Study on ecology and pathogenicity of Newcastle disease viruses

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Newcastle disease virus (NDV) comprises a variety of strains which differ in pathogenicity for chicken, such as velogenic, mesogenic, and lentogenic forms of the disease. NDV bears two types of glycoprotein, hemagglutinin-neuraminidase (HN) and fusion (F) proteins, on the surface of the particle. The F protein is initially synthesized as a precursor polypeptide F0, and then proteolytically cleaved to two polypeptides, F1 and F2 to acquire biological activities. It is generally accepted that the cleavability of the F protein is a critical determinant of the pathogenicity of NDV. Namely, the F protein of virulent strains is cleaved in a variety of cells, and virulent viruses, therefore, can be disseminated throughout their host and cause extensive disease. In contrast, the F protein of avirulent strains is cleaved only in limited types of cells (e.g., epithelia in intestinal, or respiratory tract). The cleavage site of the F protein of virulent strains consists of a pair of dibasic amino acid residues (RRQRR or RRQKR), while the corresponding region of avirulent strains is monobasic (GRQGR). Only difference of cleavability of the F protein could not be explained variety of pathogenesis by NDVs to the chickens. Namely, variety of pathogenesis by NDVs involve other factor except cleavability of the F.

In the present study, to provide information on the mechanism of attenuation of a Newcastle disease (ND) vaccine strain, TCND was compared with the parental virulent strain California 11914 (CAL) biologically and genetically. TCND possessed the F protein of virulent type, consisting of a pair of dibasic amino acid residues at the cleavage site. The F protein of TCND was cleaved by cellular proteases as well as that of CAL. However, TCND was restricted to grow at 41.5°C, indicating that it was a *ts* mutant. A small amount of functional HN proteins were expressed in TCND-infected cell surface and, hence virions lacking the HN protein were released from the cells at nonpermissive temperature. The revertants, which replicated at nonpermissive temperature as well as CAL, exhibited lower activity in fusion assay than CAL and recovered virulence to chicken only in part. The results indicate that the *ts* mutation of TCND in association with the defect of the HN protein transport is a mechanism of the attenuation, and in addition, some other factors such as fusion activity should be involved in the loss of virulence of CAL to chickens.

NDV has been isolated from a variety of wild, domestic, and cage birds around the world. The majority of NDV strains isolated from waterfowls are lentogenic, causing no obvious disease. However, little is known how those viruses are maintained in nature. To provide information how NDVs are maintained in duck population in their breeding places and how they are involved in ND outbreak in poultry, phylogenetic analyses of the genes of the isolates were carried out and virulence of isolates was assessed by pathogenicity test. NDV strains isolated from fecal samples of waterfowls in the neasting areas were analyzed for their virulence. Although many isolates were virulent character in chicken embryos, none of the isolates were virulent for

chickens. Some isolates possessed the F protein of a virulent type, which contained a pair of dibasic amino acids at the cleavage site. The present results suggest that potentially virulent strains, which showed avirulent character, of NDV are maintained in migratory waerfowl populations. Some of those may transmit to domestic poultry and acquire pathogenicity during passages in chicken population.

These results indicate that pathogenicity of NDV strains was not necessarily correlated to the amino acid sequence at the cleavage site of the F protein. Pathogenicity, i.e. the ability of a virus to induce disease in the infected organism, is the results of a complex interplay of a multitude of factors that are determined by the biological, biochemical, and genetic characteristics. Thus, a molecular basis for viral pathogenicity is not easy to define. It is reasonable, however, to assume that disease becomes manifest, if the infecting virus has the ability to multiply to high amounts and to spread in the organism, and if cells containing vital functions are altered or killed by the virus. Since cell tropism represents primarily an interaction between the surface components of the virus and receptors of the host cell, it is obvious that the surface structure of a virus determines pathogenicity.

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Functional and Structural Analysis of Pseudorabies Virus Early Protein 0

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Early protein 0 (EP0) of pseudorabies virus (PRV) has been identified to be a homolog of ICP0 of herpes simplex virus 1 (HSV-1). EP0 contains the RING finger domain near its amino terminus with homology to, besides of ICP0 of HSV-1, ORF61 of varicella-zoster virus, BICP0 of bovine herpesvirus 1, and EICP0 of equine herpesvirus 1 which belong to alphaherpesvirus. These ICP0 homologs are thought to be involved in transcriptional regulation, suggesting that EP0 may also have transcriptional regulatory activities. In the present study, the function, structure/function relationship of EP0, and regulatory mechanism of EP0 gene expression were analyzed by transient expression assays.

To characterize the function of EP0 in gene expression of PRV, a plasmid expressing EP0 was constructed. EP0 was detected by indirect immunofluorescence assay in the nuclei of the cells transfected with an EP0 expression plasmid as is the case in the cells infected with PRV. In transient expression assay, the effect of EP0 on transcription under the control of PRV immediate early (IE), thymidine kinase (TK), and glycoprotein X (gX) promoters was analyzed. In result, EP0 transactivated these promoters, indicating that EP0, like ICP0 of HSV-1, is a transactivating protein.

To map the functional domains on EP0 molecule, a series of truncated forms of the EP0 gene were constructed. The amino-terminal region containing the RING finger domain, amino