fluorescent protein-expressing virus, BX1, were used to infect breast cancer cell lines, which were then examined for EBV gene expression and viral genome copy number. Reverse transcription-PCR analyses revealed that the cells supported a mixture of latency II and lytic EBV gene expression. Lytic Zta and BMRF1 protein expression was detected by immunohistochemistry, and DNA PCR analyses estimated an EBV copy number of 300 to 600 genomes per infected cell. Evidence for lytic EBV expression was also found in breast tissue, where reverse transcription-PCR analyses detected lytic Zta transcripts in 7 of 10 breast carcinoma tissues and 4 of 10 normal tissues from the same patients. Scattered cells immunoreactive for Zta protein were also detectable in breast carcinoma. Quantitative real-time PCR analysis of EBV-positive breast carcinoma tissues suggested that less than 0.1% of the cells contained viral genomes. We suggest that sporadic lytic EBV infection may contribute to PCR-based detection of EBV in traditionally nonvirally associated epithelial malignancies.
Antiviral resistance is a significant obstacle in the treatment of human immunodeficiency virus type 1 (HIV-1)-infected individuals. Because nonnucleoside reverse transcriptase inhibitors (NNRTIs) specifically target HIV-1 reverse transcriptase (RT) and do not effectively inhibit simian immunodeficiency virus (SIV) RT, the development of animal models to study the evolution of antiviral resistance has been problematic. To facilitate in vivo studies of NNRTI resistance, we examined whether a SIV that causes immunopathogenesis in pigtail macaques could be made sensitive to NNRTIs. Two simian-human immunodeficiency viruses (SHIVs) were derived from the genetic background of SIVmne: SIV-RT-YY contains RT substitutions intended to confer NNRTI susceptibility (V181Y and L188Y), and RT-SHIVmne contains the entire HIV-1 RT coding region. Both mutant viruses grew to high titers in vitro but had reduced fitness relative to wild-type SIVmne. Although the HIV-1 RT was properly processed into p66 and p51 subunits in RT-SHIVmne particles, the RT-SHIVmne virions had lower levels of RT per viral genomic RNA than HIV-1. Correspondingly, there was decreased RT activity in RT-SHIVmne and SIV-RT-YY particles. HIV-1 and RT-SHIVmne were similarly susceptible to the NNRTIs efavirenz, nevirapine, and UC781. However, SIV-RT-YY was less sensitive to NNRTIs than HIV-1 or RT-SHIVmne. Classical NNRTI resistance mutations were selected in RT-SHIVmne after in vitro drug treatment and were monitored in a sensitive allele-specific real-time RT-PCR assay. Collectively, these results indicate that RT-SHIVmne may be a useful model in macaques for the preclinical evaluation of NNRTIs and for studies of the development of drug resistance in vivo.


Ten antibody escape mutants of coxsackievirus B3 (CVB3) were used to identify nucleotide substitutions that determine viral virulence for the heart and pancreas. The P1 region, encoding the structural genes of each mutant, was sequenced to identify mutations associated with the lack of neutralization. Eight mutants were found to have a lysine-to-arginine mutation in the puff region of VP2, while two had a glutamate-to-glycine substitution in the knob of VP3. Two mutants, EM1 and EM10, representing each of these mutations, were further analyzed, initially by determining their entire sequence. In addition to the mutations in P1, EM1 was found to have two mutations in the 3D polymerase, while EM10 had a mutation in stem-loop II of the 5’ nontranslated region (5’NTR). The pathogenesis of the mutants relative to that of CVB3 strain RK [CVB3(RK)] then was examined in A/J mice. Both mutants were found to be less cardiotropic than the parental strain, with a 40-fold (EM1) or a 100- to 1,000-fold (EM10) reduction in viral titers in the heart relative to the titers of CVB3(RK). The mutations in VP2, VP3, and the 5’NTR were introduced independently into the RK infectious clone, and the phenotypes of the progeny viruses were determined. The results substantiated that the VP2 and VP3 mutations reduced cardiovirulence, while the 5’NTR mutation in EM10 was associated with a more virulent phenotype when expressed on its own. Stereographic imaging of the two mutations in the capsomer showed that they lie in close proximity on either side of a narrow cleft between the puff and the knob, forming a conformational epitope that is part of the putative binding site for coreceptor DAF.

Inefficient adenoviral vector (AdV)-mediated gene transfer to the ciliated respiratory epithelium has hindered gene transfer strategies for the treatment of cystic fibrosis lung disease. In part, the inefficiency is due to an absence of the coxsackie B and adenovirus type 2 and 5 receptor (CAR) from the apical membranes of polarized epithelia. In this study, using an in vitro model of human ciliated airway epithelium, we show that providing a glycosylphosphatidylinositol (GPI)-linked AdV receptor (GPI-CAR) at the apical surface did not significantly improve AdV gene transfer efficiency because the luminal surface glycocalyx limited the access of AdV to apical GPI-CAR. The highly glycosylated tethered mucins were considered to be significant glycocalyx components that restricted AdV access because proteolytic digestion and inhibitors of O-linked glycosylation enhanced AdV gene transfer. To determine whether these in vitro observations are relevant to the in vivo situation, we generated transgenic mice expressing GPI-CAR at the surface of the airway epithelium, crossbred these mice with mice that were genetically devoid of tethered mucin type 1 (Muc1), and tested the efficiency of gene transfer to murine airways expressing apical GPI-human CAR (GPI-hCAR) in the presence and absence of Muc1. We determined that AdV gene transfer to the murine airway epithelium was inefficient even in GPI-hCAR transgenic mice but that the gene transfer efficiency improved in the absence of Muc1. However, the inability to achieve a high gene transfer efficiency, even in mice with a deletion of Muc1, suggested that other glycocalyx components, possibly other tethered mucin types, also provide a significant barrier to AdV interacting with the airway luminal surface.


Administration of either lamivudine (2'-deoxy-3'-thiacytidine) or L-FMAU (2'-fluoro-5-methyl-(beta)-L-arabinofuranosyluracil) to woodchucks chronically infected with woodchuck hepatitis virus (WHV) induces a transient decline in virus titers. However, within 6 to 12 months, virus titers begin to increase towards pretreatment levels. This is associated with the emergence of virus strains with mutations of the B and C regions of the viral DNA polymerase (T. Zhou et al., Antimicrob. Agents Chemother. 43:1947-1954, 1999; Y. Zhu et al., J. Virol. 75:311-322, 2001). The present study was carried out to determine which of the mutants that we have identified conferred resistance to lamivudine and/or to L-FMAU. When inserted into a laboratory strain of WHV, each of the mutations, or combinations of mutations, of regions B and C produced a DNA replication-competent virus and typically conferred resistance to both nucleoside analogs in cell culture. Sequencing of the polymerase active site also occasionally revealed other mutations, but these did not appear to contribute to drug resistance. Moreover, in transfected cells, most of the mutants synthesized viral DNA nearly as efficiently as wild-type WHV. Computational models suggested that persistence of several of the WHV mutants as prevalent species in the serum and, by inference, liver for up to 6 months following drug withdrawal required a replication efficiency of at least 10 to 30% of that of the wild type. However, their delayed emergence during therapy suggested replication efficiency in the presence of the drug that was still well below that of wild-type WHV in the absence of the drug.

This study analyzes the effect of highly active antiretroviral therapy (HAART), and thus immunologic status, on hepatitis C virus (HCV) load and quasispecies diversity in patients coinfected with the human immunodeficiency virus (HIV) and HCV. Three cohorts of coinfected patients were analyzed retrospectively over a period of 7 to 10 months: group A was antiretroviral drug naive at baseline and then on HAART for the remainder of the study, group B did not receive antiretroviral therapy at any point, and group C was on HAART for the entire study. HCV quasispecies diversity was analyzed by sequencing hypervariable region 1. In a longitudinal analysis, there was no significant change from baseline in any immunologic, virologic, or quasispecies parameter in any of the three groups. However, in comparison to groups A and B, group C had significantly higher CD4+- and CD8+-cell counts, a trend toward a higher HCV load, and significantly increased number of HCV clones, entropy, genetic distance, and ratio of nonsynonymous substitutions per nonsynonymous site to synonymous substitutions per synonymous site (Ka/Ks). In addition, CD4+-cell count was positively correlated with HCV load, genetic distance, and Ka. Interestingly, patients infected with HCV genotype 2 or 3 had a significantly higher CD4+-cell count, HCV load, genetic distance, and Ka/Ks than those infected with genotype 1. These results suggest that there is no immediate effect of HAART on HCV but that, with prolonged HAART, immune restoration results in an increase in HCV load and quasispecies diversity.


http://jvi.asm.org/cgi/content/abstract/77/3/2010

In the context of the Rous sarcoma virus Gag polyprotein, only the nucleocapsid (NC) domain is required to mediate the specificity of genomic RNA packaging. We have previously showed that the Saccharomyces cerevisiae three-hybrid system provides a rapid genetic assay to analyze the RNA and protein components of the avian retroviral RNA-Gag interactions necessary for specific encapsidation. In this study, using both site-directed mutagenesis and in vivo random screening in the yeast three-hybrid binding assay, we have examined the amino acids in NC required for genomic RNA binding. We found that we could delete either of the two Cys-His boxes without greatly abrogating either RNA binding or packaging, although the two Cys-His boxes are likely to be required for efficient viral assembly and release. In contrast, substitutions for the Zn-coordinating residues within the boxes did prevent RNA binding, suggesting changes in the overall conformation of the protein. In the basic region between the two Cys-His boxes, three positively charged residues, as well as basic residues flanking the two boxes, were necessary for both binding and packaging. Our results suggest that the stretches of positively charged residues within NC that need to be in a proper conformation appear to be responsible for selective recognition and binding to the packaging signal ((Psi))-containing RNAs.


http://jvi.asm.org/cgi/content/abstract/77/3/1840

Infection with the Epstein-Barr virus (EBV) is often subclinical in the presence of a healthy immune response; thus, asymptomatic infection is largely uncharacterized. This study analyzed the nature of EBV infection in 20 asymptomatic immunocompetent hosts over time through the identification of EBV strain variants in the peripheral blood and oral cavity. A heteroduplex tracking assay specific for the EBV gene LMP1 precisely identified the presence of multiple EBV strains in each subject. The strains present in the peripheral blood and oral cavity were often completely discordant, indicating the existence of distinct infections, and the strains present and
their relative abundance changed considerably between time points. The possible transmission of strains between the oral cavity and peripheral blood compartments could be tracked within subjects, suggesting that reactivation in the oral cavity and subsequent reinfection of B lymphocytes that reenter the periphery contribute to the maintenance of persistence. In addition, distinct virus strains persisted in the oral cavity over many time points, suggesting an important role for epithelial cells in the maintenance of persistence. Asymptomatic individuals without tonsillar tissue, which is believed to be an important source of virus for the oral cavity, also exhibited multiple strains and a cyclic pattern of transmission between compartments. This study revealed that the majority of patients with infectious mononucleosis were infected with multiple strains of EBV that were also compartmentalized, suggesting that primary infection involves the transmission of multiple strains. Both the primary and carrier states of infection with EBV are more complex than previously thought.


http://jvi.asm.org/cgi/content/abstract/77/3/2182

Cytomegalovirus (CMV) infections have been shown to dramatically affect solid organ transplant graft survival in both human and animal models. Recently, it was demonstrated that rat CMV (RCMV) infection accelerates the development of transplant vascular sclerosis (TVS) in both rat heart and small bowel graft transplants. However, the mechanisms involved in this process are still unclear. In the present study, we determined the kinetics of RCMV-accelerated TVS in a rat heart transplant model. Acute RCMV infection enhances the development of TVS in rat heart allografts, and this process is initiated between 21 and 24 days posttransplantation. The virus is consistently detected in the heart grafts from day 7 until day 35 posttransplantation but is rarely found at the time of graft rejection (day 45 posttransplantation). Grafts from RCMV-infected recipients had upregulation of chemokine expression compared to uninfected controls, and the timing of this increased expression paralleled that of RCMV-accelerated neointimal formation. In addition, graft vessels from RCMV-infected grafts demonstrate the increased infiltration of T cells and macrophages during periods of highest chemokine expression. These results suggest that CMV-induced acceleration of TVS involves the increased graft vascular infiltration of inflammatory cells through enhanced chemokine expression.


http://jvi.asm.org/cgi/content/abstract/77/3/2134

Hepatitis C virus (HCV) is a major cause of chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma. Studies of HCV replication and pathogenesis have so far been hampered by the lack of an efficient tissue culture system for propagating HCV in vitro. Although HCV is primarily a hepatotrophic virus, an increasing body of evidence suggests that HCV also replicates in extrahepatic tissues in natural infection. In this study, we established a B-cell line (SB) from an HCV-infected non-Hodgkin's B-cell lymphoma. HCV RNA and proteins were detectable by RNase protection assay and immunoblotting. The cell line continuously produces infectious HCV virions in culture. The virus particles produced from the culture had a buoyant density of 1.13 to 1.15 g/ml in sucrose and could infect primary human hepatocytes, peripheral blood mononuclear cells (PBMCs), and an established B-cell line (Raji cells) in vitro. The virus from SB cells belongs to genotype 2b. Single-stranded conformational polymorphism and sequence analysis of the viral
RNA quasispecies indicated that the virus present in SB cells most likely originated from the patient's spleen and had an HCV RNA quasispecies pattern distinct from that in the serum. The virus production from the infected primary hepatocytes showed cyclic variations. In addition, we have succeeded in establishing several Epstein-Barr virus-immortalized B-cell lines from PBMCs of HCV-positive patients. Two of these cell lines are positive for HCV RNA as detected by reverse transcriptase PCR and for the nonstructural protein NS3 by immunofluorescence staining. These observations unequivocally establish that HCV infects B cells in vivo and in vitro. HCV-infected cell lines show significantly enhanced apoptosis. These B-cell lines provide a reproducible cell culture system for studying the complete replication cycle and biology of HCV infections.


http://jvi.asm.org/cgi/content/abstract/77/3/1868

The 4-oxo-dihydroquinolines (PNU-182171 and PNU-183792) are nonnucleoside inhibitors of herpesvirus polymerases (R. J. Brideau et al., Antiviral Res. 54:19-28, 2002; N. L. Oien et al., Antimicrob. Agents Chemother. 46:724-730, 2002). In cell culture these compounds inhibit herpes simplex virus type 1 (HSV-1), HSV-2, human cytomegalovirus (HCMV), varicella-zoster virus (VZV), and human herpesvirus 8 (HHV-8) replication. HSV-1 and HSV-2 mutants resistant to these drugs were isolated and the resistance mutation was mapped to the DNA polymerase gene. Drug resistance correlated with a point mutation in conserved domain III that resulted in a V823A change in the HSV-1 or the equivalent amino acid in the HSV-2 DNA polymerase. Resistance of HCMV was also found to correlate with amino acid changes in conserved domain III (V823A+V824L). V823 is conserved in the DNA polymerases of six (HSV-1, HSV-2, HCMV, VZV, Epstein-Barr virus, and HHV-8) of the eight human herpesviruses; the HHV-6 and HHV-7 polymerases contain an alanine at this amino acid. In vitro polymerase assays demonstrated that HSV-1, HSV-2, HCMV, VZV, and HHV-8 polymerases were inhibited by PNU-183792, whereas the HHV-6 polymerase was not. Changing this amino acid from valine to alanine in the HSV-1, HCMV, and HHV-8 polymerases alters the polymerase activity so that it is less sensitive to drug inhibition. In contrast, changing the equivalent amino acid in the HHV-6 polymerase from alanine to valine alters polymerase activity so that PNU-183792 inhibits this enzyme. The HSV-1, HSV-2, and HCMV drug-resistant mutants were not altered in their susceptibilities to nucleoside analogs; in fact, some of the mutants were hypersensitive to several of the drugs. These results support a mechanism where PNU-183792 inhibits herpesviruses by interacting with a binding determinant on the viral DNA polymerase that is less important for the binding of nucleoside analogs and deoxynucleoside triphosphates.


http://jvi.asm.org/cgi/content/abstract/78/3/1109

Poxviruses and gamma-2 herpesviruses share the K3 family of viral immune evasion proteins that inhibit the surface expression of glycoproteins such as major histocompatibility complex class I (MHC-I), B7.2, ICAM-1, and CD95(Fas). K3 family proteins contain an amino-terminal PHD/LAP or RING-CH domain followed by two transmembrane domains. To examine whether human homologues are functionally related to the viral immunoevasins, we studied seven membrane-associated RING-CH (MARCH) proteins. All MARCH proteins located to subcellular membranes, and several MARCH proteins reduced surface levels of known substrates of the viral K3 family.
Two closely related proteins, MARCH-IV and MARCH-IX, reduced surface expression of MHC-I molecules. In the presence of MARCH-IV or MARCH-IX, MHC-I was ubiquitinated and rapidly internalized by endocytosis, whereas MHC-I molecules lacking lysines in their cytoplasmic tail were resistant to downregulation. The amino-terminal regions containing the RING-CH domain of several MARCH proteins examined catalyzed multiubiquitin formation in vitro, suggesting that MARCH proteins are ubiquitin ligases. The functional similarity of the MARCH family and the K3 family suggests that the viral immune evasion proteins were derived from MARCH proteins, a novel family of transmembrane ubiquitin ligases that seems to target glycoproteins for lysosomal destruction via ubiquitination of the cytoplasmic tail.


http://jvi.asm.org/cgi/content/abstract/78/3/1411

A single protein, termed Gag, is responsible for retrovirus particle assembly. After the assembled virion is released from the cell, Gag is cleaved at several sites by the viral protease (PR). The cleavages catalyzed by PR bring about a wide variety of physical changes in the particle, collectively termed maturation, and convert the particle into an infectious virion. In murine leukemia virus (MLV) maturation, Gag is cleaved at three sites, resulting in formation of the matrix (MA), p12, capsid (CA), and nucleocapsid (NC) proteins. We introduced mutations into MLV that inhibited cleavage at individual sites in Gag. All mutants had lost the intensely staining ring characteristic of immature particles; thus, no single cleavage event is required for this feature of maturation. Mutant virions in which MA was not cleaved from p12 were still infectious, with a specific infectivity only [-]10-fold below that of the wild type. Particles in which p12 and CA could not be separated from each other were noninfectious and lacked a well-delineated core despite the presence of dense material in their interiors. In both of these mutants, the dimeric viral RNA had undergone the stabilization normally associated with maturation, suggesting that this change may depend upon the separation of CA from NC. Alteration of the C-terminal end of CA blocked CA-NC cleavage but also reduced the efficiency of particle formation and, in some cases, severely disrupted the ability of Gag to assemble into regular structures. This observation highlights the critical role of this region of Gag in assembly.


