Poxviruses are remarkable for the wide variety of virulence factors that they encode, many of which are linked to the antiviral pathway triggered by interferons (IFNs). The signaling pathway triggered by IFN lead to an increased expression of the double-stranded RNA dependent protein kinase (PKR) and 2'-5' oligo (A) synthetase, inhibiting protein synthesis in infected cells. Vacccinia virus (VACV) genes that encode inhibitors of IFN-related pathways are involved in the virus IFN-resistant phenotype. Therefore, our goal is to study the involvement of these proteins in several aspects of virus-cell interaction during infection. First, by Western-blot and metabolic labeling assays followed by SDS-PAGE we evaluated the time-course progression of VACV infection when the action of IFN-related pathways was not counteracted by the virus. We observed that VACV completed only the initial phase of the viral cycle in monkey cells expressing high levels of PKR, but concluded all phases of the cycle in hamster cells, which present low levels of the enzyme. This is consistent with the data from viral growth curves under similar conditions. By immunofluorescence assay we observed the formation of round virossomes in cells infected by VACV that did not counteract the antiviral host response, in contrast to expanded virossomes and late protein detection when virus inhibited the IFN-related antiviral pathways of the cells. We also assessed the intracellular localization of lysosomal membrane protein-2 during vaccinia virus infection in monkey cells. Lysosomes participate in protein degradation pathway when cells are under distinct stress conditions. We observed the punctate pattern and the progressive redistribution of the protein from a perinuclear sites to cytoplasmic clusters after 8 hours post-infection in monkey cells. This pattern was not observed in control infection when host antiviral response was restrained.

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BV63 - ISOLATION OF CAPRINE ARTHRITIS ENCEPHALITIS VIRUS FROM INFECTED MACROPHAGE SUBMITTED TO CULTURES.

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Small ruminant lentivirosis (SRLV) are caused by caprine arthritis encephalitis (CAE) and Maedi Visna (MV) virii and are the major viral diseases of goats and sheep. The caprine arthritis encephalitis virus (CAEV) has tropism for monocytes / macrophages and viral replication is dependent on the maturation process of macrophages from monocytes. The objective of this study is to standardize in vitro goat macrophage cultures obtained from infected animals, with subsequent co-cultivation with cells from goat synovial membrane (GSM) for isolation of CAEV. Blood samples were collected from seven positive animals as previously determined by Western blot. The samples were centrifuged to obtain leukocytes which were washed with 0.84% ammonium chloride and PBS followed by further centrifugation. These were resuspended in RPMI medium (5% FBS, 2% penicillin and streptomycin and 1% Fungison) and incubated at 37 oC and 5% CO2. The culture was supplemented with 1 volume of fresh media after 24 hours of incubation. After one week the media was removed and substituted by MEM medium (2x macrophage starter culture volume) containing GSM cells. Cultures were incubated up to 42 days and were checked daily for cytopathic effects (CPE). Cultures were sampled for DNA extraction and viral isolation was confirmed by Nested PCR targeting part of the gag gene. CAEV was isolated from all seven samples from different herds from Minas Gerais state. Of these, two were capable of inducing syncytium (multinucleate cells) and another had a very strong lytic effect. We concluded that this method is efficient in the isolation of CAEV from infected animals.

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BV64 - ACTION OF A Lentinula edodes SULFATED POLYSACCHARIDE IN THE REPLICATION OF POLIOVIRUS-1, BOVINE HERPESVIRUS-1 AND HERPES SIMPLEX VIRUS-1

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Bioactive compounds isolated from natural sources have drawn much attention in the field of pharmacology. Basidiomycetes sulfated polysaccharides present in the fruiting body have shown antiviral properties mainly attributed to the blockade of the initial stage of the viral replication cycle. The Lentinula edodes, commonly known as Shiitake, is the second most cultivated mushroom in the world and has several biological activities such as antimicrobial, antitumor, immunomodulatory and antiviral. The aim of this study was to evaluate the antiviral activity of sulfated polysaccharide of L. edodes (PSLe) in the replication of poliovirus type 1 (PV-1), bovine herpesvirus type 1 (BoHV-1) and herpes simplex virus type 1 in HEp-2 cells. Antiviral activity was determined by adding the PSLe before (-2 and -1h), during (0h) and after (1 and 2h) viral