1. J Virol. 2004 May;78(10):5458-65.

Properties of replication-competent vesicular stomatitis virus vectors expressing glycoproteins of filoviruses and arenaviruses.

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Replication-competent recombinant vesicular stomatitis viruses (rVSVs) expressing the type I transmembrane glycoproteins and selected soluble glycoproteins of several viral hemorrhagic fever agents (Marburg virus, Ebola virus, and Lassa virus) were generated and characterized. All recombinant viruses exhibited rhabdovirus morphology and replicated cytolytically in tissue culture. Unlike the rVSVs with an additional transcription unit expressing the soluble glycoproteins, the viruses carrying the foreign transmembrane glycoproteins in replacement of the VSV glycoprotein were slightly attenuated in growth. Biosynthesis and processing of the foreign glycoproteins were authentic, and the cell tropism was defined by the transmembrane glycoprotein. None of the rVSVs displayed pathogenic potential in animals. The rVSV expressing the Zaire Ebola virus transmembrane glycoprotein mediated protection in mice against a lethal Zaire Ebola virus challenge. Our data suggest that the recombinant VSV can be used to study the role of the viral glycoproteins in virus replication, immune response, and pathogenesis.

PMCID: PMC400370
PMID: 15113924 [PubMed - indexed for MEDLINE]

2. J Infect Dis. 2004 May 1;189 Suppl 1:S171-6.

Genotyping of measles virus in Canada: 1979-2002.

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Genotyping is an important component of measles surveillance. In this study, we report the genotypes of 30 measles viruses from cases in Canada; 6 of these were collected between 1979 and 1996 and 24 were collected from 1997 through 2002.

Many measles virus genotypes were found (C1, C2, D3, D4, D5, D6, D7, D8, E, and H1). These data indicate that the predominant measles virus genotypes detected from 1979 to 1997 in Canada are no longer commonly found. Since the implementation of a routine second dose of measles vaccine and catch-up campaigns in 1996-1997, the wide variety of measles virus genotypes found supports epidemiological data showing that importation of measles is the source of current measles cases in Canada.

PMID: 15106107 [PubMed - indexed for MEDLINE]

3. Virology. 2003 Dec 20;317(2):191-6.

New insights into the evolutionary relationships between arenaviruses provided by comparative analysis of small and large segment sequences.

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Arenaviruses are rodent-borne negative-stranded bisegmented RNA viruses. Five arenaviruses are etiologic agents of hemorrhagic fever in humans and are potential agents of bioterrorism. They are classified as Biosafety level 4 agents and listed in the category A of the Pathogen Agents edited by the Center for Disease Control and Prevention. To date, evolution and phylogeny of arenaviruses have been based on the analysis of sequences derived from structural genes (small RNA segment) exclusively, due to the lack of sequences available for the large RNA segment. In this study, partial sequences of the polymerase gene were determined for 18 species of arenaviruses and used to investigate phylogenetic relationships. Comparative analysis of topologies obtained from polymerase and structural gene analyses permitted us to determine the evolutionary origin of the major parent of the North American recombinant arenaviruses, and to investigate the role of genetic exchange (reassortment and recombination) in the evolutionary mechanisms driving the evolution of the genus Arenavirus.

PMID: 14698659 [PubMed - indexed for MEDLINE]

4. Virology. 2003 Sep 15;314(1):443-50.

Structure of replicating intermediates of human herpesvirus type 6.

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We have studied the structure of the replicative intermediates of human herpesvirus 6 (HHV-6) using pulsed-field gel electrophoresis, partial digestion, two-dimensional gel electrophoresis, and sedimentation centrifugation. The results show that DNA replication of HHV-6 produces head-to-tail concatemeric intermediates as well as approximately equal amounts of circular monomers or oligomers. Unlike the situation in herpes simplex virus, the intermediates of human herpesvirus 6 replication are not highly branched, suggesting a difference in the mechanism of replication or a lower frequency of homologous recombination in human herpesvirus 6 compared to herpes simplex virus.