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Rearrangements of the JC virus (JCV) regulatory region (RR) are consistently found in the brains of patients with progressive multifocal leukoencephalopathy (PML), whereas the archetype RR is present in their kidneys. In addition, the C terminus of the large T antigen (T-Ag) shows greater variability in PML than does the rest of the coding region. To determine whether similar changes in simian virus 40 (SV40) are necessary for disease induction in monkeys, we sequenced the SV40 RR and the C terminus of the T-Ag from the brain of simian/human immunodeficiency virus (SHIV)-infected monkey 18429, which presented spontaneously with an SV40-associated PML-like disease, as well as from the peripheral blood mononuclear cells (PBMC), kidneys, and brains of SV40-seronegative, SHIV-infected monkeys 21289 and 21306, which were inoculated with the 18429 brain SV40 isolate. These animals developed both SV40-associated PML and meningoencephalitis. Thirteen types of SV40 RR were characterized. Compared to the SV40 archetype, we identified RRs with variable deletions in either the origin of replication, the 21-bp repeat elements, or the late promoter, as well as deletions or duplications of the 72-bp enhancer.
The archetype was the most prominent RR in the brain of monkey 18429. Shortly after inoculation, a wide range of RRs could be found in the PBMC of monkeys 21289 and 21306. However, the archetype RR became the predominant type in their blood, kidneys, and brains at the time of sacrifice. On the contrary, the T-Ag C termini remained identical in all compartments of the three animals. These results indicate that unlike JCV in humans, rearrangements of SV40 RR are not required for brain disease induction in immunosuppressed monkeys.


http://jvi.asm.org/cgi/content/abstract/79/3/1569

The hepatitis C virus (HCV) causes chronic hepatitis, which often results in liver cirrhosis and hepatocellular carcinoma. We have previously shown that HCV nonstructural proteins induce activation of STAT-3 via oxidative stress and Ca2+ signaling (G. Gong, G. Waris, R. Tanveer, and A. Siddiqui, Proc. Natl. Acad. Sci. USA 98:9599-9604, 2001). In this study, we focus on the signaling pathway leading to STAT-3 activation in response to oxidative stress induced by HCV translation and replication activities. Here, we demonstrate the constitutive activation of STAT-3 in HCV replicon-expressing cells. The HCV-induced STAT-3 activation was inhibited in the presence of antioxidant (pyrrolidine dithiocarbamate) and Ca2+ chelators (BAPTA-AM and TMB-8). Previous studies have shown that maximum STAT-3 transactivation requires Ser727 phosphorylation in addition to tyrosine phosphorylation. Using a series of inhibitors and dominant negative mutants, we show that HCV-induced activation of STAT-3 is mediated by oxidative stress and influenced by the activation of cellular kinases, including p38 mitogen-activated protein kinase, JNK, JAK-2, and Src. Our results also suggest a potential role of STAT-3 in HCV RNA replication. We also observed the constitutive activation of STAT-3 in the liver biopsy of an HCV-infected patient. These studies provide an insight into the mechanisms by which HCV induces intracellular events relevant to liver pathogenesis associated with the viral infection.


http://jvi.asm.org/cgi/content/abstract/79/3/1480

Coreceptor specificity of human immunodeficiency virus type 1 (HIV-1) strains is generally defined in vitro in cell lines expressing CCR5 or CXCR4, but lymphocytes and macrophages are the principal targets in vivo. CCR5-using (R5) variants dominate early in infection, but strains that use CXCR4 emerge later in a substantial minority of subjects. Many or most CXCR4-using variants can use both CXCR4 and CCR5 (R5X4), but the pathways that are actually used to cause infection in primary cells and in vivo are unknown. We examined several R5X4 prototype and primary isolates and found that they all were largely or completely restricted to CXCR4-mediated entry in primary lymphocytes, even though lymphocytes are permissive for CCR5-mediated entry by R5 strains. In contrast, in primary macrophages R5X4 isolates used both CCR5 and CXCR4. The R5X4 strains were also more sensitive than R5 strains to CCR5 blocking, suggesting that interactions between the R5X4 strains and CCR5 are less efficient. These results indicate that coreceptor phenotyping in transformed cells does not necessarily predict utilization in primary cells, that variability exists among HIV-1 isolates in the ability to use CCR5 expressed on lymphocytes, and that many or most strains characterized as R5X4 are functionally X4 in primary lymphocytes. Less efficient interactions between R5X4 strains and CCR5 may be responsible for the inability to use CCR5 on lymphocytes, which express relatively low CCR5 levels. Since isolates that acquire CXCR4 utilization retain the capacity to use CCR5 on macrophages despite their inability to use it on lymphocytes, these results also raise the
possibility that a CCR5-mediated macrophage reservoir is required for sustained infection in vivo.


http://jvi.asm.org/cgi/content/abstract/76/4/1971

A heteroduplex mobility assay was used to identify variants of varicella-zoster virus circulating in the United Kingdom and elsewhere. Within the United Kingdom, 58 segregating sites were found out of the 23,266 examined (0.25%), and nucleotide diversity was estimated to be 0.00063. These are an order of magnitude smaller than comparable estimates from herpes simplex virus type 1. Sixteen substitutions were nonsynonymous, the majority of which were clustered within surface-expressed proteins. Extensive genetic correlation between widely spaced sites indicated that recombination has been rare. Phylogenetic analysis of varicella-zoster viruses from four continents distinguished at least three major genetic clades. Most geographical regions contained only one of these three strains, apart from the United Kingdom and Brazil, where two or more strains were found. There was minimal genetic differentiation (one or fewer substitutions in 1,895 bases surveyed) between the samples collected from Africa (Guinea Bissau, Zambia) and the Indian subcontinent (Bangladesh, South India), suggesting recent rapid spread and/or low mutation rates. The geographic pattern of strain distribution would favor a major influence of the former. The genetic uniformity of most virus populations makes recombination difficult to detect. However, at least one probable recombinant between two of the major strains was found among the samples originating from Brazil, where mixtures of genotypes co-occur.


http://jvi.asm.org/cgi/content/abstract/76/4/1781

To analyze the compatibility of avian influenza A virus hemagglutinins (HAs) and human influenza A virus matrix (M) proteins M1 and M2, we doubly infected Madin-Darby canine kidney cells with amantadine (1-aminoadamantane hydrochloride)-resistant human viruses and amantadine-sensitive avian strains. By using antisera against the human virus HAs and amantadine, we selected reassortants containing the human virus M gene and the avian virus HA gene. In our system, high virus yields and large, well-defined plaques indicated that the avian HAs and the human M gene products could cooperate effectively; low virus yields and small, turbid plaques indicated that cooperation was poor. The M gene products are among the primary components that determine the species specificities of influenza A viruses. Therefore, our system also indicated whether the avian HA genes effectively reassorted into the genome and replaced the HA gene of the prevailing human influenza A viruses. Most of the avian HAs that we tested efficiently cooperated with the M gene products of the early human A/PR/8/34 (H1N1) virus; however, the avian HAs did not effectively cooperate with the most recently isolated human virus that we tested, A/Nanchang/933/95 (H3N2). Cooperation between the avian HAs and the M proteins of the human A/Singapore/57 (H2N2) virus was moderate. These results suggest that the currently prevailing human influenza A viruses might have lost their ability to undergo antigenic shift and therefore are unable to form new pandemic viruses that contain an avian HA, a finding that is of great interest for pandemic planning.

Human immunodeficiency virus (HIV) gp120 induces multiple cellular signaling pathways, including the phosphatidylinositol 3-kinase (PI3-kinase) pathway. The role of the PI3-kinase pathway in HIV-1 replication is not understood. Here we examined whether HIV-1 gp120 upregulates the PI3-kinase pathway and whether PI3-kinase activity plays a role in virus replication in primary human CD4+ T cells and macrophages. Soluble and virion-associated HIV-1 gp120 induced calcium mobilization and phosphorylation of the PI3-kinase downstream effectors PKB/Akt and p70 S6 kinase. gp120-induced PI3-kinase activity and calcium mobilization were inhibited by pertussis toxin and blocking antibodies directed against CCR5 and CXCR4, suggesting that the signaling is mediated through the chemokine receptor. The PI3-kinase inhibitor LY294002 inhibited infection of CD4+ T cells and macrophages with X4 and R5 HIV-1-pseudotyped viruses at concentrations that did not induce cell toxicity or downregulate HIV-1 coreceptor expression. When gp120-induced signaling was bypassed with the vesicular stomatitis virus G envelope protein, infection was still sensitive to PI3-kinase inhibition, suggesting that basal PI3-kinase activity is required for infection. LY294002 inhibited HIV-1 infection when added after viral entry and did not affect formation of the HIV-1 reverse transcriptase products R/U5 and long terminal repeat/Gag in the presence of the inhibitor. However, when the inhibitor was added after viral integration had occurred, no inhibition of HIV infection was observed. Our studies show that inhibition of the PI3-kinase signaling pathway suppresses virus infection post-viral entry and post-reverse transcription but prior to HIV gene expression. This type of host-virus interaction has implications for anti-HIV therapeutics that target cellular signaling machinery.


The KwaZulu-Natal region of South Africa is experiencing an explosive outbreak of human immunodeficiency virus type 1 (HIV-1) subtype C infections. Understanding the genetic diversity of C viruses and the biological consequences of this diversity is important for the design of effective control strategies. We analyzed the protease gene, the first 935 nucleotides of reverse transcriptase, and the C2V5 envelope region of a representative set of 72 treatment-naive patients from KwaZulu-Natal and correlated the results with amino acid signature and resistance patterns. Phylogenetic analysis revealed multiple clusters or "lineages" of HIV-1 subtype C that segregated with other C viruses from southern Africa. The same pattern was observed for both black and Indian subgroups and for retrospective specimens collected prior to 1990, indicating that multiple sublineages of HIV-1 C have been present in KwaZulu-Natal since the early stages of the epidemic. With the exception of three nonnucleoside reverse transcriptase inhibitor mutations, no primary resistance mutations were identified. Numerous accessory polymorphisms were present in the protease, but none were located at drug-binding or active sites of the enzyme. One frequent polymorphism, I93L, was located near the protease/reverse transcriptase cleavage site. In the envelope, disruption of the glycosylation motif at the beginning of V3 was associated with the presence of an extra protein kinase C phosphorylation site at codon 11. Many polymorphisms were embedded within cytotoxic T lymphocyte or overlapping cytotoxic T-lymphocyte/T-helper epitopes, as defined for subtype B. This work forms a baseline for future studies aimed at understanding the impact of genetic diversity on vaccine efficacy and on natural susceptibility to antiretroviral drugs.
Muscle is an attractive target for gene delivery because of its mass and because vectors can be delivered in a noninvasive fashion. Adeno-associated virus (AAV) has been shown to be effective for muscle-targeted gene transfer. Recent progress in characterization of AAV serotype 1 (AAV1) and AAV6 demonstrated that these two AAV serotypes are far more efficient in transducing muscle than is the traditionally used AAV2. Since all cis elements are identical in these vectors, the potential determinants for their differences in transducing muscle appear to be located within the AAV capsid proteins. In the present study, a series of AAV capsid mutants were generated to identify the major regions affecting AAV transduction efficiency in muscle. Replacement of amino acids 350 to 736 of AAV2 VP1 with the corresponding amino acids from VP1 of AAV1 resulted in a hybrid vector that behaved very similarly to AAV1 in vitro and in vivo in muscle. Characterization of additional mutants carrying smaller regions of the AAV1 VP1 amino acid sequence in the AAV2 capsid protein suggested that amino acids 350 to 430 of VP1 function as a major tissue tropism determinant. Further analysis showed that the heparin binding domain and the major antigenic determinants in the AAV capsid region were not necessary for the efficiency of AAV1 transduction of muscle.


Influenza A viruses are the cause of annual epidemics of human disease with occasional outbreaks of pandemic proportions. The zoonotic nature of the disease and the vast viral reservoirs in the aquatic birds of the world mean that influenza will not easily be eradicated and that vaccines will continue to be needed. Recent technological advances in reverse genetics methods and limitations of the conventional production of vaccines by using eggs have led to a push to develop cell-based strategies to produce influenza vaccine. Although cell-based systems are being developed, barriers remain that need to be overcome if the potential of these systems is to be fully realized. These barriers include, but are not limited to, potentially poor reproducibility of viral rescue with reverse genetics systems and poor growth kinetics and yields. In this study we present a modified A/Puerto Rico/8/34 (PR8) influenza virus master strain that has improved viral rescue and growth properties in the African green monkey kidney cell line, Vero. The improved properties were mediated by the substitution of the PR8 NS gene for that of a Vero-adapted reassortant virus. The Vero growth kinetics of viruses with H1N1, H3N2, H6N1, and H9N2 hemagglutinin and neuraminidase combinations rescued on the new master strain were significantly enhanced in comparison to those of viruses with the same combinations rescued on the standard PR8 master strain. These improvements pave the way for the reproducible generation of high-yielding human and animal influenza vaccines by reverse genetics methods. Such a means of production has particular relevance to epidemic and pandemic use.


http://jvi.asm.org/cgi/content/abstract/79/4/2631
Human immunodeficiency virus type 2 (HIV-2) originated from simian immunodeficiency viruses (SIVs) that naturally infect sooty mangabeys (SMs; Cercocebus atys). In order to further investigate the relationship between HIV-2 and SIVsm, the SIV specific to the SM, we characterized seven new SIVsm strains from SMs sold in Sierra Leone markets as bush meat. The gag, pol, and env sequences showed that, while the viruses of all seven SMs belonged to the SIVsm-HIV-2 lineage, they were highly divergent viruses, in spite of the fact that most of the samples originated from the same geographical region. They clustered in three lineages, two of which have been previously reported. Two of the new SIVsm strains clustered differently in gag and env phylogenetic trees, suggesting SIVsm recombination that had occurred in the past. In spite of the fact that our study doubles the number of known SIVsm strains from wild SMs, none of the simian strains were close to the groups in which HIV-2 was epidemic (groups A and B).


http://jvi.asm.org/cgi/content/abstract/79/4/2643

The origin and biological significance of deletions at the 3' end of the Epstein-Barr virus (EBV)-encoded latent membrane protein 1 (LMP-1) gene are still controversial. We herein demonstrate that LMP-1 deletion mutants are highly associated with human immunodeficiency virus-related Hodgkin's lymphoma (HIV-HL) of Italian patients (29 of 31 cases; 93.5%), a phenomenon that is not due to a peculiar distribution of EBV strains in this area. In fact, although HIV-HL patients are infected by multiple EBV variants, we demonstrate that LMP-1 deletion mutants preferentially accumulate within neoplastic tissues. Subcloning and sequencing of the 3' LMP-1 ends of two HIV-HL genes in which both variants were present showed the presence of molecular signatures suggestive of a likely derivation of the LMP-1 deletion mutant from a nondeletion ancestor. This phenomenon likely occurs within tumor cells in vivo, as shown by the detection of both LMP-1 variants in single microdissected Reed-Sternberg cells, and may at least in part explain the high prevalence of LMP-1 deletions associated with HIV-HL.


http://jvi.asm.org/cgi/content/abstract/79/4/2001

Exotic wildlife can act as reservoirs of diseases that are endemic in the area or can be the source of new emerging diseases through interspecies transmission. The recent emergence of severe acute respiratory syndrome-associated coronavirus (SARS-CoV) highlights the importance of virus surveillance in wild animals. Here, we report the identification of a novel bat coronavirus through surveillance of coronaviruses in wildlife. Analyses of the RNA sequence from the ORF1b and S-gene regions indicated that the virus is a group 1 coronavirus. The virus was detected in fecal and respiratory samples from three bat species (Miniopterus spp.). In particular, 63% (12 of 19) of fecal samples from Miniopterus pusillus were positive for the virus. These findings suggest that this virus might be commonly circulating in M. pusillus in Hong Kong.

