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COMPARATIVE ANALYSIS OF ATTENUATED VACCINE STRAIN TCND AND THE PARENTAL NEWCASTLE DISEASE VIRUS

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The cleavability of the fusion (F) protein of Newcastle disease virus (NDV) is a critical determinant of pathogenicity. The F protein of virulent strains is cleaved in a wide variety of cells both *in vivo* and *in vitro*, whereas that of avirulent strains is cleaved only in limited types of cells such as epithelial cells lining the upper respiratory and intestinal tracts. Virulent NDV strains thus have biologically active F proteins and are able to grow in cells of various organs *in vivo* and cause extensive disease. In the present study, it was found by protein analysis that attenuated vaccine strain TCND bore the cleaved F protein. TCND virus formed plaques in chicken embryo cells without addition of trypsin. Sequence analysis revealed that there were two pairs of dibasic amino acids at the cleavage site of the F protein of TCND, which matched the consensus sequence of those of virulent strains. These findings indicate that some factor(s) other than cleavability of the F protein should be involved in the attenuation of TCND.

Biological and genetic comparison between TCND and the parental virulent strain, California 11914 (CAL) revealed that TCND was temperature-sensitive; it did not grow at 41.5°C, whereas CAL extensively replicated. In the infected cells, viral gene transcription and protein synthesis of TCND occurred in a manner similar to that of the parental strain even at the nonpermissive temperature. By immunofluorescence assay, the HN protein was scarcely detected on the surface of TCND-infected cells at the nonpermissive temperature. These results suggest that transport of the HN protein is limited to the exocytic pathway at the normal body temperature of chickens, resulting in attenuation of TCND.

Revertants that grew at the nonpermissive temperature were obtained by egg passage of TCND for 4 days at 41.5°C. They showed virulence comparable to the parental CAL in a test using chicken embryos. For 6-week-old chickens, on the other hand, the revertants showed only slightly higher virulence than TCND. These results suggest some other factor(s) is involved in the attenuation of TCND viruses in addition to its temperature sensitivity.