PMID: 14517096 [PubMed - indexed for MEDLINE]

5. Science. 2003 May 30;300(5624):1399-404. Epub 2003 May 1.

The Genome sequence of the SARS-associated coronavirus.

Marra MA(1), Jones SJ, Astell CR, Holt RA, Brooks-Wilson A, Butterfield YS, Khattra J, Asano JK, Barber SA, Chan SY, Cloutier A, Coughlin SM, Freeman D, Girn N, Griffith OL, Leach SR, Mayo M, McDonald H, Montgomery SB, Pandoh PK, Petrescu AS, Robertson AG, Schein JE, Siddiqui A, Smailus DE, Stott JM, Yang GS, Plummer F, Andonov A, Artsob H, Bastien N, Bernard K, Booth TF, Bowness D, Czub M, Drebot M, Fernando L, Flick R, Garbutt M, Gray M, Grolla A, Jones S, Feldmann H, Meyers A, Kabani A, Li Y, Normand S, Stroher U, Tipples GA, Tyler S, Vogrig R, Ward D, Watson B, Brunham RC, Krajden M, Petric M, Skowronski DM, Upton C, Roper RL.

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Comment in
Science. 2003 May 30;300(5624):1377-8.

We sequenced the 29,751-base genome of the severe acute respiratory syndrome (SARS)-associated coronavirus known as the Tor2 isolate. The genome sequence reveals that this coronavirus is only moderately related to other known coronaviruses, including two human coronaviruses, HCoV-OC43 and HCoV-229E. Phylogenetic analysis of the predicted viral proteins indicates that the virus does not closely resemble any of the three previously known groups of coronaviruses. The genome sequence will aid in the diagnosis of SARS virus

infection in humans and potential animal hosts (using polymerase chain reaction and immunological tests), in the development of antivirals (including neutralizing antibodies), and in the identification of putative epitopes for vaccine development.

PMID: 12730501 [PubMed - indexed for MEDLINE]

6. Med Microbiol Immunol. 2002 Oct;191(2):63-74. Epub 2002 Sep 3.

Emerging and re-emerging infectious diseases.

Feldmann H, Czub M, Jones S, Dick D, Garbutt M, Grolla A, Artsob H.

In human history, numerous infectious diseases have emerged and re-emerged. Aside from many others, the so-called 'exotic' agents in particular are a threat to our public health systems due to limited experience in case management and lack of appropriate resources. Many of these agents are zoonotic in origin and transmitted from animals to man either directly or via vectors. The reservoirs are often infected subclinically or asymptomatically and the distribution of the diseases basically reflects the range and the population dynamics of their reservoir hosts. As examples, emergence/re-emergence is discussed here for diseases caused by filoviruses, hantaviruses, paramyxoviruses, flaviviruses and Yersinia pestis. In addition, bioterrorism is addressed as one factor which has now to be considered in infectious disease emergence/re-emergence. Preparedness for known and unknown infectious diseases will be a top priority for our public health systems in the beginning of the millennium.

PMID: 12410344 [PubMed - indexed for MEDLINE]

7. Biochem J. 2002 Feb 1;361(Pt 3):653-61.

Cell-permeable ceramides preferentially inhibit coated vesicle formation and exocytosis in Chinese hamster ovary compared with Madin-Darby canine kidney cells by preventing the membrane association of ADP-ribosylation factor.

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Differential effects of acetyl(C2-) ceramide (N-acetylsphingosine) were studied on coated vesicle formation from Golgi-enriched membranes of Chinese hamster ovary (CHO) and Madin-Darby canine kidney (MDCK) cells. C2-ceramide blocked the