A study was conducted to evaluate the prevalence and diversity of simian T-cell lymphotropic virus (STLV) isolates within the long-established Tulane National Primate Research Center (TNPRC) colony of sooty mangabeys (SMs; Cercocebus atys). Serological analysis determined that 22 of 39 animals (56%) were positive for STLV type 1 (STLV-1). A second group of thirteen SM bush meat samples from Sierra Leone in Africa was also included and tested only by PCR. Twenty-two of 39 captive animals (56%) and 3 of 13 bush meat samples (23%) were positive for STLV-1, as shown by testing with PCR. Nucleotide sequencing and phylogenetic analysis of viral strains obtained demonstrated that STLV-1 strains from SMs (STLV-1sm strains) from the TNPRC colony and Sierra Leone formed a single cluster together with the previously reported STLV-1sm strain from the Yerkes National Primate Research Center. These data confirm that Africa is the origin for TNPRC STLV-1sm and suggest that Sierra Leone is the origin for the SM colonies in the United States. The TNPRC STLV-1sm strains further divided into two subclusters, suggesting STLV-1sm infection of two original founder SMs at the time of their importation into the United States. STLV-1sm diversity in the TNPRC colony matches the high diversity of SIVsm in the already reported colony. The lack of correlation between the lineage of the simian immunodeficiency virus from SMs (SIVsm) and the STLV-1sm subcluster distribution of the TNPRC strains suggests that intracolony transmissions of both viruses were independent events.


The complete DNA sequence of grouper iridovirus (GIV) was determined using a whole-genome shotgun approach on virion DNA. The circular form genome was 139,793 bp in length with a 49% G+C content. It contained 120 predicted open reading frames (ORFs) with coding capacities ranging from 62 to 1,268 amino acids. A total of 21% (25 of 120) of GIV ORFs are conserved in the other five sequenced iridovirus genomes, including DNA replication, transcription, nucleotide metabolism, protein modification, viral structure, and virus-host interaction genes. The whole-genome nucleotide pairwise comparison showed that GIV virus was partially colinear with counterparts of previously sequenced ranaviruses (ATV and TFV). Besides, sequence analysis revealed that GIV possesses several unique features which are different from those of other complete sequenced iridovirus genomes: (i) GIV is the first ranavirus-like virus which has been sequenced completely and which infects fish other than amphibians, (ii) GIV is the only vertebrate iridovirus without CpG sequence methylation and lacking DNA methyltransferase, (iii) GIV contains a purine nucleoside phosphorylase gene which is not found in other iridoviruses or in any other viruses, (iv) GIV contains 17 sets of repeat sequence, with basic unit sizes ranging from 9 to 63 bp, dispersed throughout the whole genome. These distinctive features of GIV further extend our understanding of molecular events taking place between ranavirus and its hosts and the iridovirus evolution.


Marker rescue, the restoration of gene function by replacement of a defective gene with a normal one by recombination, has been utilized to produce novel adeno-associated virus (AAV) vectors.
AAV serotype 2 (AAV2) clones containing wild-type terminal repeats, an intact rep gene, and a mutated cap gene, served as the template for marker rescue. When transfected alone in 293 cells, these AAV2 mutant plasmids produced noninfectious AAV virions that could not bind heparin sulfate after infection with adenovirus dl309 helper virus. However, the mutation in the cap gene was corrected after cotransfection with AAV serotype 3 (AAV3) capsid DNA fragments, resulting in the production of AAV2/AAV3 chimeric viruses. The cap genes from several independent marker rescue experiments were PCR amplified, cloned, and then sequenced. Sequencing results confirmed not only that homologous recombination occurred but, more importantly, that a mixed population of AAV chimeras carrying 16 to 2,200 bp throughout different regions of the type 3 cap gene were generated in a single marker rescue experiment. A 100% correlation was observed between infectivity and the ability of the chimeric virus to bind heparin sulfate. In addition, many of the AAV2/AAV3 chimeras examined exhibited differences at both the nucleotide and amino acid levels, suggesting that these chimeras may also exhibit unique infectious properties. Furthermore, AAV helper plasmids containing these chimeric cap genes were able to function in the triple transfection method to generate recombinant AAV. Together, the results suggest that DNA from other AAV serotypes can rescue AAV capsid mutants and that marker rescue may be a powerful, yet simple, technique to map, as well as develop, chimeric AAV capsids that display different serotype-specific properties.


http://jvi.asm.org/cgi/content/abstract/77/1/744

In order to study primate lentivirus evolution in the Colobinae subfamily, in which only one simian immunodeficiency virus (SIV) has been described to date, we screened additional species from the three different genera of African colobus monkeys for SIV infection. Blood was obtained from 13 West African colobids, and HIV cross-reactive antibodies were observed in 5 of 10 Piliocolobus badius, 1 of 2 Procolobus verus, and 0 of 1 Colobus polykomos specimens. Phylogenetic analyses of partial pol sequences revealed that the new SIVs were more closely related to each other than to the other SIVs and especially did not cluster with the previously described SIVcol from Colobus guereza. This study presents evidence that the three genera of African colobus monkeys are naturally infected with an SIV and indicates also that there was no coevolution between virus and hosts at the level of the Colobinae subfamily.


http://jvi.asm.org/cgi/content/abstract/78/1/240

We used a panel of monoclonal antibodies to H9 hemagglutinin to select 18 escape mutants of mouse-adapted influenza A/Swine/Hong Kong/9/98 (H9N2) virus. Cross-reactions of the mutants with the antibodies and the sequencing of hemagglutinin genes revealed two minimally overlapping epitopes. We mapped the amino acid changes to two areas of the recently reported three-dimensional structure of A/Swine/Hong Kong/9/98 hemagglutinin. The grouping of the antigenically relevant amino acid positions in H9 hemagglutinin differs from the pattern observed in H3 and H5 hemagglutinins. Several positions in site B of H3 hemagglutinin are distributed in two sites of H9 hemagglutinin. Unlike any subtype analyzed so far, H9 hemagglutinin does not contain an antigenic site corresponding to site A in H3 hemagglutinin. Positions 145 and 193 (H3 numbering), which in H3 hemagglutinin belong to sites A and B, respectively, are within one site
in H9 hemagglutinin. This finding is consistent with the peculiarity of the three-dimensional structure of the H9 molecule, that is, the absence from H9 hemagglutinin of the lateral loop that forms site A in H3 and the equivalent site in H5 hemagglutinins. The escape mutants analyzed displayed phenotypic variations, including decreased virulence for mice and changes in affinity for sialyl substrates. Our results demonstrate a correlation between intersubtype differences in three-dimensional structure and variations among subtypes in the distribution of antigenic areas. Our findings also suggest that covariation and pleiotropic effects of antibody-selected mutations may be important in the evolution of H9 influenza virus, a possible causative agent of a future pandemic.


http://jvi.asm.org/cgi/content/abstract/76/2/541

Infection of animals with a molecular viral clone is critical to study the genetic determinants of viral replication and virulence in the host. Type 2 porcine circovirus (PCV2) has been incriminated as the cause of postweaning multisystemic wasting syndrome (PMWS), an emerging disease in pigs. We report here for the first time the construction and use of an infectious molecular DNA clone of PCV2 to characterize the disease and pathologic lesions associated with PCV2 infection by direct in vivo transfection of pigs with the molecular clone. The PCV2 molecular clone was generated by ligating two copies of the complete PCV2 genome in tandem into the pBluescript SK (pSK) vector and was shown to be infectious in vitro when transfected into PK-15 cells. Forty specific-pathogen-free pigs at 4 weeks of age were randomly assigned to four groups of 10 each. Group 1 pigs served as uninoculated controls. Pigs in group 2 were each inoculated intranasally with about $1.9 \times 10^5$ 50% tissue culture infective doses of a homogeneous PCV2 live virus stock derived from the molecular clone. Pigs in group 3 were each injected intrahepatically with 200 {micro}g of the cloned PCV2 plasmid DNA, and pigs in group 4 were each injected into the superficial iliac lymph nodes with 200 {micro}g of the cloned PCV2 plasmid DNA. Animals injected with the cloned PCV2 plasmid DNA developed infection resembling that induced by intranasal inoculation with PCV2 live virus stock. Seroconversion to PCV2-specific antibody was detected in the majority of pigs from the three inoculated groups at 35 days postinoculation (DPI). Viremia, beginning at 14 DPI and lasting 2 to 4 weeks, was detected in the majority of the pigs from all three inoculated groups. There were no remarkable clinical signs of PMWS in control or any of the inoculated pigs. Gross lesions in pigs of the three inoculated groups were similar and were characterized by systemically enlarged, tan lymph nodes and lungs that failed to collapse. Histopathological lesions and PCV2-specific antigen were detected in numerous tissues and organs, including brain, lung, heart, kidney, tonsil, lymph nodes, spleen, ileum, and liver of infected pigs. This study more definitively characterizes the clinical course and pathologic lesions exclusively attributable to PCV2 infection. The data from this study indicate that the cloned PCV2 genomic DNA may replace infectious virus for future PCV2 pathogenesis and immunization studies. The data also suggest that PCV2, although essential for development of PMWS, may require other factors or agents to induce the full spectrum of clinical signs and lesions associated with advanced cases of PMWS.


http://jvi.asm.org/cgi/content/abstract/76/2/644
The human serum human immunodeficiency virus type 1 (HIV-1)-neutralizing serum 2 (HNS2) neutralizes many primary isolates of different clades of HIV-1, and virus expressing envelope from the same donor, clone R2, is neutralized cross-reactively by HIV-immune human sera. The basis for this cross-reactivity was investigated. It was found that a rare mutation in the proximal limb of variable region 3 (V3), 313-4 PM, caused virus pseudotyped with the R2 envelope to be highly sensitive to neutralization by monoclonal antibodies (MAbs) directed against conformation-sensitive epitopes at the tip of the V3 loop, such as 19b, and moderately sensitive to MAbs against CD4 binding site (CD4bs) and CD4-induced (CD4i) epitopes, soluble CD4 (sCD4), and HNS2. In addition, introduction of this sequence by mutagenesis caused enhanced sensitivity to neutralization by 19b, anti-CD4i MAb, and HNS2 in three other primary HIV-1 envelopes and by anti-CD4bs MAb and sCD4 in one of the three. The 313-4 PM sequence also conferred increased infectivity for CD4+ CCR5+ cells and the ability to infect CCR5+ cells upon all of these four and two of these four HIV-1 envelopes, respectively. Neutralization of R2 by HNS2 was substantially inhibited by the cyclized R2 V3 35-mer synthetic peptide. Similarly, the peptide also had some lesser efficacy in blocking neutralization of R2 by other sera or of neutralization of other primary viruses by HNS2. Together, these results indicate that the unusual V3 mutation in the R2 clone accounts for its uncommon neutralization sensitivity phenotype and its capacity to mediate CD4-independent infection, both of which could relate to immunogenicity and the neutralizing activity of HNS2. This is also the first primary HIV-1 isolate envelope glycoprotein found to be competent for CD4-independent infection.


http://jvi.asm.org/cgi/content/abstract/77/2/1469

Human immunodeficiency virus type 1 (HIV-1) containing mutations in the nucleocapsid (NC) Zn2+ finger domains have greatly reduced infectivity, even though genome packaging is largely unaffected in certain cases. To examine replication defects, viral DNA (vDNA) was isolated from cells infected with viruses containing His-to-Cys changes in their Zn2+ fingers (NCH23C and NCH44C), an integrase mutant (IND116N), a double mutant (NCH23C/IND116N), or wild-type HIV-1. In vitro assays have established potential roles for NC in reverse transcription and integration. In vivo results for these processes were obtained by quantitative PCR, cloning of PCR products, and comparison of the quantity and composition of vDNA generated at discrete points during reverse transcription. Quantitative analysis of the reverse transcription intermediates for these species strongly suggests decreased stability of the DNA produced. Both Zn2+ finger mutants appear to be defective in DNA synthesis, with the minus- and plus-strand transfer processes being affected while interior portions of the vDNA remain more intact. Sequences obtained from PCR amplification and cloning of 2-LTR circle junction fragments revealed that the NC mutants had a phenotype similar to the IN mutant; removal of the terminal CA dinucleotides necessary for integration of the vDNA is disabled by the NC mutations. Thus, the loss of infectivity in these NC mutants in vivo appears to result from defective reverse transcription and integration processes stemming from decreased protection of the full-length vDNA. Finally, these results indicate that the chaperone activity of NC extends from the management of viral RNA through to the full-length vDNA.


http://jvi.asm.org/cgi/content/abstract/77/2/1512
Suboptimal treatment of human immunodeficiency virus type 1 (HIV-1) infection with nonnucleoside reverse transcriptase inhibitors (NNRTI) often results in the rapid selection of drug-resistant virus. Several amino acid substitutions at position 190 of reverse transcriptase (RT) have been associated with reduced susceptibility to the NNRTI, especially nevirapine (NVP) and efavirenz (EFV). In the present study, the effects of various 190 substitutions observed in viruses obtained from NNRTI-experienced patients were characterized with patient-derived HIV isolates and confirmed with a panel of isogenic viruses. Compared to wild-type HIV, which has a glycine at position 190 (G190), viruses with 190 substitutions (A, C, Q, S, V, E, or T, collectively referred to as G190X substitutions) were markedly less susceptible to NVP and EFV. In contrast, delavirdine (DLV) susceptibility of these G190X viruses increased from 3 to 300-fold (hypersusceptible) or was only slightly decreased. The replication capacity of viruses with certain 190 substitutions (C, Q, V, T, and E) was severely impaired and was correlated with reduced virion-associated RT activity and incomplete protease (PR) processing of the viral p55gag polyprotein. These defects were the result of inadequate p160gagpol incorporation into virions. Compensatory mutations within RT and PR improved replication capacity, p55gag processing, and RT activity, presumably through increased incorporation of p160gagpol into virions. We observe an inverse relationship between the degree of NVP and EFV resistance and the impairment of viral replication in viruses with substitutions at 190 in RT. These observations may have important implications for the future design and development of antiretroviral drugs that restrict the outgrowth of resistant variants with high replication capacity.


http://jvi.asm.org/cgi/content/abstract/77/2/1105

All currently licensed yellow fever (YF) vaccines are propagated in chicken embryos. Recent studies of chick cell-derived measles and mumps vaccines show evidence of two types of retrovirus particles, the endogenous avian retrovirus (EAV) and the endogenous avian leukosis virus (ALV-E), which originate from the chicken embryonic fibroblast substrates. In this study, we investigated substrate-derived avian retrovirus contamination in YF vaccines currently produced by three manufacturers (YF-vax [Connaught Laboratories], Stamaril [Aventis], and YF-FIOCRUZ [FIOCRUZ-Bio-Manguinhos]). Testing for reverse transcriptase (RT) activity was not possible because of assay inhibition. However, Western blot analysis of virus pellets with anti-ALV RT antiserum detected three distinct RT proteins in all vaccines, indicating that more than one source is responsible for the RTs present in the vaccines. PCR analysis of both chicken substrate DNA and particle-associated RNA from the YF vaccines showed no evidence of the long terminal repeat sequences of exogenous ALV subgroups A to D in any of the vaccines. In contrast, both ALV-E and EAV particle-associated RNA were detected at equivalent titers in each vaccine by RT-PCR. Quantitative real-time RT-PCR revealed 61,600, 348,000, and 1,665,000 ALV-E RNA copies per dose of Stamaril, YF-FIOCRUZ, and YF-vax vaccines, respectively. ev locus-specific PCR testing of the vaccine-associated chicken substrate DNA was positive both for the nondefective ev-12 locus in two vaccines and for the defective ev-1 locus in all three vaccines. Both intact and ev-1 pol sequences were also identified in the particle-associated RNA. To investigate the risks of transmission, serum samples from 43 YF vaccine recipients were studied. None of the samples were seropositive by an ALV-E-based Western blot assay or had detectable EAV or ALV-E RNA sequences by RT-PCR. YF vaccines produced by the three manufacturers all have particles containing EAV genomes and various levels of defective or nondefective ALV-E sequences. The absence of evidence of infection with ALV-E or EAV in 43 YF vaccine recipients suggests low risks for transmission of these viruses, further supporting the safety of these vaccines.
Seven distinct sequence variants of the Epstein-Barr virus latent membrane protein 1 (LMP1) have been identified by distinguishing amino acid changes in the carboxy-terminal domain. In this study the transmembrane domains are shown to segregate identically with the distinct carboxy-terminal amino acid sequences. Since strains of LMP1 have been shown to differ in abundance between blood and throat washes, nasopharyngeal carcinomas (NPCs) from areas of endemicity and nonendemicity with matching blood were analyzed by using a heteroduplex tracking assay to distinguish LMP1 variants. Striking differences were found between the compartments with the Ch1 strain prevalent in the NPCs from areas of endemicity and nonendemicity and the B958 strain prevalent in the blood of the endemic samples, whereas multiple strains of LMP1 were prevalent in the blood of the nonendemic samples. The possible selection against the B958 strain appearing in the tumor was highly significant (P < 0.0001). Sequence analysis of the full-length LMP1 variants revealed changes in many of the known and computer-predicted HLA-restricted epitopes with changes in key positions in multiple, potential epitopes for the specific HLA of the patients. These amino acid substitutions at key positions in the LMP1 epitopes may result in a reduced cytotoxic-T-lymphocyte response. These data indicate that strains with specific variants of LMP1 are more likely to be found in NPC. The predominance of specific LMP1 variants in NPC could reflect differences in the biologic or molecular properties of the distinct forms of LMP1 or possible immune selection.

