patterns activate receptors which induce production of pro-inflammatory cytokines and signals to activate inflammation. In addition, these receptors are required for an adaptive immune response. Innate and adaptive immune responses act as two interlocking defense lines. Following a rabies exposure, virus may be initially suppressed by innate immunity accompanied by effector T cell recruitment for activation of adaptive immunity. Alternatively, the uniquely high mortality rate of successful rabies virus infection may be due to virus-host interactions that remain largely a mystery. The accurate and precise measurement of an adaptive immune response may be defined by experimental methods or well-described methods, such as the rapid fluorescent focus antibody test, as performed and fully validated in some laboratories. The interpretation of findings based on these various methods should be in relation to clinical observations and collaborative investigation of unique, isolated, novel findings. The fine-tuning, interaction, and timing of innate and adaptive host responses and the methods used for detection and measurement, will require dedicated investigation towards optimal disease prevention.

CO.12
DIFFERENCE IN INTRACELLULAR LOCALIZATION AND EXPRESSION LEVEL OF RECOMBINANT RABIES G-PROTEINS OF STREET VIRUS (KYOTO STRAIN) AND FIXED VIRUS (CVS-26 STRAIN) EXPRESSED IN HEK293T CELLS

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Virulence of rabies virus (RABV) has been mainly studied with fixed viruses (laboratory strains) having different degrees of pathogenicity, however, virulence is much different between fixed viruses and street viruses (wild strains). Highly attenuated fixed strains of RABV does not cause lethal infection with profound inflammation accompanied with apoptosis and neural degeneration in the central nervous system (CNS). Induction of innate immune responses in CNS is a hallmark of infection with highly attenuated strains, whereas neural damage is absent or minimal and innate immune responses are not induced in animals infected with street virus. In the street virus infected cells, intracytoplasmic virion maturation taken place in the ER/Golgi apparatus is commonly observed and budding of virus from cellular plasma membrane is less frequent than in the fixed virus infected cells. Since RABV G-protein is critical for induction of virus neutralizing antibody, profound expression and budding of virions on cellular surface in fixed virus infected cells might be a major target of the host immune system. Thus different pathogenicity between street virus and fixed virus might be associated with different localization of virion maturation. However, little is known about molecular mechanism of virion maturation both of fixed and street viruses. To elucidate this, we have expressed G-proteins of CVS-26 strain (fixed virus) and Kyoto strain (street virus) in HEL293T cells upon transfection of the pCAGGS (CXN2) plasmid bearing G-genes of CVS-26 and Kyoto strains, respectively. Intracellular expression and localization of G-protein of each strain was then examined by fluorescence antibody technique and Western blot analysis using anti-G mAb (No.#7-1- 9, kindly provided from Dr.Kawai). Confocal laser scanning microscopy showed CVS- 26 G-protein was mainly localized on plasma membrane, while Kyoto G-protein was predominantly localized at perinuclear membrane region. CVS-26 G-protein was shown to be expressed in abundance than Kyoto G-protein in HEK293T cells by Western blot analysis. These results indicate maturation site of infectious RABV solely determined by localization of G-protein. Four amino acids in a signal peptide (SP) of G-protein of CVS-26 were distinct from those of Kyoto. The number of putative N-linked glycosylation sites was 3 and 2 on G-proteins of CVS-26 and Kyoto, respectively. Further analysis is required to elucidate whether these differences affect intracellular localization and expression level of RABV G-protein.

CO.13
ROLE OF MITOCHONDRIA IN RABIES VIRUS-INDUCED OXIDATIVE STRESS

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Neuronal process degeneration occurs in an experimental mouse model of rabies with hindlimb footpad inoculation of the challenge virus standard-11 (CVS) strain. CVS infection of primary mouse and rat dorsal root ganglion (DRG) neurons has associated axonal degeneration with axonal swellings and neurite outgrowth reduction. The CVSinduced axonal swellings feature protein adducts of 4-hydroxy-2-nonenal (4-HNE), a marker for lipid peroxidation, indicating a critical role of oxidative stress. Western immunoblotting analysis indicated that adducts of 4-HNE expression is also increased in the CVS-infected rat adrenal medulla (PC12) cell line. Mitochondrial dysfunction is one of the most important causes for overproduction and accumulation of reactive oxygen species (ROS). We investigated the effects of CVS infection on several mitochondrial parameters in different cell types (DRG primary neurons, PC12, mouse neuroblastoma (MNA), and baby hamster kidney (BHK-S13) cells) at 72 hrs post infection. The biochemical activity of electron transport system (ETS) complexes (I, IIIII, and IV) and Krebs cycle enzymes (citrate synthase and malate dehydrogenase) were evaluated using a spectrophotometric approach. Krebs cycle enzyme activities were not affected in CVS- versus mock-infected cells. Complex I activity was significantly increased in all CVS-infected cells versus mock-infected controls. Complex I was increased by 30-35% in CVS-infected DRG and PC12 cells, whereas it was increased by 65-75% in MNA and BHK-S13 cells. These values were proportional to the susceptibility of the cells to CVS infection suggesting a direct effect of the CVS infection on Complex I. Complex II-III activity was normal in the infected cells. Complex IV activity was upregulated in all types of CVS-infected cells. However, the increase did not relate to the susceptibility of the cells to the infection, suggesting an indirect effect. We postulate that enhanced Complex IV activity in CVS-infected cells may play a role in avoiding apoptosis. NADH, which is a Complex I-substrate, level was significantly higher in CVS-infected versus mock-infected PC12 cells. NAD+ level in CVS-infected PC12 cells was similar to that in mock-infected controls. Despite the increased activity of ETS complexes, CVS infection reduced the intracellular level of ATP in PC12 cells. The reduced ATP level in CVS-infected DRG neurons may explain, at least in part, the reduction in the neurite outgrowth that was previously observed. We predict that a high mitochondrial inner membrane potential is generated in CVS infection because of increased proton pumping across the mitochondrial inner membrane due to higher activity of Complex I and IV, and decreased proton consumption as indicated by reduced intracellular ATP level. Induction of a high mitochondrial membrane potential promotes electron leakage, primarily at the Complex I site, leading to ROS overgeneration and oxidative stress.