translocation of ADP-ribosylation factor-1 (ARF-1) and protein kinase C-alpha (PKC-alpha) to the membranes from CHO cells, but not those of MDCK cells. Consequently, C2-ceramide blocked the stimulation of phospholipase D1 (PLD1) by the cytosol and guanosine 5'-[gamma-thio]triphosphate (GTP[S]) in membranes from CHO cells. Basal specific activity of PLD1 and the concentration of ARF-1 were 3-4 times higher in Golgi-enriched membranes from MDCK cells compared with CHO cells. Moreover, PLD1 activity in MDCK cells was stimulated less by cytosol and GTP[S]. PLD2 was not detectable in the Golgi-enriched membranes. Incubation of intact CHO cells or their Golgi-enriched membranes with C2-ceramide also inhibited COP1 vesicle formation by membranes from CHO, but not MDCK, cells. Specificity was demonstrated, since dihydro-C2-ceramide had no significant effect on ARF-1 translocation, PLD1 activation or vesicle formation in membranes from both cell types. C2-ceramide also decreased the secretion of virus-like particles to a greater extent in CHO compared with MDCK cells, whereas dihydro-C2-ceramide had no significant effect. The results demonstrate a biological effect of C2-ceramide in CHO cells by decreasing ARF-1 and PKC-alpha binding to Golgi-enriched membranes, thereby preventing COP1 vesicle formation.

PMCID: PMC1222349
PMID: 11802796 [PubMed - indexed for MEDLINE]

8. Virology. 1999 Sep 1;261(2):340-6.

Secretion of rubella virions and virus-like particles in cultured epithelial cells.

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Rubella virus (RV) is an enveloped RNA virus that causes systemic infections in humans. More importantly, first trimester in utero infection leads to a collection of devastating birth defects known as congenital rubella syndrome. Epithelial cells are the first line of defense against viruses and consequently, the polarity of virus secretion is an important factor affecting viral spread. As a first step toward understanding how RV interacts with epithelial cells, we have examined the release of RV-like particles and virions from polarized cells in culture. RV structural proteins were targeted to the Golgi complex and virus particle formation occurred on intracellular membranes in three different polarized epithelial cells. Polarized cells could be infected from the apical and basal membranes, indicating that receptors are not confined to one surface. The secretion of virus-like particles and infectious virions varied according to cell type. In two of the three polarized cell lines examined, virus was released primarily from the apical surface, but significant quantities were also secreted

from the basolateral membrane. Release of virus from the apical surface may facilitate virus spread from person to person, whereas basolateral secretion could be important for establishing a systemic infection and/or crossing the placenta prior to fetal infection.

PMID: 10497119 [PubMed - indexed for MEDLINE]

9. J Virol. 1999 May;73(5):3524-33.

Role of rubella virus glycoprotein domains in assembly of virus-like particles.

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Rubella virus is a small enveloped positive-strand RNA virus that assembles on intracellular membranes in a variety of cell types. The virus structural proteins contain all of the information necessary to mediate the assembly of virus-like particles in the Golgi complex. We have recently identified intracellular retention signals within the two viral envelope glycoproteins. E2 contains a Golgi retention signal in its transmembrane domain, whereas a signal for retention in the endoplasmic reticulum has been localized to the transmembrane and cytoplasmic domains of E1 (T. C. Hobman, L. Woodward, and M. G. Farquhar, Mol. Biol. Cell 6:7-20, 1995; T. C. Hobman, H. F. Lemon, and K. Jewell, J. Virol. 71:7670-7680, 1997). In the present study, we have analyzed the role of these retention signals in the assembly of rubella virus-like particles. Deletion or replacement of these domains with analogous regions from other type I membrane glycoproteins resulted in failure of rubella virus-like particles to be secreted from transfected cells. The E1 transmembrane and cytoplasmic domains were not required for targeting of the structural proteins to the Golgi complex and, surprisingly, assembly and budding of virus particles into the lumen of this organelle; however, the resultant particles were not secreted. In contrast, replacement or alteration of the E2 transmembrane or cytoplasmic domain, respectively, abrogated the targeting of the structural proteins to the budding site, and consequently, no virion formation was observed. These results indicate that the transmembrane and cytoplasmic domains of E2 and E1 are required for early and late steps respectively in the viral assembly pathway and that rubella virus morphogenesis is very different from that of the structurally similar alphaviruses.

PMCID: PMC104124

PMID: 10196241 [PubMed - indexed for MEDLINE]

10. Atherosclerosis. 1996 Jan 5;119(1):17-41.

Development of an avian model for restenosis.