RNA virus genomes contain cis-acting sequence and structural elements that participate in viral replication. We previously identified a bulged stem-loop secondary structure at the upstream end of the 3' untranslated region (3' UTR) of the genome of the coronavirus mouse hepatitis virus (MHV). This element, beginning immediately downstream of the nucleocapsid gene stop codon, was shown to be essential for virus replication. Other investigators discovered an adjacent downstream pseudoknot in the 3' UTR of the closely related bovine coronavirus (BCoV). This pseudoknot was also shown to be essential for replication, and it has a conserved counterpart in every group 1 and group 2 coronavirus. In MHV and BCoV, the bulged stem-loop and pseudoknot are, in part, mutually exclusive, because of the overlap of the last segment of the stem-loop and stem 1 of the pseudoknot. This led us to hypothesize that they form a molecular switch, possibly regulating a transition occurring during viral RNA synthesis. We have now performed an extensive genetic analysis of the two components of this proposed switch. Our results define essential and nonessential components of these structures and establish the limits to which essential parts of each element can be destabilized prior to loss of function. Most notably, we have confirmed the interrelationship of the two putative switch elements. Additionally, we have identified a pseudoknot loop insertion mutation that appears to point to a genetic interaction between the pseudoknot and a distant region of the genome.

Dengue is the most common mosquito-borne viral disease in humans. The spread of both mosquito vectors and viruses has led to the resurgence of epidemic dengue fever (a self-limited flu-like syndrome) and the emergence of dengue hemorrhagic fever (severe dengue with bleeding abnormalities) in urban centers of the tropics. There are no animal or laboratory models of dengue disease; indirect evidence suggests that dengue viruses differ in virulence, including their pathogenicities for humans and epidemic potential. We developed two assay systems (using human dendritic cells and *Aedes aegypti* mosquitoes) for measuring differences in virus replication that correlate with the potential to cause hemorrhagic dengue and increased virus transmission. Infection and growth experiments showed that dengue serotype 2 viruses causing dengue hemorrhagic fever epidemics (Southeast Asian genotype) can outcompete viruses that cause dengue fever only (American genotype). This fact implies that Southeast Asian genotype viruses will continue to displace other viruses, causing more hemorrhagic dengue epidemics.


Two acidic domains of the Potato leafroll virus (PLRV) coat protein, separated by 55 amino acids and predicted to be adjacent surface features on the virion, were the focus of a mutational analysis. Eleven site-directed mutants were generated from a cloned infectious cDNA of PLRV and delivered to plants by Agrobacterium-mediated mechanical inoculation. Alanine substitutions of any of the three amino acids of the sequence EWH (amino acids 170 to 172) or of D177 disrupted the ability of the coat protein to assemble stable particles and the ability of the viral RNA to move systemically in four host plant species. Alanine substitution of E109, D173, or E176 reduced the accumulation of virus in agrobacterium-infiltred tissues, the efficiency of systemic infection, and the efficiency of aphid transmission relative to wild-type virus, but the mutations did not affect virion stability. A structural model of the PLRV capsid predicted that the amino acids critical for virion assembly were located within a depression at the center of a coat protein trimer. The other amino acids that affected plant infection and/or aphid transmission were predicted to be located around the perimeter of the depression. PLRV virions play key roles in phloem-limited virus movement in plant hosts as well as in transport and persistence in the aphid vectors. These results identified amino acid residues in a surface-oriented loop of the coat protein that are critical for virus assembly and stability, systemic infection of plants, and movement of virus through aphid vectors.


Successful human immunodeficiency virus (HIV) vaccines will need to induce effective T-cell immunity. We studied immunodominant simian immunodeficiency virus (SIV) Gag-specific T-cell responses and their restricting major histocompatibility complex (MHC) class I alleles in pigtail macaques (*Macaca nemestrina*), an increasingly common primate model for the study of HIV infection of humans. CD8+ T-cell responses to an SIV epitope, Gag164-172KP9, were present in at least 15 of 36 outbred pigtail macaques. The immunodominant KP9-specific response accounted for the majority (mean, 63%) of the SIV Gag response. Sequencing from six
macaques identified 7 new Mane-A and 13 new Mane-B MHC class I alleles. One new allele, Mane-A*10, was common to four macaques that responded to the KP9 epitope. We adapted reference strand-mediated conformational analysis (RSCA) to MHC class I genotype M. nemestrina. Mane-A*10 was detected in macaques presenting KP9 studied by RSCA but was absent from non-KP9-presenting macaques. Expressed on class I-deficient cells, Mane-A*10, but not other pigtail macaque MHC class I molecules, efficiently presented KP9 to responder T cells, confirming that Mane-A*10 restricts the KP9 epitope. Importantly, naive pigtail macaques infected with SIVmac251 that respond to KP9 had significantly reduced plasma SIV viral levels (log10 0.87 copies/ml; P = 0.025) compared to those of macaques not responding to KP9. The identification of this common M. nemestrina MHC class I allele restricting a functionally important immunodominant SIV Gag epitope establishes a basis for studying CD8+ T-cell responses against AIDS in an important, widely available nonhuman primate species.


http://jvi.asm.org/cgi/content/abstract/77/13/7225

The adenovirus (Ad) fiber protein mediates Ad binding to the coxsackievirus and Ad receptor (CAR) and is thus a major determinant of viral tropism. The fiber contains three domains: an N-terminal tail that anchors the fiber to the viral capsid, a central shaft region of variable length and flexibility, and a C-terminal knob domain that binds to cell receptors. Ad type 37 (Ad37), a subgroup D virus associated with severe ocular infections, is unable to use CAR efficiently to infect host cells, despite containing a CAR binding site in its fiber knob. We hypothesized that the relatively short, inflexible Ad37 fiber protein restricts interactions with CAR at the cell surface. To test this hypothesis, we analyzed the infectivity and binding of recombinant Ad particles containing modified Ad37 or Ad5 fiber proteins. Ad5 particles equipped with a truncated Ad5 fiber or with a chimeric fiber protein comprised of the Ad5 knob fused to the short, rigid Ad37 shaft domain had significantly reduced infectivity and attachment. In contrast, placing the Ad37 knob onto the long, flexible Ad5 shaft allowed CAR-dependent virus infection and cell attachment, demonstrating the importance of the shaft domain in receptor usage. Increasing fiber rigidity by substituting the predicted flexibility modules in the Ad5 shaft with the corresponding regions of the rigid Ad37 fiber dramatically reduced both virus infection and cell attachment. Cryoelectron microscopy (cryo-EM) single-particle analysis demonstrated the increased rigidity of this chimeric fiber. These studies demonstrate that both length and flexibility of the fiber shaft regulate CAR interaction and provide a molecular explanation for the use of alternative receptors by subgroup D Ad with ocular tropism. We present a molecular model for Ad-CAR interactions at the cell surface that explains the significance of fiber flexibility in cell attachment.


http://jvi.asm.org/cgi/content/abstract/76/14/7000

The emergence of antiretroviral (ART) drug-resistant human immunodeficiency virus type 1 (HIV-1) quasispecies is a major cause of treatment failure. These variants are usually replaced by drug-sensitive ones when the selective pressure of the drugs is removed, as the former have reduced fitness in a drug-free environment. This was the rationale for the design of structured ART treatment interruption (STI) studies for the management of HIV-1 patients with treatment failure. We have studied the origin of drug-sensitive HIV-1 quasispecies emerging after STI in patients with treatment failure due to ART drug resistance. Plasma and peripheral blood
mononuclear cell samples were obtained the day of treatment interruption (day 0) and 30 and 60 days afterwards. HIV-1 pol and env were partially amplified, cloned, and sequenced. At day 60 drug-resistant variants were replaced by completely or partially sensitive quasispecies. Phylogenetic analyses of pol revealed that drug-sensitive variants emerging after STI were not related to their immediate temporal ancestors but formed a separate cluster, demonstrating that STI leads to the recrudescence and reemergence of a sequestered viral population rather than leading to the back mutation of drug-resistant forms. No evidence for concomitant changes in viral tropism was seen, as deduced from env sequences. This study demonstrates the important role that the reemergence of quasispecies plays in HIV-1 population dynamics and points out the difficulties that may be found when recycling ARV therapies with patients with treatment failure.


http://jvi.asm.org/cgi/content/abstract/76/14/7140

Pattern recognition proteins such as lipopolysaccharide and (beta)-1,3-glucan binding protein (LGBP) play an important role in the innate immune response of crustaceans and insects. Random sequencing of cDNA clones from a hepatopancreas cDNA library of white spot virus (WSV)-infected shrimp provided a partial cDNA (PsEST-289) that showed similarity to the LGBP gene of crayfish and insects. Subsequently full-length cDNA was cloned by the 5'-RACE (rapid amplification of cDNA ends) technique and sequenced. The shrimp LGBP gene is 1,352 bases in length and is capable of encoding a polypeptide of 376 amino acids that showed significant similarity to homologous genes from crayfish, insects, earthworms, and sea urchins. Analysis of the shrimp LGBP deduced amino acid sequence identified conserved features of this gene family including a potential recognition motif for (beta)-(1-[gt])3 linkage of polysaccharides and putative RGD cell adhesion sites. It is known that LGBP gene expression is upregulated in bacterial and fungal infection and that the binding of lipopolysaccharide and (beta)-1,3-glucan to LGBP activates the prophenoloxidase (proPO) cascade. The temporal expression of LGBP and proPO genes in healthy and WSV-challenged Penaeus stylirostris shrimp was measured by real-time quantitative reverse transcription-PCR, and we showed that LGBP gene expression in shrimp was upregulated as the WSV infection progressed. Interestingly, the proPO expression was upregulated initially after infection followed by a downregulation as the viral infection progressed. The downward trend in the expression of proPO coincided with the detection of WSV in the infected shrimp. Our data suggest that shrimp LGBP is an inducible acute-phase protein that may play a critical role in shrimp-WSV interaction and that the WSV infection regulates the activation and/or activity of the proPO cascade in a novel way.


http://jvi.asm.org/cgi/content/abstract/77/14/7914

Hepatitis C virus (HCV) infection is thought to mostly become chronic and rarely resolves. HCV infection was serologically screened in 4,984 samples from Ghanaian blood donors, and 1.3% prevalence was found. At least 53% of confirmed anti-HCV carriers had no detectable viral RNA and were considered to have cleared the virus and recovered from the infection. Confirmation was authenticated by the presence of antibodies specific to at least two viral antigens, mostly NS3 and E2. Reactivity to HCV core antigens was lower in Ghanaian than United Kingdom blood donors. The minority of chronically infected donors carried a viral load significantly lower than an unselected comparative group of United Kingdom blood donors (2.5 x 10^5 versus 2.9 x 10^6 IU/ml;
P = 0.004). HCV genotype 2 was largely predominant (87%). Sequence clustering was similarly broad in the E1/E2 and NS5 regions. The phylogenetic diversity and the incapacity to distinguish subtypes within genotype 2 in our and others’ West African strains suggested that West Africa may be the origin of HCV genotype 2. The genetic diversity extended to the identification of strains clearly separated from known subtypes of genotype 2 and genotype 1. One strain appears to be part of a new HCV genotype. HCV infection in Ghana is characterized by a high rate of recovery and the predominance of broadly divergent genotype 2 strains.


http://jvi.asm.org/cgi/content/abstract/78/14/7427

The phenylmethylthiazolylthiourea (PETT) derivative MSK-076 shows, besides high potency against human immunodeficiency virus type 1 (HIV-1), marked activity against HIV-2 (50% effective concentration, 0.63 \( \mu \)M) in cell culture. Time-of-addition experiments pointed to HIV-2 reverse transcriptase (RT) as the target of action of MSK-076. Recombinant HIV-2 RT was inhibited by MSK-076 at 23 \( \mu \)M. As was also found for HIV-1 RT, MSK-076 inhibited HIV-2 RT in a noncompetitive manner with respect to dGTP and poly(rC)poly(dG) as the substrate and template-primer, respectively. MSK-076 selected for A101P and G112E mutations in HIV-2 RT and for K101E, Y181C, and G190R mutations in HIV-1 RT. The selected mutated strains of HIV-2 were fully resistant to MSK-076, and the mutant HIV-2 RT enzymes into which the A101P and/or G112E mutation was introduced by site-directed mutagenesis showed more than 50-fold resistance to MSK-076. Mapping of the resistance mutations to the HIV-2 RT structure ascertained that A101P is located at a position equivalent to the nonnucleoside RT inhibitor (NNRTI)-binding site of HIV-1 RT. G112E, however, is distal to the putative NNRTI-binding site in HIV-2 RT but close to the active site, implying a novel molecular mode of action and mechanism of resistance. Our findings have important implications for the development of new NNRTIs with pronounced activity against a wider range of lentiviruses.


http://jvi.asm.org/cgi/content/abstract/76/11/5588

The more severe form of dengue virus infection, dengue hemorrhagic fever, is characterized by plasma leakage and derangements in hemostasis. As elevated interleukin-8 (IL-8) levels have been observed in sera from patients with more severe disease manifestations, a study was initiated to look at the effect of dengue virus infection in vitro on proinflammatory cytokine secretion and expression. A significant increase in IL-8 levels in the culture supernatant of primary human monocytes infected with dengue 2 virus (D2V) New Guinea C (NGC) was found by enzyme-linked immunosorbent assay. Additionally, by reverse transcriptase PCR, the mRNA was also augmented. Among the proinflammatory cytokines and their mRNAs measured (IL-6, IL-1{beta}, IL-8, and tumor necrosis factor alpha), IL-8 showed the greatest change following D2V infection. Similarly, two cell lines, 293T (a human epithelial cell line) and ECV304 (an endothelial cell line), were permissive to D2V NGC and responded to the infection by increasing the synthesis of IL-8. Nuclear factor kappa B (NF-\( \kappa \)B) and nuclear factor IL-6 (NFIL-6) are primary mediators of IL-8 expression. We studied the transcriptional regulation of IL-8 in the ECV304 and 293T cell lines and found that the induction of IL-8 gene expression involved the activation of NF-\( \kappa \)B (P = 0.001) and, to a lesser extent, the activation of NFIL-6 in ECV304.
cells only. We next observed by the chromatin immunoprecipitation procedure in vivo acetylation of core histones bound to the IL-8 promoter after D2V infection. IL-8 produced by infected monocytes and also IL-8 that may be produced by endothelial or other epithelial cells is associated with the hyperacetylation of histones bound to the IL-8 promoter in addition to the activation of transcription by NF-(kappa)B. We hypothesize that the overall increase in IL-8 synthesis observed in this in vitro study may play a role in the pathogenesis of the plasma leakage seen in dengue hemorrhagic fever and dengue shock syndrome.


http://jvi.asm.org/cgi/content/abstract/76/11/5540

Upon retroviral infection, the genomic RNA is reverse transcribed to make proviral DNA, which is then integrated into the host chromosome. Although the viral elements required for successful integration have been extensively characterized, little is known about the host DNA structure constituting preferred targets for proviral integration. In order to elucidate the mechanism for the target selection, comparison of host DNA sequences at proviral integration sites may be useful. To achieve simultaneous analysis of the upstream and downstream host DNA sequences flanking each proviral integration site, a Moloney murine leukemia virus-based retroviral vector was designed so that its integrated provirus could be removed by Cre-loxP homologous recombination, leaving a solo long terminal repeat (LTR). Taking advantage of the solo LTR, inverse PCR was carried out to amplify both the upstream and downstream cellular flanking DNA. The method called solo LTR inverse PCR, or SLIP, proved useful for simultaneously cloning the upstream and downstream flanking sequences of individual proviral integration sites from the polyclonal population of cells harboring provirus at different chromosomal sites. By the SLIP method, nucleotide sequences corresponding to 38 independent proviral integration targets were determined and, interestingly, atypical virus-host DNA junction structures were found in more than 20% of the cases. Characterization of retroviral integration sites using the SLIP method may provide useful insights into the mechanism for proviral integration and its target selection.


http://jvi.asm.org/cgi/content/abstract/77/11/6227

Infection with genital human papillomaviruses (HPVs) is the primary cause of cervical cancer. The infection is widespread, and little is known about the secondary factors associated with progression from subclinical infection to invasive carcinoma. Here we report that HPV genomes are efficiently targeted in vivo by CpG methylation, a well-known mechanism of transcriptional repression. Indeed, it has been shown previously that in vitro-methylated HPV type 16 (HPV-16) DNA is transcriptionally repressed after transfection into cell cultures. By using a scan with the restriction enzyme MrcBC, we observed a conserved profile of CpG hyper- and hypomethylation throughout the HPV-16 genomes of the tumor-derived cell lines SiHa and CaSki. Methylation is particularly high in genomic segments overlying the late genes, while the long control region (LCR) and the oncogenes are unmethylated in the single HPV-16 copy in SiHa cells. In 81 patients from two different cohorts, the LCR and the E6 gene of HPV-16 DNA were found to be hypermethylated in 52% of asymptomatic smears, 21.7% of precursor lesions, and 6.1% of invasive carcinomas. This suggests that neoplastic transformation may be suppressed by CpG methylation, while demethylation occurs as the cause of or concomitant with neoplastic progression. These prevalences of hyper- and hypomethylation also indicate that CpG
methylation plays an important role in the papillomavirus life cycle, which takes place in asymptomatic infections and precursor lesions but not in carcinomas. Bisulfite modification revealed that in most of the HPV-16 genomes of CaSki cells and of asymptomatic patients, all 11 CpG dinucleotides that overlap with the enhancer and the promoter were methylated, while in SiHa cells and cervical lesions, the same 11 or a subset of CpGs remained unmethylated. Our report introduces papillomaviruses as models to study the mechanism of CpG methylation, opens research on the importance of this mechanism during the viral life cycle, and provides a marker relevant for the etiology and diagnosis of cervical cancer.


http://jvi.asm.org/cgi/content/abstract/77/11/6178

The accumulation of cellular transcripts from cells infected with herpes simplex virus 1 (HSV-1) as measured with the aid of Affymetrix microchips has been reported elsewhere. Among these transcripts were genes that respond to stress and that could have a noxious effect on viral replication. We have selected the stress-inducible cellular gene encoding the immediate-early response protein IEX-1 to verify and determine the significance of the accumulation of these transcripts in infected cells. We report that we verified the increase in accumulation of IEX-1 transcripts after infection by Northern analyses and real-time PCR. These transcripts reach peak levels between 3 and 7 h after infection and decrease thereafter. However, IEX-1 protein was detected in cells 1 h after infection but not at later intervals. Studies designed to elucidate the failure of IEX-1 protein to be synthesized revealed the following points. (i) IEX-1 RNA transported to the cytoplasm after 1 h of infection consisted of at least two populations, a partially degraded population and a population consisting of unspliced IEX-1 RNA. Neither of these RNAs could translate the authentic IEX-1 protein. (ii) The partially degraded IEX-1 RNA was not detected in the cytoplasm of cells infected with a mutant virus lacking the UL41 gene encoding the virion host shutoff protein (vhs). Although degradation of RNA mediated by vhs was reported to be 5’ to 3’, the partially degraded IEX-1 RNA lacked the 3’ sequences rather than the 5’ sequences. (iii) The unspliced pre-RNA form containing the IEX-1 intron sequences was detected in the cytoplasm of cell infected with wild-type virus but not in those infected with a mutant lacking the [alpha]27 gene encoding the infected cell protein No. 27. (iv) Overexpression of IEX-1 protein by transduction of the gene prior to infection with 1 PFU of HSV-1 per cell had no effect on the accumulation of late genes and virus yield. We conclude that the failure of IEX-1 to express its protein reflects the numerous mechanisms by which the virus thwarts the cells from expressing its genes after infection.


http://jvi.asm.org/cgi/content/abstract/76/12/6131

Eight hepatitis B virus (HBV) isolates of genotype G were recovered from patients and sequenced over the entire genome. Six of them had a genomic length of 3,248 bp and two had genomic lengths of 3,239 bp (USG15) and 3,113 bp (USG18) due to deletions. The 10 HBV/G isolates, including the 8 sequenced isolates as well as the original isolate (AF160501) and another isolate (B1-89), had a close sequence homology of 99.3 to 99.8% among themselves (excluding USG18 with a long deletion) but of <88.7% to any of the 68 HBV isolates of the other six genotypes with the full-length sequence known. The eight HBV/G isolates possessed an insertion of 36 bp in the
core gene and two stop codons in the precore region, as did the AF160501 and B1-89 isolates. The 10 HBV/G isolates clustered on a branch separate from those bearing the other six genotypes (A through F [A-F]) in the phylogenetic tree constructed from full-length sequences of 78 HBV isolates as well as in those constructed from the core, polymerase, X, and envelope genes. Despite two stop codons in the precore region that prohibited the translation of the HBV e antigen (HBeAg), all of the eight patients with HBV/G infection possessed the HBeAg in serum. By restriction fragment length polymorphism of the surface gene, all of the eight patients were found to be coinfected with HBV of genotype A (HBV/A), which would be responsible for the expression of HBeAg in them. It is worthy of examination to determine how coinfection occurs and whether HBV/G needs HBV/A for replication.


http://jvi.asm.org/cgi/content/abstract/78/12/6409

Complement plays a pivotal role in the regulation of innate and adaptive immunity. It has been shown that the binding of C1q, a natural ligand of gC1qR, on T cells inhibits their proliferation. Here, we demonstrate that direct binding of the hepatitis C virus (HCV) core to gC1qR on T cells leads to impaired Lck/Akt activation and T-cell function. The HCV core associates with the surface of T cells specifically via gC1qR, as this binding is inhibited by the addition of either anti-gC1qR antibody or soluble gC1qR. The binding affinity constant of core protein for gC1qR, as determined by BIAcore analysis, is 3.8 x 10^-7 M. The specificity of the HCV core-gC1qR interaction is confirmed by reduced core binding on Molt-4 T cells treated with gC1qR-silencing small interfering RNA and enhanced core binding on GPC-16 guinea pig cells transfected with human gC1qR. Interestingly, gC1qR is expressed at higher levels on CD8+ than on CD4+ T cells, resulting in more severe core-induced suppression of the CD8+-T-cell population. Importantly, T-cell receptor-mediated activation of the Src kinases Lck and ZAP-70 but not Fyn and the phosphorylation of Akt are impaired by the HCV core, suggesting that it inhibits the very early events of T-cell activation.


http://jvi.asm.org/cgi/content/abstract/77/5/3148

H9 influenza viruses have become endemic in land-based domestic poultry in Asia and have sporadically crossed to pigs and humans. To understand the molecular determinants of their adaptation to land-based birds, we tested the replication and transmission of several 1970s duck H9 viruses in chickens and quail. Quail were more susceptible than chickens to these viruses, and generation of recombinant H9 viruses by reverse genetics showed that changes in the HA gene are sufficient to initiate efficient replication and transmission in quail. Seven amino acid positions on the HA molecule corresponded to adaptation to land-based birds. In quail H9 viruses, the pattern of amino acids at these seven positions is intermediate between those of duck and chicken viruses; this fact may explain the susceptibility of quail to duck H9 viruses. Our findings suggest that quail provide an environment in which the adaptation of influenza viruses from ducks generates novel variants that can cross the species barrier.

http://jvi.asm.org/cgi/content/abstract/78/5/2478

The molecular clones pSPeiav19 and p19/wenv17 of equine infectious anemia virus (EIAV) differ in env and long terminal repeats (LTRs) and produce viruses (EIAV19 and EIAV17, respectively) of dramatically different virulence phenotypes. These constructs were used to generate a series of chimeric clones to test the individual contributions of LTR, surface (SU), and transmembrane (TM)/Rev regions to the disease potential of the highly virulent EIAV17. The LTRs of EIAV19 and EIAV17 differ by 16 nucleotides in the transcriptional enhancer region. The two viruses differ by 30 amino acids in SU, by 17 amino acids in TM, and by 8 amino acids in Rev. Results from in vivo infections with chimeric clones indicate that both LTR and env of EIAV17 are required for the development of severe acute disease. In the context of the EIAV17 LTR, SU appears to have a greater impact on virulence than does TM. EIAV17SU, containing only the TM/Rev region from the avirulent parent, induced acute disease in two animals, while a similar infectious dose of EIAV17TM (which derives SU from the avirulent parent) did not. Neither EIAV17SU nor EIAV17TM produced lethal disease when administered at infectious doses that were 6- to 30-fold higher than a lethal dose of the parental EIAV17. All chimeric clones replicated in primary equine monocyte-derived macrophages, and there was no apparent correlation between macrophage tropism and virulence phenotype.


http://jvi.asm.org/cgi/content/abstract/78/5/2502

The potential transmission of porcine endogenous retroviruses (PERVs) has raised concern in the development of porcine xenotransplantation products. Our previous studies have resulted in the identification of animals within a research herd of inbred miniature swine that lack the capacity to transmit PERV to human cells in vitro. In contrast, other animals were capable of PERV transmission. The PERVs that were transmitted to human cells are recombinants between PERV-A and PERV-C in the post-VRA region of the envelope (B. A. Oldmixon, J. C. Wood, T. A. Ericsson, C. A. Wilson, M. E. White-Scharf, G. Andersson, J. L. Greenstein, H. J. Schuurman, and C. Patience, J. Virol. 76:3045-3048, 2002); these viruses we term PERV-A/C. This observation prompted us to determine whether these human-tropic replication-competent (HTRC) PERV-A/C recombinants were present in the genomic DNA of these miniature swine. Genomic DNA libraries were generated from one miniature swine that transmitted HTRC PERV as well as from one miniature swine that did not transmit HTRC PERV. HTRC PERV-A/C proviruses were not identified in the germ line DNAs of these pigs by using genomic mapping. Similarly, although PERV-A loci were identified in both libraries that possessed long env open reading frames, the Env proteins encoded by these loci were nonfunctional according to pseudotype assays. In the absence of a germ line source for HTRC PERV, further studies are warranted to assess the mechanisms by which HTRC PERV can be generated. Once identified, it may prove possible to generate animals with further reduced potential to produce HTRC PERV.

We describe the development of a selectable, bi-cistronic subgenomic replicon for bovine viral diarrhea virus (BVDV) in Huh-7 cells, similar to that established for hepatitis C virus (HCV). The selection marker and reporter (Luc-Ubi-Neo) in the BVDV replicon was fused with the amino-terminal protease Npro, and expression of the nonstructural proteins (NS3 to NS5B) was driven by an encephalomyocarditis virus internal ribosome entry site. This BVDV replicon allows us to compare RNA replication of these two related viruses in a similar cellular background and to identify antiviral molecules specific for HCV RNA replication. The BVDV replicon showed similar sensitivity as the HCV replicon to interferons (alpha, beta, and gamma) and 2'-{beta}-C-methyl ribonucleoside inhibitors. Known nonnucleoside inhibitor molecules specific for either HCV or BVDV can be easily distinguished by using the parallel replicon systems. The HCV replicon has been shown to block, via the NS3/4A serine protease, Sendai virus-induced activation of interferon regulatory factor 3 (IRF-3), a key antiviral signaling molecule. Similar suppression of IRF-3-mediated responses was also observed with the Huh-7-BVDV replicon but was independent of NS3/4A protease activity. Instead, the amino-terminal cysteine protease Npro of BVDV appears to be, at least partly, responsible for suppressing IRF-3 activation induced by Sendai virus infection. This result suggests that different viruses, including those closely related, may have developed unique mechanisms for evading host antiviral responses. The parallel BVDV and HCV replicon systems provide robust counterscreens to distinguish viral specificity of small-molecule inhibitors of viral replication and to study the interactions of the viral replication machinery with the host cell innate immune system.


Following the introduction of highly active antiretroviral therapy (HAART), the incidence of Kaposi's sarcoma (KS) has significantly declined in human immunodeficiency virus type 1 (HIV-1)-positive (HIV-1+) individuals and clinical remission is often observed. We hypothesize that these effects are partly due to anti-KS-associated herpesvirus (KSHV) immune restoration. Here, 15-mer overlapping peptides from proteins K12 and K8.1 were used to identify novel KSHV-specific cytotoxic T-lymphocyte epitopes. Three immunogenic peptides, two lytic and one latent, were subsequently used to monitor the anti-KSHV CD8+ T-cell responses in a cohort of 19 HIV-1+ KSHV+/- KS+/- individuals during 52 weeks of HAART. KSHV and HIV-1 loads, KSHV antibody titers, and both CD4+ and CD8+ T-lymphocyte counts were enumerated. Prior to HAART, the total number of spot-forming cells (SFC) for all three peptides correlated with both CD4+ and CD8+ T-lymphocyte counts (P \[\leq\] 0.05) in the KSHV-positive KS-positive cohort (n = 11). Following 52 weeks of HAART, significant decreases in HIV-1 and KSHV loads were associated with significant increases in CD4+ T-lymphocyte counts and number of SFC for the three KSHV-specific peptides. Although these increases were modest in comparison to the number of SFC observed with the HIV-1 gag peptide SLYNTVATL, they represented a fourfold increase from the baseline, continuing an upward trend to week 52.


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Kaposi’s sarcoma-associated herpesvirus (also called human herpesvirus type 8 [HHV8]) latently infects a number of cell types. Reactivation of latent virus can occur by treatment with the phorbol ester tetradecanoyl phorbol acetate (TPA) or with the transfection of plasmids expressing the lytic switch activator protein K-Rta, the gene product of ORF50. K-Rta expression is sufficient for the activation of the entire lytic cycle and the transactivation of viral genes necessary for DNA replication. In addition, recent evidence has suggested that K-Rta may participate directly in the initiation of lytic DNA synthesis. We have now generated a recombinant HHV8 bacterial artificial chromosome (BAC) with a large deletion within the ORF50 locus. This BAC, BAC36(Delta)50, failed to produce infectious virus upon treatment with TPA and was defective for DNA synthesis. Expression of K-Rta in trans in BAC36(Delta)50-containing cells was able to abolish both defects. Real-time PCR revealed that K-bZIP, ORF40/41, and K8.1 were not expressed when BAC36(Delta)50-containing cells were induced with TPA. However, the mRNA levels of ORF57 were over fivefold higher in TPA-treated BAC36(Delta)50-containing cells than those observed in similarly treated wild-type BAC-containing cells. In addition, immunohistochemical analysis showed that while the latency-associated nuclear antigen (LANA) was expressed in the mutant BAC-containing cells, ORF59 and K8.1 expression was not detected in TPA-induced BAC36(Delta)50-containing cells. These results showed that K-Rta is essential for lytic viral reactivation and transactivation of viral genes contributing to DNA replication.


http://jvi.asm.org/cgi/content/abstract/76/9/4612

Since targeting of recombinant adenovirus vectors to defined cell types in vivo is a major challenge in gene therapy and vaccinology, we explored the natural diversity in human adenovirus tissue tropism. Hereto, we constructed a library of Ad5 vectors carrying fibers from other human serotypes. From this library, we identified vectors that efficiently infect human cells that are important for diverse gene therapy approaches and for induction of immunity. For several medical applications (prenatal diagnosis, artificial bone, vaccination, and cardiovascular disease), we demonstrate the applicability of these novel vectors. In addition, screening cell types derived from different species revealed that cellular receptors for human subgroup B adenoviruses are not conserved between rodents and primates. These results provide a rationale for utilizing elements of human adenovirus serotypes to generate chimeric vectors that improve our knowledge concerning adenovirus biology and widen the therapeutic window for vaccination and many different gene transfer applications.


http://jvi.asm.org/cgi/content/abstract/77/9/5065

Mus spicilegus is an Eastern European wild mouse species that has previously been reported to harbor an unusual infectious ecotropic murine leukemia virus (MLV) and proviral envelope genes of a novel MLV subgroup. In the present study, M. spicilegus neonates were inoculated with Moloney ecotropic MLV (MoMLV). All 17 inoculated mice produced infectious ecotropic virus after 8 to 14 weeks, and two unusual phenotypes distinguished the isolates from MoMLV. First, most of the M. spicilegus isolates grew to equal titers on M. dunnii and SC-1 cells, although MoMLV does not efficiently infect M. dunnii cells. The deduced amino acid sequence of a representative clone differed from MoMLV by insertion of two serine residues within the VRA of SUenv. Modification of a molecular clone of MoMLV by the addition of these serines produced a virus that grows to high titer in M. dunnii cells, establishing a role for these two serine residues in host
A second unusual phenotype was found in only one of the M. spicilegus isolates, Spl574. Spl574 produces large syncytia of multinucleated giant cells in M. dunni cells, but its replication is restricted in other mouse cell lines. Sequencing and mutagenesis demonstrated that syncytium formation could be attributed to a single amino acid substitution within VRA, S82F. Thus, viruses with altered growth properties are selected during growth in M. spicilegus. The mutations associated with the host range and syncytium-inducing variants map to a key region of VRA known to govern interactions with the cell surface receptor, suggesting that the associated phenotypes may result from altered interactions with the unusual ecotropic virus mCAT1 receptor carried by M. dunni.


http://jvi.asm.org/cgi/content/abstract/77/9/5084

We previously identified retroperitoneal fibromatosis-associated herpesvirus (RFHV) as a simian homolog of Kaposi's sarcoma-associated herpesvirus (KSHV) in a fibroproliferative malignancy of macaques that has similarities to Kaposi's sarcoma. In this report, we cloned 4.3 kb of divergent locus B (DL-B) flanking the DNA polymerase gene from two variants of RFHV from different species of macaque with a consensus degenerate hybrid oligonucleotide primer approach. Within the DL-B region of RFHV, viral homologs of the cellular interleukin-6, dihydrofolate reductase, and thymidylate synthase genes were identified, along with a homolog of the gammaherpesvirus open reading frame (ORF) 10. In addition, a homolog of the KSHV ORF K3, the modulator of immune recognition-1, was identified. Our data show a close similarity in sequence conservation, gene content, and genomic structure between RFHV and KSHV which strongly supports the grouping of these viral species within the same RV-1 rhadinovirus lineage and the hypothesis that RFHV is the macaque homolog of KSHV.