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Recurrence of atherosclerotic plaque growth after interventional therapy, restenosis, is a significant clinical problem occurring in 20%-50% of cases. We have developed a new avian model for the investigation of restenosis after arterial injury in cholesterol fed White Leghorn roosters. Atherosclerotic plaque growth 1-30 weeks after angioplasty balloon mediated endothelial injury in the abdominal aorta was studied in 37 roosters. Roosters were maintained on either normal poultry diet or high cholesterol diet. Twelve cholesterol fed roosters were also fed a hormone supplemented diet in order to modify plaque morphology. The procedural success rate was high. Angiographic stenoses (mean 36% with maximum of 74%) were detectable in cholesterol fed roosters after balloon angioplasty with associated histological evidence of plaque growth ($P < 0.017$). Cholesterol feeding enhanced fatty plaque growth; hormone manipulation increased calcific and ulcerated plaque but with high associated morbidity. Three interventional devices were subsequently examined in 32 roosters (16 laser angioplasty, 7 atherectomy, and 9 stent implant). Plaque development was again assessed by contrast angiography and histological analysis. We conclude that balloon mediated arterial injury in cholesterol fed roosters produces early proliferative and late, complex atherosclerotic lesions providing an inexpensive model for plaque development after intimal injury.

PMID: 8929254 [PubMed - indexed for MEDLINE]

11. Virology. 1996 Jan 1;215(1):17-30.

Myxoma virus M-T7, a secreted homolog of the interferon-gamma receptor, is a critical virulence factor for the development of myxomatosis in European rabbits.

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Myxoma virus is a leporipoxvirus of New World rabbits (Sylvilagus sp.) that induces a rapidly lethal infection known as myxomatosis in the European rabbit (Oryctolagus cuniculus). Like all poxviruses, myxoma virus encodes a plethora of proteins to circumvent or inhibit a variety of host antiviral immune mechanisms. M-T7, the most abundantly secreted protein of myxoma virus-infected cells, was originally identified as an interferon-gamma receptor homolog (Upton, Mossman, and McFadden, Science 258, 1369-1372, 1992). Here, we demonstrate that M-T7 is dispensable for virus replication in cultured cells but is a critical virulence factor for virus pathogenesis in European rabbits. Disruption of both copies of the M-T7 gene in myxoma virus was achieved by the deletion of 372 bp of M-T7 coding sequences, replacement with a selectable marker, p7.5Ecogpt, and selection of a recombinant virus (vMyxlac-T7gpt) resistant to mycophenolic acid. vMyxlac-T7gpt expressed no detectable M-T7 protein and infected cells supernatants were devoid of any detectable interferon-gamma binding activities. Immunohistochemical staining with anti-beta-galactosidase and anti-CD43 antibodies demonstrated that in vMyxlac-T7gpt-infected rabbits the loss of M-T7 not only caused a dramatic reduction in disease symptoms and viral dissemination to secondary sites, but also dramatically influenced host leukocyte behavior. Notably, primary lesions in wild-type virus infections were generally underlayed by large masses of inflammatory cells that did not effectively migrate into the dermal sites of viral replication, whereas in vMyxlac-T7gpt infections this apparent block to leukocyte influx was relieved. A second major phenotypic distinction noted for the M-T7 knockout virus was the extensive activation of lymphocytes in secondary immune organs, particularly the spleen and lymph nodes, by Day 4 of the infection. This is in stark contrast to infection by wild-type myxoma virus, which results in relatively little, if any, cellular activation of germinal centers of spleen and lymph node by Day 4. We conclude that M-T7 functions early in infection to (1) retard inflammatory cell migration into infected tissues and (2) disrupt the communication between sentinel immune cells at the site of primary virus infection in the subdermis and lymphocytes in the secondary lymphoid organs, thereby disabling the host from mounting an effective cellular immune response. To summarize, in addition to neutralizing host interferon-gamma at infected sites, we propose that M-T7 protein also modifies leukocyte traffic in the vicinity of virus lesions, thus effectively severing the link between antigen presenting cells of the infected tissue and the effector lymphocytes of the peripheral immune organs.

PMID: 8553583 [PubMed - indexed for MEDLINE]