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Three types of human T-cell leukemia virus (HTLV)-simian T-cell leukemia virus (STLV) (collectively called primate T-cell leukemia viruses [PTLVs]) have been characterized, with evidence for zoonotic origin from primates for HTLV type 1 (HTLV-1) and HTLV-2 in Africa. To assess human exposure to STLVs in western Central Africa, we screened for STLV infection in primates hunted in the rain forests of Cameroon. Blood was obtained from 524 animals representing 18 different species. All the animals were wild caught between 1999 and 2002; 328 animals were sampled as bush meat and 196 were pets. Overall, 59 (11.2%) of the primates had antibodies cross-reacting with HTLV-1 and/or HTLV-2 antigens; HTLV-1 infection was confirmed in 37 animals, HTLV-2 infection was confirmed in 9, dual HTLV-1 and HTLV-2 infection was confirmed in 10, and results for 3 animals were indeterminate. Prevalences of infection were significantly lower in pets than in bush meat, 1.5 versus 17.0%, respectively. Discriminatory PCRs identified STLV-1, STLV-3, and STLV-1 and STLV-3 in HTLV-1, HTLV-2, and HTLV-1- and HTLV-2-cross-reactive samples, respectively. We identified for the first time STLV-1 sequences in mustached monkeys (Cercopithecus cephus), talapoin (Miopithecus ogouensis), and gorillas (Gorilla gorilla) and confirmed STLV-1 infection in mandrills, African green monkeys, agile mangabeys, and crested mona and greater spot-nosed monkeys. STLV-1 long terminal repeat
(LTR) and env sequences revealed that the strains belonged to different PTLV-1 subtypes. A high prevalence of PTLV infection was observed among agile mangabeys (Cercocebus agilis); 89% of bush meat was infected with STLV. Cocirculation of STLV-1 and STLV-3 and STLV-1-STLV-3 coinfections were identified among the agile mangabeys. Phylogenetic analyses of partial LTR sequences indicated that the agile mangabey STLV-3 strains were more related to the STLV-3 CTO604 strain isolated from a red-capped mangabey (Cercocebus torquatus) from Cameroon than to the STLV-3 PH969 strain from an Eritrean baboon or the PPA-F3 strain from a baboon in Senegal. Our study documents for the first time that (i) a substantial proportion of wild-living monkeys in Cameroon is STLV infected, (ii) STLV-1 and STLV-3 cocirculate in the same primate species, (iii) coinfection with STLV-1 and STLV-3 occurs in agile mangabeys, and (iv) humans are exposed to different STLV-1 and STLV-3 subtypes through handling primates as bush meat.


http://jvi.asm.org/cgi/content/abstract/78/9/4408

AIDS-related B-cell non-Hodgkin's lymphoma (AIDS-NHL) is a significant cause of morbidity and mortality among individuals infected with human immunodeficiency virus type 1 (HIV-1). AIDS-NHL is clinically and histologically heterogeneous, but common features include an aggressive clinical course and frequent extranodal presentation. HIV-1 infection of nonimmune cells that interact with malignant B cells at extranodal sites may influence both the development and the clinical presentation of disease. Our previous studies have shown that coculture of B-lymphoma (BL) cells with HIV-1-infected endothelial cells (EC) leads to contact activation of EC and firm BL-cell adhesion. The key event promoting EC-BL-cell adhesion was HIV-1 upregulation of endothelial CD40, which allowed induction of vascular cell adhesion molecule 1 (VCAM-1) in a CD40-dependent manner. The present study was designed to identify the HIV-1 protein(s) that influence EC-BL-cell adhesion. When HIV-1 proteins were individually expressed in EC by using recombinant adenoviruses, cultured BL cells adhered exclusively to Vpu-transduced EC. As with HIV-infected EC, adhesive properties were linked to the capacity of Vpu to upregulate CD40, which in turn allowed efficient expression of VCAM-1. When EC were infected with an HIV-1 pseudotype lacking the Vpu gene, CD40 upregulation and BL-cell adhesive properties were lost, indicating an essential role for Vpu in EC-BL-cell interactions. Thus, these data reveal a novel function for HIV-1 Vpu and further suggest a role for Vpu in the development of AIDS-NHL at EC-rich extranodal sites.


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Resistance to enfuvirtide (ENF; T-20), a fusion inhibitor of human immunodeficiency virus type 1 (HIV-1), is conferred by mutations in the first heptad repeat of the gp41 ectodomain. The replicative fitness of recombinant viruses carrying ENF resistance mutations was studied in growth competition assays. ENF resistance mutations, selected in vitro or in vivo, were introduced into the env gene of HIV-1NL4-3 by site-directed mutagenesis and expressed in HIV-1 recombinants carrying sequence tags in nef. The doubling time of ENF-resistant viruses was highly correlated with decreasing ENF susceptibility ($R^2 = 0.859; P < 0.001$). Initial fitness experiments focused on mutants identified by in vitro selection in the presence of ENF (L. T. Rimsky, D. C. Shugars, and T. J. Matthews, J. Virol. 72:986-993, 1998). In the absence of drug, these mutants displayed reduced fitness compared to wild-type virus with a relative order of
fitness of wild type > I37T > V38 M > D36S/V38 M; this order was reversed in the presence of ENF. Likewise, recombinant viruses carrying ENF resistance mutations selected in vivo displayed reduced fitness in the absence of ENF with a relative order of wild type > N42T > V38A > N42T/N43K (approx) N42T/N43S > V38A/N42D (approx) V38A/N42T. Fitness and ENF susceptibility were inversely correlated ($r = -0.988; P < 0.001$). Similar results were obtained with recombinants expressing molecularly cloned full-length env genes obtained from patient-derived HIV-1 isolates before and after ENF treatment. Further studies are needed to determine whether the reduced fitness of ENF-resistant viruses alters their pathogenicity in vivo.


http://jvi.asm.org/cgi/content/abstract/78/9/4665

A safe and effective dengue vaccine is still not available. Passive immunization with monoclonal antibodies from humans or nonhuman primates represents an attractive alternative for the prevention of dengue virus infection. Fab monoclonal antibodies to dengue type 4 virus (DENV-4) were recovered by repertoire cloning of bone marrow mRNAs from an immune chimpanzee and analyzed for antigen binding specificity, VH and VL sequences, and neutralizing activity against DENV-4 in vitro. Fabs 5A7, 3C1, 3E4, and 7G4 were isolated from a library constructed from a chimpanzee following intrahepatic transfection with infectious DENV-4 RNA. Fabs 5H2 and 5D9, which had nearly identical VH sequences but varied in their VL sequences, were recovered from a library constructed from the same chimpanzee after superinfection with a mixture of DENV-1, DENV-2, and DENV-3. In radioimmunoprecipitation, Fab 5A7 precipitated only DENV-4 prM, and Fabs 3E4, 7G4, 5D9, and 5H2 precipitated DENV-4 E but little or no prM. Fab 3E4 and Fab 7G4 competed with each other for binding to DENV-4 in an enzyme-linked immunosorbent assay, as did Fab 3C1 and Fab 5A7. Fab 5H2 recognized an epitope on DENV-4 that was separate from the epitope(s) recognized by other Fabs. Both Fab 5H2 and Fab 5D9 neutralized DENV-4 efficiently with a titer of 0.24 to 0.58 (micro)g/ml by plaque reduction neutralization test (PRNT), whereas DENV-4-neutralizing activity of other Fabs was low or not detected. Fab 5H2 was converted to full-length immunoglobulin G1 (IgG1) by combining it with human sequences. The humanized chimpanzee antibody IgG1 5H2 produced in CHO cells neutralized DENV-4 strains from different geographical origins at a similar 50% plaque reduction (PRNT50) titer of 0.03 to 0.05 (micro)g/ml. The DENV-4 binding affinities were 0.42 nM for Fab 5H2 and 0.24 nM for full-length IgG1 5H2. Monoclonal antibody IgG1 5H2 may prove valuable for passive immunoprophylaxis against dengue virus in humans.


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The role of DC-SIGN on human rectal mucosal dendritic cells is unknown. Using highly purified human rectal mucosal DC-SIGN+ cells and an ultrasensitive real-time reverse transcription-PCR assay to quantify virus binding, we found that HLA-DR+/DC-SIGN+ cells can bind and transfer more virus than the HLA-DR+/DC-SIGN- cells. Greater than 90% of the virus bound to total mucosal mononuclear cells (MMCs) was accounted for by the DC-SIGN+ cells, which comprise only 1 to 5% of total MMCs. Significantly, anti-DC-SIGN antibodies blocked 90% of the virus binding when more-physiologic amounts of virus inoculum were used. DC-SIGN expression in the rectal mucosa was significantly correlated with the interleukin-10 (IL-10)/IL-12 ratio ($r = 0.58, P < 0.002; n = 26$) among human immunodeficiency virus (HIV)-positive patients. Ex vivo and in vitro
data implicate the role of IL-10 in upregulating DC-SIGN expression and downregulating expression of the costimulatory molecules CD80/CD86. Dendritic cells derived from monocytes (MDDCs) in the presence of IL-10 render the MDDCs less responsive to maturation stimuli, such as lipopolysaccharide and tumor necrosis factor alpha, and migration to the CCR7 ligand macrophage inflammatory protein 3{beta}. Thus, an increased IL-10 environment could render DC-SIGN+ cells less immunostimulatory and migratory, thereby dampening an effective immune response. DC-SIGN and the IL-10/IL-12 axis may play significant roles in the mucosal transmission and pathogenesis of HIV type 1.

http://jvi.asm.org/cgi/content/abstract/79/9/5288

The expression of the genomic information of severe acute respiratory syndrome coronavirus (SARS CoV) involves synthesis of a nested set of subgenomic RNAs (sgRNAs) by discontinuous transcription. In SARS CoV-infected cells, 10 sgRNAs, including 2 novel ones, were identified, which were predicted to be functional in the expression of 12 open reading frames located in the 3' one-third of the genome. Surprisingly, one new sgRNA could lead to production of a truncated spike protein. Sequence analysis of the leader-body fusion sites of each sgRNA showed that the junction sequences and the corresponding transcription-regulatory sequence (TRS) are unique for each species of sgRNA and are consistent after virus passages. For the two novel sgRNAs, each used a variant of the TRS that has one nucleotide mismatch in the conserved hexanucleotide core (ACGAAC) in the TRS. Coexistence of both plus and minus strands of SARS CoV sgRNAs and evidence for derivation of the sgRNA core sequence from the body core sequence favor the model of discontinuous transcription during minus-strand synthesis. Moreover, one rare species of sgRNA has the junction sequence AAA, indicating that its transcription could result from a noncanonical transcription signal. Taken together, these results provide more insight into the molecular mechanisms of genome expression and subgenomic transcription of SARS CoV.

http://jvi.asm.org/cgi/content/abstract/79/9/5455

Historically, the adenoviral E3 region was found to be nonessential for viral replication in vitro. In addition, adenoviruses whose genome was more than approximately 105% the size of the native genome were inefficiently packaged. These profound observations were used experimentally to insert transgenes into the adenoviral backbone. More recently, however, the reintroduction of the E3 region into oncolytic adenoviruses has been found to positively influence antitumor efficacy in preclinical models and clinical trials. In the studies reported here, the granulocyte-macrophage colony-stimulating factor (GM-CSF) cDNA sequence has been substituted for the E3-gp19 gene in oncolytic adenoviruses that otherwise retained the E3 region. Five viruses that differed slightly in the method of transgene insertion were generated and compared to Ar6pAE2fGmF (E2F/GM/(Delta)E3), a previously described E3-deleted oncolytic adenovirus encoding GM-CSF. In all of the viruses, the human E2F-1 promoter regulated E1A expression and GM-CSF expression was under the control of the adenoviral E3 promoter and the packaging signal was relocated immediately upstream from the right terminal repeat. The E3-gp19-deleted viruses had similar cytolytic properties, as measured in vitro by cytotoxicity assays, but differed markedly in their capacity to express and secrete GM-CSF. Ar15pAE2fGmF (E2F/GM/E3b), the virus that
produced the highest levels of GM-CSF and retained the native GM-CSF leader sequence, was selected for further analysis. The E2F/GM/E3b and E2F/GM/(Delta)E3 viruses exhibited similar cytotoxic activity and GM-CSF production in several tumor cell lines in vitro. However, when compared in vivo in nude mouse xenograft tumor models, E2F/GM/E3b spread through tumors to a greater extent, resulted in higher peak GM-CSF and total exposure levels in both tumor and serum, and was more efficacious than the E3-deleted virus. Using the matched WI-38 (parental) and WI-38-VA13 (simian virus 40 large T antigen transformed) cell pair, GM-CSF was shown to be selectively produced in cells expressing high levels of E2F, indicating that the tumor-selective E2F promoter controlled E1A and GM-CSF expression.


The coronavirus membrane (M) protein is the most abundant virion protein and the key component in viral assembly and morphogenesis. The M protein of mouse hepatitis virus (MHV) is an integral membrane protein with a short ectodomain, three transmembrane segments, and a large carboxy-terminal endodomain facing the interior of the viral envelope. The carboxy terminus of MHV M has previously been shown to be extremely sensitive to mutation, both in a virus-like particle expression system and in the intact virion. We have constructed a mutant, M(Delta)2, containing a two-amino-acid truncation of the M protein that was previously thought to be lethal. This mutant was isolated by means of targeted RNA recombination with a powerful host range-based selection allowed by the interspecies chimeric virus fMHV (MHV containing the ectodomain of the feline infectious peritonitis virus S protein). Analysis of multiple second-site revertants of the M(Delta)2 mutant has revealed changes in regions of both the M protein and the nucleocapsid (N) protein that can compensate for the loss of the last two residues of the M protein. Our data thus provide the first genetic evidence for a structural interaction between the carboxy termini of the M and N proteins of MHV. In addition, this work demonstrates the efficacy of targeted recombination with fMHV for the systematic genetic analysis of coronavirus structural protein interactions.


We have investigated the induction of protective mucosal immunity to human immunodeficiency virus type 1 (HIV-1) isolate 89.6 by intranasal (i.n.) immunization of mice with gp120 and gp140 together with interleukin-12 (IL-12) and cholera toxin subunit B (CTB) as adjuvants. It was found that both IL-12 and CTB were required to elicit mucosal antibody responses and that i.n. immunization resulted in increased total, immunoglobulin G1 (IgG1), and IgG2a anti-HIV-1 antibody levels in serum; increased total, IgG1, IgG2a, and IgA antibody expression in bronchoalveolar lavage fluids; and increased IgA antibody levels in vaginal washes. Levels of anti-HIV-1 antibodies in both sera and secretions were higher in groups immunized with gp140 than in those immunized with gp120. However, only gp120-specific mucosal antibodies demonstrated neutralizing activity against HIV-1 89.6. Taken together, the results show that IL-12 and CTB act synergistically to enhance both systemic and local mucosal antibody responses to HIV-1 glycoproteins and that even though gp140 induces higher antibody titers than gp120, only gp120-specific mucosal antibodies interfere with virus infectivity.
Replication of human immunodeficiency virus type 1 (HIV-1) in primary blood lymphocytes, certain T-cell lines (nonpermissive cells), and most likely in vivo is highly dependent on the virally encoded Vif protein. Evidence suggests that Vif acts late in the viral life cycle during assembly, budding, and/or maturation to counteract the antiviral activity of the CEM15 protein and possibly other antiviral factors. Because HIV-1 virions produced in the absence of Vif are severely restricted at a postentry, preintegration step of infection, it is presumed that such virions differ from wild-type virions in some way. In the present study, we established a protocol for producing large quantities of vif-deficient HIV-1 (HIV-1/Δvif) from an acute infection of nonpermissive T cells and performed a thorough examination of the defect in these virions. Aside from the expected lack of Vif, we observed no apparent abnormalities in the packaging, modification, processing, or function of proteins in Δvif virions. In addition, we found no consistent defect in the ability of Δvif virions to perform intravirion reverse transcription under a variety of assay conditions, suggesting that the reverse transcription complexes in these particles can behave normally under cell-free conditions. Consistent with this finding, neither the placement of the primer tRNA3Lys nor its ability to promote reverse transcription in an in vitro assay was affected by a lack of Vif. Based on the inability of this comprehensive analysis to uncover molecular defects in Δvif virions, we speculate that such defects are likely to be subtle and/or rare.

Herpesvirus saimiri (HVS), a T-lymphotropic tumor virus of neotropical primates, and the Kaposi's sarcoma-associated human herpesvirus 8 (KSHV) belong to the gamma-2-herpesvirus (Rhadinovirus) subfamily and share numerous features of genome structure and organization. The KSHV latency-associated nuclear antigen (LANA) protein appears to be relevant for viral persistence, latency, and transformation. It binds to DNA, colocalizes with viral episomal DNA, and presumably mediates efficient persistence of viral genomes. LANA further represses the transcriptional and proapoptotic activities of the p53 tumor suppressor protein. Here we report on the ORF73 gene of HVS strain C488, which is the positional and structural homolog of KSHV LANA. The ORF73 gene in OMK cells can encode a 62-kDa protein that localizes to the nucleus in a pattern similar to that of LANA. We show that the ORF73 gene product can regulate viral gene expression by acting as a transcriptional modulator of latent and lytic viral promoters. To define the HVS ORF73 function in the background of a replication-competent virus, we constructed a viral mutant that expresses ORF73 under the transcriptional control of a mifepristone (RU-486)-inducible promoter. The HVS ORF73 gene product efficiently suppresses lytic viral replication in permissive cells, indicating that it defines a critical control point between viral persistence and lytic replication.
The innate immune response is a key barrier against pathogenic microorganisms such as human immunodeficiency virus type 1 (HIV-1). Because HIV-1 is rarely transmitted orally, we hypothesized that oral epithelial cells participate in the innate immune defense against this virus. We further hypothesized that secretory leukocyte protease inhibitor (SLPI), a 12-kDa mucosal antiviral protein, is a component of the host immune response to this virus. Here we demonstrated constitutive expression and production of SLPI in immortalized human oral keratinocytes. Brief exposure of cells to HIV-1 BaL and HXB2 significantly increased SLPI mRNA and protein production compared to that in mock-exposed cells (P < 0.01), as evaluated by real-time quantitative reverse transcription-PCR and enzyme-linked immunosorbent assay. HIV-1-mediated stimulation of SLPI occurred at the transcriptional level, was dose and time dependent, was elicited by heat-inactivated and infectious viruses, and did not depend on cellular infection. Experiments with purified retroviral proteins showed that the stimulatory effect was induced specifically by external envelope glycoproteins from HIV-1 and simian immunodeficiency virus. SLPI responsiveness to HIV-1 was also observed in an unrelated oral epithelial cell line and in normal (nonimmortalized) human oral epithelial cells isolated from healthy uninfected gingival tissues. In this first report of SLPI regulation by HIV-1, we show that the expression and production of the antimicrobial and anti-inflammatory protein can be stimulated in oral epithelial cells by the virus through interactions with gp120 in the absence of direct infection. These findings indicate that SLPI is a component of the oral mucosal response to HIV-1.


Differences in hepatitis C virus (HCV) variants of the highly conserved 5' untranslated region (UTR) have been observed between plasma and peripheral blood mononuclear cells (PBMC). The prevalence and the mechanisms of this compartmentalization are unknown. Plasma and PBMC HCV variants were compared by single-strand conformation polymorphism (SSCP) and by cloning or by genotyping with a line probe assay (LiPA) in 116 chronically infected patients, including 44 liver transplant recipients. SSCP patterns differed between compartments in 43/109 analyzable patients (39%). Differences were significantly more frequent in patients with transplants (21/38 [55%] versus 22/71 [31%]; P < 0.01) and in those who acquired HCV through multiple transfusions before 1991 (15/20; 75%) or through drug injection (16/31; 52%) than in those infected through an unknown route (7/29; 24%) or through a single transfusion (5/29; 17%; P < 0.001). Cloning of the 5' UTR, LiPA analysis, and nonstructural region 5B sequencing revealed different genotypes in the two compartments from 10 patients (9%). In nine patients, the genotype detected in PBMC was not detected in plasma and was weak or undetectable in the liver in three cases. This genotypic compartmentalization persisted for years in three patients and after liver transplantation in two. The present study shows that a significant proportion of HCV-infected subjects harbor in their PBMC highly divergent variants which were likely acquired through superinfections.

During studies to determine a role for tumor necrosis factor (TNF) in herpes simplex virus type 1 (HSV-1) infection using TNF receptor null mutant mice, we discovered a genetic locus, closely linked to the TNF p55 receptor (Tnfrsf1a) gene on mouse chromosome 6 (c6), that determines resistance or susceptibility to HSV-1. We named this locus the herpes resistance locus, Hrl, and showed that it also mediates resistance to HSV-2. Hrl has at least two alleles, Hrlr, expressed by resistant strains like C57BL/6 (B6), and Hrls, expressed by susceptible strains like 129S6 (129) and BALB/c. Although Hrl is inherited as an autosomal dominant gene, resistance to HSV-1 is strongly sex biased such that female mice are significantly more resistant than male mice. Analysis of backcrosses between resistant B6 and susceptible 129 mice revealed that a second locus, tentatively named the sex modifier locus, Sml, functions to augment resistance of female mice. Besides determining resistance, Hrl is one of several genes involved in the control of HSV-1 replication in the eye and ganglion. Remarkably, Hrl also affects reactivation of HSV-1, possibly by interaction with some unknown gene(s). We showed that Hrl is distinct from Cmv1, the gene that determines resistance to murine cytomegalovirus, which is encoded in the major NK cell complex just distal of p55 on c6. Hrl has been mapped to a roughly 5-centimorgan interval on c6, and current efforts are focused on obtaining a high-resolution map for Hrl.

http://jvi.asm.org/cgi/content/abstract/77/21/11849

Mice infected with myopathic coxsackievirus B1 Tucson (CVB1T) develop chronic inflammatory myopathy (CIM) consisting of hind limb weakness and inflammation. Amyopathic virus variants are infectious but attenuated for CIM. In this report, viral clones, chimeras, and sequencing were used to identify viral determinants of CIM. Chimeras identified several regions involved in CIM and localized a weakness determinant to nucleotides 2493 to 3200 of VP1. Sequencing of multiple clones and viruses identified five candidate determinants that were strictly conserved in myopathic viruses with one located in the 5' untranslated region (UTR), three in the VP1 capsid, and one in the 3C protease. Taken together, these studies implicate Tyr-87 and/or Val-136 as candidate determinants of weakness. They also indicate that there are at least two determinants of inflammation and one additional determinant of weakness encoded by myopathic CVB1T.

http://jvi.asm.org/cgi/content/abstract/77/21/11822

Endothelial cells are permissive to dengue virus (DV) infection in vitro, although their importance as targets of DV infection in vivo remains a subject of debate. To analyze the virus-host interaction, we studied the effect of DV infection on gene expression in human umbilical vein endothelial cells (HUVECs) by using differential display reverse transcription-PCR (DD-RTPCR), quantitative RT-PCR, and Affymetrix oligonucleotide microarrays. DD identified eight differentially expressed cDNAs, including inhibitor of apoptosis-1, 2'5' oligoadenylate synthetase (OAS), a 2'-5' OAS-like (OASL) gene, galectin-9, myxovirus protein A (MxA), regulator of G-protein signaling, endothelial and smooth muscle cell-derived neuropilin-like protein, and phospholipid scramblase 1. Microarray analysis of 22,000 human genes confirmed these findings and identified an additional 269 genes that were induced and 126 that were repressed more than fourfold after DV infection. Broad functional responses that were activated included the stress, defense, immune, cell adhesion, wounding, inflammatory, and antiviral pathways. These changes in gene expression were seen after infection of HUVECs with either laboratory-adapted virus or with virus isolated directly from plasma of DV-infected patients. Tumor necrosis factor alpha, OASL, and
MxA and h-IAP1 genes were induced within the first 8 to 12 h after infection, suggesting a direct effect of DV infection. These global analyses of DV effects on cellular gene expression identify potentially novel mechanisms involved in dengue disease manifestations such as hemostatic disturbance.


http://jvi.asm.org/cgi/content/abstract/78/21/11506

A unique opportunity for the study of the role of serial passage and cross-species transmission was offered by a series of experiments carried out at the Tulane National Primate Research Center in 1990. To develop an animal model for leprosy, three black mangabeys (BkMs) (Lophocebus aterrimus) were inoculated with lepromatous tissue that had been serially passaged in four sooty mangabeys (SMs) (Cercocebus atys). All three BkMs became infected with simian immunodeficiency virus from SMs (SIVsm) by day 30 postinoculation (p.i.) with lepromatous tissue. One (BkMG140) died 2 years p.i. from causes unrelated to SIV, one (BkMG139) survived for 10 years, whereas the third (BkMG138) was euthanized with AIDS after 5 years. Histopathology revealed a high number of giant cells in tissues from BkMG138, but no SIV-related lesions were found in the remaining two BkMs. Four-color immunofluorescence revealed high levels of SIVsm associated with both giant cells and T lymphocytes in BkMG138 and no detectable SIV in the remaining two. Serum viral load (VL) showed a significant increase (>1 log) during the late stage of the disease in BkMG138, as opposed to a continuous decline in VL in the remaining two BkMs. With the progression to AIDS, neopterin levels increased in BkMG138. This study took on new significance when phylogenetic analysis unexpectedly showed that all four serially inoculated SMs were infected with different SIVsm lineages prior to the beginning of the experiment. Furthermore, the strain infecting the BkMs originated from the last SM in the series. Therefore, the virus infecting BkMs has not been serially passaged. In conclusion, we present the first compelling evidence that direct cross-species transmission of SIV may induce AIDS in heterologous African nonhuman primate (NHP) species. The results showed that cross-species-transmitted SIVsm was well controlled in two of three BkMs for 2 and 10 years, respectively. Finally, this case of AIDS in an African monkey suggests that the dogma of SIV nonpathogenicity in African NHP hosts should be reconsidered.


http://jvi.asm.org/cgi/content/abstract/78/21/11656

The chromosomal features that influence retroviral integration site selection are not well understood. Here, we report the mapping of 226 avian sarcoma virus (ASV) integration sites in the human genome. The results show that the sites are distributed over all chromosomes, and no global bias for integration site selection was detected. However, RNA polymerase II transcription units (protein-encoding genes) appear to be favored targets of ASV integration. The integration frequency within genes is similar to that previously described for murine leukemia virus but distinct from the higher frequency observed with human immunodeficiency virus type 1. We found no evidence for preferred ASV integration sites over the length of genes and immediate flanking regions. Microarray analysis of uninfected HeLa cells revealed that the expression levels of ASV target genes were similar to the median level for all genes represented in the array. Although expressed genes were targets for integration, we found no preference for integration into highly
expressed genes. Our results provide a more detailed description of the chromosomal features that may influence ASV integration and support the idea that distinct, virus-specific mechanisms mediate integration site selection. Such differences may be relevant to viral pathogenesis and provide utility in retroviral vector design.


http://jvi.asm.org/cgi/content/abstract/78/21/11615

Earlier we reported that NF-(kappa)B is activated by protein kinase R (PKR) in herpes simplex virus 1-infected cells. Here we report that in PKR-/- cells the yields of wild-type virus are 10-fold higher than in PKR+/+ cells. In cells lacking NF-(kappa)B p50 (nfb1), p65 (relA), or both p50 and p65, the yields of virus were reduced 10-fold. Neither wild-type nor mutant cells undergo apoptosis following infection with wild-type virus. Whereas PKR+/+ and NF-(kappa)B+/+ control cell lines undergo apoptosis induced by the d120 (Delta(alpha4)) mutant of HSV-1, the mutant PKR-/- and NF-(kappa)B-/- cell lines were resistant. The evidence suggests that the stress-induced apoptosis resulting from d120 infection requires activation of NF-(kappa)B and that this proapoptotic pathway is blocked in cells in which NF-(kappa)B is not activated or absent. Activation of NF-(kappa)B in the course of viral infection may have dual roles of attempting to curtail viral replication by rendering the cell susceptible to apoptosis induced by the virus and by inducing the synthesis of proteins that enhance viral replication.


http://jvi.asm.org/cgi/content/abstract/76/22/11291

Human papillomaviruses (HPVs) cause a number of human tumors and malignancies, including cervical cancers. Epithelial differentiation is required for the complete HPV life cycle and can be achieved using the organotypic (raft) culture system. The CIN-612 9E cell line maintains episomal copies of HPV type 31b (HPV31b), an HPV type associated with cervical cancers. When grown in the raft system, CIN-612 9E cells form a differentiated epithelium such that infectious virions can be synthesized. Many aspects of the later stages of the HPV31b life cycle have been investigated in CIN-612 9E raft tissues. We used a biologically contained homogenization system for efficient virion extraction from raft epithelial tissues. Purified HPV31b virions were used to infect low-passage-number human foreskin keratinocytes and a variety of epithelial cell lines. Newly synthesized, spliced HPV31b transcripts were detected by reverse transcription and PCR (RT-PCR) following HPV31b infection. HPV31b infection was most efficient and reproducible in HaCaT cells. The onset of viral transcription following infection was also investigated using RT-PCR techniques. Spliced E1*E1,E2 RNAs were present as early as 4 h postinfection (p.i.), whereas the other major viral transcripts were detected by 8 to 10 h p.i. Furthermore, we characterized the structures and temporal expression of seven novel spliced early transcripts expressed following infection.

The presence of human erythrovirus DNA in 2,440 blood donations from the United Kingdom and sub-Saharan Africa (Ghana, Malawi, and South Africa) was screened. Sensitive qualitative and real-time quantitative PCR assays revealed a higher prevalence of persistent infection with the simultaneous presence of immunoglobulin G (IgG) and viral DNA (0.55 to 1.3%) than previously reported. This condition was characterized by a low viral load (median, 558 IU/ml; range, 42 to 135,000 IU/ml), antibody-complexed virus, free specific IgG, and potentially infectious free virus. Human erythrovirus genotype 1 (formerly parvovirus B19) was prevalent in the United Kingdom, Malawi, and South Africa. In contrast, only human erythrovirus genotype 3 (erythrovirus variant V9) was prevalent in Ghana. Genotype 3 had considerable genetic diversity, clustering in two probable subtypes. Genotype 1-based antibody assays failed to detect 38.5% of Ghanaian samples containing antibodies to genotype 3 virus but did not fail to detect cases of persistent infection. This study indicates a potential African origin of genotype 3 human erythrovirus and considerable shortcomings in the tools currently used to diagnose erythrovirus infection.


We measured the quantity of plasma feline immunodeficiency virus (FIV) RNA using a real-time sequence detecting system. Plasma viral RNA load was shown to correlate with the clinical stage, survival time, and disease progression in naturally FIV-infected cats. The present study indicates that the plasma viral RNA load can be used as a clinical marker representing the impairment of the immune system and predicting the clinical outcome in FIV-infected cats.


Epstein-Barr virus (EBV) strains can be distinguished by specific sequence variations in the LMP1 gene. In this study, a heteroduplex tracking assay (HTA) specific for LMP1 was developed to precisely identify the prototypic undeleted strain B958, other undeleted strains (Ch2, AL, NC, and Med-), and strains with the 30-bp deletion (Med+ and Ch1). This technique also provides an estimate of the relative abundance of strains in patient samples. In this study, EBV strains were identified in 25 hairy leukoplakia (HLP) biopsies and six matched peripheral blood samples and throat washes with the LMP1-HTA. To investigate the relationship of the virus found in the peripheral blood to that in the HLP lesion, the strain variants in the peripheral blood B lymphocytes and those present within the epithelial cells in the HLP lesion and in throat washes were identified. In many of the subjects, compartmental differences in the EBV strain profiles in the oral cavity and peripheral blood were readily apparent. The throat wash specimens usually had a strain profile similar to that within the corresponding HLP sample, which was distinct from the strain profile detected in the peripheral blood. These analyses reveal that the nature of EBV infection can be very dynamic, with changes in relative strain abundance over time as well as the appearance of new strains. The patterns of abundance in the blood and oral cavity provide evidence for compartmentalization and for the transmission of strains between the blood and oropharynx.

http://jvi.asm.org/cgi/content/abstract/77/19/10504

A complex interaction between the retroviral envelope glycoproteins and a specific cell surface protein initiates viral entry into cells. The avian leukemia-sarcoma virus (ALV) group of retroviruses provides a useful experimental system for studying the retroviral entry process and the evolution of receptor usage. In this study, we demonstrate that evolutionary pressure on subgroup A ALV (ALV(A)) entry exerted by the presence of a competitive inhibitor, a soluble form of the ALV(A) Tva receptor linked to a mouse immunoglobulin G tag (quail sTva-mIgG), can select different populations of escape variants. This escape population contained three abundant ALV(A) variant viruses, all with mutations in the surface glycoprotein hypervariable regions: a previously identified variant containing the Y142N mutation in the hr1 region; a new variant with two mutations, W141G in hr1 and K261E in vr3; and another new variant with two mutations, W145R in hr1 and K261E. The W141G K261E and W145R K261E viruses escape primarily by lowering their binding affinities for the quail Tva receptor competitive inhibitor while retaining wild-type levels of binding affinity for the chicken Tva receptor. A secondary phenotype of the new variants was an alteration in receptor interference patterns from that of wild-type ALV(A), indicating that the mutant glycoproteins are possibly interacting with other cellular proteins. One result of these altered interactions was that the variants caused a transient period of cytotoxicity. We could also directly demonstrate that the W141G K261E variant glycoproteins bound significant levels of a soluble form of the TvbS3 ALV receptor in a binding assay. Alterations in the normally extreme specificity of the ALV(A) glycoproteins for Tva may represent an evolutionary first step toward expanding viral receptor usage in response to inefficient viral entry.


http://jvi.asm.org/cgi/content/abstract/77/19/10295

Open reading frame 73 (ORF 73) is conserved among the gamma-2-herpesviruses (rhadinoviruses) and, in Kaposi's sarcoma-associated herpesvirus (KSHV) and herpesvirus saimiri (HVS), has been shown to encode a latency-associated nuclear antigen (LANA). The KSHV and HVS LANAs have also been shown to be required for maintenance of the viral genome as an episome during latency. LANA binds both the viral latency-associated origin of replication and the host cell chromosome, thereby ensuring efficient partitioning of viral genomes to daughter cells during mitosis of a latently infected cell. In gammaherpesvirus 68 ((gamma)HV68), the role of the LANA homolog in viral infection has not been analyzed. Here we report the construction of a (gamma)HV68 mutant containing a translation termination codon in the LANA ORF (73.STOP). The 73.STOP mutant virus replicated normally in vitro, in both proliferating and quiescent murine fibroblasts. In addition, there was no difference between wild-type (WT) and 73.STOP virus in the kinetics of induction of lethality in mice lacking B and T cells (Rag 1-/-) infected with 1,000 PFU of virus. However, compared to WT virus, the 73.STOP mutant exhibited delayed kinetics of replication in the lungs of immunocompetent C57BL/6 mice. In addition, the 73.STOP mutant exhibited a severe defect in the establishment of latency in the spleen of C57BL/6 mice. Increasing the inoculum of 73.STOP virus partially overcame the acute replication defect observed in the lungs at day 4 postinfection but did not ameliorate the severe defect in the establishment of splenic latency. Thus, consistent with its proposed role in replication of the latent viral episome, LANA appears to be a critical determinant in the
establishment of \( \gamma \text{HV68} \) latency in the spleen post-intranasal infection.


http://jvi.asm.org/cgi/content/abstract/76/20/10099

There are several forms of human immunodeficiency virus type 1 (HIV-1) DNA in peripheral blood T cells and lymph nodes in untreated HIV-1-infected individuals and in patients whose plasma HIV-1 RNA levels are suppressed by long-term combination antiretroviral therapy. However, it remains to be established whether the concentration of HIV-1 DNA in cells predicts the clinical outcome of HIV-1 infection. In this report, we measured the concentration of HIV-1 DNA forms which has undergone the second template switch (STS DNA) and 2-long-terminal-repeat DNA circles in peripheral blood mononuclear cell (PBMC) samples. To do this, we used molecular-beacon-based real-time PCR assays and studied 130 patients with hemophilia in the Multicenter Hemophilia Cohort Study. We assessed the influence of baseline HIV-1 STS DNA levels on the progression of HIV-1 disease in the absence of combination antiretroviral therapy by Kaplan-Meier and Cox regression analysis. Among the patients who progressed to AIDS, the median levels (interquartile ranges) of STS HIV-1 DNA in PBMC were significantly higher than those of patients who remained AIDS free during the 16 years of follow-up (1,017 [235 to 6,059] and 286 [31 to 732] copies per 106 PBMC, respectively; \( P < 0.0001 \)). Rates of progression to death and development of AIDS varied significantly (log rank \( P < 0.001 \)) by quartile distribution of HIV-1 STS DNA levels. After adjustment for age at seroconversion, baseline CD4+ T-cell counts, plasma viral load, and T-cell-receptor excision circles, the relative hazards (RH) of death and AIDS were significantly increased with higher HIV-1 STS DNA levels (adjusted RH, 1.84 [95% confidence interval (CI), 1.30 to 2.59] and 2.62 [95% CI, 1.75 to 3.93] per 10-fold increase per 106 PBMC, respectively). HIV-1 STS DNA levels in each individual remained steady in longitudinal PBMC samples during 16 years of follow-up. Our findings show that the concentration of HIV-1 STS DNA in PBMC complements the HIV-1 RNA load in plasma in predicting the clinical outcome of HIV-1 disease. This parameter may have important implications for understanding the virological response to combination antiretroviral therapy.


http://jvi.asm.org/cgi/content/abstract/76/20/10147

In a recent vaccine trial, we showed efficient control of a virulent simian-human immunodeficiency virus SHIV-89.6P challenge by priming with a Gag-Pol-Env-expressing DNA and boosting with a Gag-Pol-Env-expressing recombinant modified vaccinia virus Ankara. Here we show that long-term control has been associated with slowly declining levels of viral RNA and DNA. In the vaccinated animals both viral DNA and RNA underwent an initial rapid decay, which was followed by a lower decay rate. Between 12 and 70 weeks postchallenge, the low decay rates have had half-lives of about 20 weeks for viral RNA in plasma and viral DNA in peripheral blood mononuclear cells and lymph nodes. In vaccinated animals the viral DNA has been mostly unintegrated and has appeared to be largely nonfunctional as evidenced by a poor ability to recover infectious virus in cocultivation assays, even after CD8 depletion. In contrast, in control animals, which have died, viral DNA was mostly integrated and a larger proportion appeared to be functional as evidenced by the recovery of infectious virus. Thus, to date, control of the challenge infection has appeared to improve with time, with the decay rates for viral DNA being at...
Porcine circovirus type 2 (PCV2) is the primary causative agent of postweaning multisystemic wasting syndrome (PMWS), whereas the ubiquitous porcine circovirus type 1 (PCV1) is nonpathogenic for pigs. We report here the construction and characterization of two chimeric infectious DNA clones of PCV1 and PCV2. The chimeric PCV1-2 clone contains the PCV2 capsid gene cloned in the backbone of the nonpathogenic PCV1 genome. A reciprocal chimeric PCV2-1 DNA clone was also constructed by replacing the PCV2 capsid gene with that of PCV1 in the backbone of the PCV2 genome. The PCV1, PCV2, and chimeric PCV1-2 and PCV2-1 DNA clones were all shown to be infectious in PK-15 cells, and their growth characteristics in vitro were determined and compared. To evaluate the immunogenicity and pathogenicity of the chimeric infectious DNA clones, 40 specific-pathogen-free (SPF) pigs were randomly assigned into five groups of eight pigs each. Group 1 pigs received phosphate-buffered saline as the negative control. Group 2 pigs were each injected in the superficial inguinal lymph nodes with 200 μg of the PCV1 infectious DNA clone. Group 3 pigs were each similarly injected with 200 μg of the PCV2 infectious DNA clone, group 4 pigs were each injected with 200 μg of the chimeric PCV1-2 infectious DNA clone, and group 5 pigs were each injected with 200 μg of the reciprocal chimeric PCV2-1 infectious DNA clone. As expected, seroconversion to antibodies to the PCV2 capsid antigen was detected in group 3 and group 4 pigs. Group 2 and 5 pigs all seroconverted to PCV1 antibody. Gross and microscopic lesions in various tissues of animals inoculated with the PCV2 infectious DNA clone were significantly more severe than those found in pigs inoculated with PCV1, chimeric PCV1-2, and reciprocal chimeric PCV2-1 infectious DNA clones. These data indicated that the chimeric PCV1-2 virus with the immunogenic ORF2 capsid gene of pathogenic PCV2 cloned into the nonpathogenic PCV1 genomic backbone induces a specific antibody response to the pathogenic PCV2 capsid antigen but is attenuated in pigs. Future studies are warranted to evaluate the usefulness of the chimeric PCV1-2 infectious DNA clone as a genetically engineered live-attenuated vaccine against PCV2 infection and PMWS.
or nonviremic donor and subsequent analysis of the virological and serological status of the recipients. Transmission of SRV and development of anti-SRV antibodies were only observed in recipients of blood from the viremic donor; transfer of SRV provirus and unintegrated circular DNA in blood from the nonviremic donor did not lead to infection of the recipients. These results indicate that a proportion of M. fascicularis are able to effectively control the replication and infectivity of SRV despite long-term persistence of viral DNA forms in infected lymphocytes.


http://jvi.asm.org/cgi/content/abstract/78/17/9400

Inactivated parapoxvirus ovis (Orf virus; PPVO) recently displayed strong immunostimulating and modulating capacities in several animal models for acute and chronic virus infections through the induction of gamma interferon (IFN-\(\gamma\)) as a key mediator of antiviral activity. The data presented in this work demonstrate that inactivated PPVO has strong effects on cytokine secretion by human immune cells, including the upregulation of inflammatory and Th1-related cytokines (IFN-\(\gamma\), tumor necrosis factor alpha [TNF-\(\alpha\)], interleukin 6 [IL-6], IL-8, IL-12, and IL-18) as well as anti-inflammatory and Th2-related cytokines (IL-4, IL-10, and IL-1 receptor antagonist [IL-1ra]). Studies on the mechanism of action revealed virus particles to be the effective components of the preparation. The virus particles activate monocytes or other antigen-presenting cells (APC), e.g., plasmacytoid dendritic cells, through signaling over CD14 and a Toll-like receptor and the intracellular presence of certain PPVO-specific components. The activation of monocytes or APC is followed by the release of early proinflammatory cytokines (TNF-\(\alpha\), IL-6, and IL-8) as well as the Th1-related cytokines IL-12 and IL-18. Both IL-18 and IL-12 are involved in PPVO-mediated IFN-\(\gamma\) release by T cells and/or NK cells. The proinflammatory response is accompanied by the induction of anti-inflammatory and Th2-related cytokines (IL-4, IL-10, and IL-1ra), which exert a limiting effect on the inflammatory response induced by PPVO. We conclude that the induction of a natural immune response with physiologically significant amounts of different cytokines and with antiviral potential might provide advantages over existing antiviral immunotherapies.


http://jvi.asm.org/cgi/content/abstract/78/17/9041

The effects of human papillomavirus type 18 (HPV-18) E6 and E7 proteins on global patterns of host gene expression in primary human keratinocytes grown in organotypic raft culture system were assessed. Primary human keratinocytes were infected with retroviruses that express the wild-type HPV-18 E6 and E7 genes from the native differentiation-dependent HPV enhancer-promoter. Total RNA was isolated from raft cultures and used to generate probes for querying Affymetrix U95A microarrays, which contain >12,500 human gene sequences. Quadruplicate arrays of each E6/E7-transduced and empty vector-transduced samples were analyzed by 16 pairwise comparisons. Transcripts altered in \([\geq]12\) comparisons were selected for further analysis. With this approach, HPV-18 E6/E7 expression significantly altered the expression of 1,381 genes. A large increase in transcripts associated with DNA and RNA metabolism was observed, with major increases noted for transcription factors, splicing factors, and DNA replication elements, among others. Multiple genes associated with protein translation were downregulated. In addition, major alterations were found in transcripts associated with the cell cycle and cell differentiation. Our study provides a systematic description of transcript changes
We report the complete sequence of a large rod-shaped DNA virus, called the Hz-1 virus. This virus persistently infects the Heliothis zea cell lines. The Hz-1 virus has a double-stranded circular DNA genome of 228,089 bp encoding 154 open reading frames (ORFs) and also expresses a persistence-associated transcript 1, PAT1. The G+C content of the Hz-1 virus genome is 41.8%, with a gene density of one gene per 1.47 kb. Sequence analysis revealed that a 9.6-kb region at 43.6 to 47.8 map units harbors five cellular genes encoding proteins with homology to dUTP pyrophosphatase, matrix metalloproteinase, deoxynucleoside kinase, glycine hydroxymethyltransferase, and ribonucleotide reductase large subunit. Other cellular homologs were also detected dispersed in the viral genome. Several baculovirus homologs were detected in the Hz-1 virus genome. These include PxOrf-70, PxOrf-29, AcOrf-81, AcOrf-96, AcOrf-22, VLF-1, RNA polymerase LEF-8 (orf50), and two structural proteins, p74 and p91. The Hz-1 virus p74 homolog shows high structural conservation with a double transmembrane domain at its C terminus. Phylogenetic analysis of the p74 revealed that the Hz-1 virus is evolutionarily distant from the baculoviruses. Another distinctive feature of the Hz-1 virus genome is a gene that is involved in insect development. However, the remainder of the ORFs (81%) encoded proteins that bear no homology to any known proteins. In conclusion, the sequence differences between the Hz-1 virus and the baculoviruses outnumber the similarities and suggest that the Hz-1 virus may form a new family of viruses distantly related to the Baculoviridae.

The infection of human fetal thymus organ cultures (FTOC) with coxsackievirus B4 E2 (CVB4 E2) was investigated. Both positive- and negative-strand viral RNA were detected by real-time quantitative reverse transcription-PCR (RT-PCR) in CVB4 E2-infected FTOC, which supported high yields of virus production ([~]106 50% tissue culture infective doses/ml), and in flow-sorted thymocyte populations for 7 days after inoculation. Cortical CD4+ CD8+ thymocytes were found to be the principal targets of infection. Inoculation of human FTOC with CVB4 E2 led to a marked and progressive depletion of immature thymocytes (CD4+ CD8+ cells) with no enhancement of Annexin V-positive cells. CVB4 E2 replication caused significant major histocompatibility complex (MHC) class I upregulation on these cells. MHC class I upregulation was correlated with positive- and negative-strand RNA quantitative detection and the release of infectious particles. In addition, chloroquine treatment of FTOC and single-thymocyte suspensions suggested that MHC class I upregulation on thymocytes was the result of direct infection rather than caused by production of soluble factors such as alpha interferon. Thus, CVB4 E2 can infect human fetal thymocytes, which subsequently results in quantitative and qualitative abnormalities of these cells.

Although it is established that the cleavage site and glycosylation patterns in the hemagglutinin (HA) play important roles in determining the pathogenicity of H5 avian influenza viruses, some viruses exist that are not highly pathogenic despite possessing the known characteristics of high pathogenicity (i.e., their HA contains multiple basic amino acids at the cleavage site and has glycosylation patterns similar to that of the highly pathogenic H5 viruses). Currently little is known about the H5N1 viruses that fall into this intermediate category of pathogenicity. We have identified strains of H5N1 avian influenza viruses that have markers typical of high pathogenicity but distinctly differ in their ability to cause disease and death in chickens. By analyzing viruses constructed by reverse-genetic methods and containing recombinant HAs, we established that amino acids 97, 108, 126, 138, 212, and 217 of HA, in addition to those within the cleavage site, affect pathogenicity. Further investigation revealed that an additional glycosylation site within the neuraminidase (NA) protein globular head contributed to the high virulence of the H5N1 virus. Our findings are in agreement with previous observations that suggest that the activities of the HA and NA proteins are functionally linked.


The coronavirus spike protein (S) forms the distinctive virion surface structures that are characteristic of this viral family, appearing in negatively stained electron microscopy as stems capped with spherical bulbs. These structures are essential for the initiation of infection through attachment of the virus to cellular receptors followed by fusion to host cell membranes. The S protein can also mediate the formation of syncytia in infected cells. The S protein is a type I transmembrane protein that is very large compared to other viral fusion proteins, and all except a short carboxy-terminal segment of the S molecule constitutes the ectodomain. For the prototype coronavirus mouse hepatitis virus (MHV), it has previously been established that S protein assembly into virions is specified by the carboxy-terminal segment, which comprises the transmembrane domain and the endodomain. We have genetically dissected these domains in the MHV S protein to localize the determinants of S incorporation into virions. Our results establish that assembly competence maps to the endodomain of S, which was shown to be sufficient to target a heterologous integral membrane protein for incorporation into MHV virions. In particular, mutational analysis indicated a major role for the charge-rich carboxy-terminal region of the endodomain. Additionally, we found that the adjacent cysteine-rich region of the endodomain is critical for fusion of infected cells, confirming results previously obtained with S protein expression systems.