Molecular Analyses of Structure-Function of the Influenza Virus Hemagglutinin

Masaki Imai

Laboratory of Microbiology, Department of Disease Control,
Graduate School of Veterinary Medicine,
Hokkaido University, Sapporo 060–0818, Japan

Molecular basis of structure-function of the H5 and H7 influenza A virus hemagglutins (HAs), correlated with their pathogenicity potential, was analyzed in the present study.

To provide information on the structure-function of the H7 HA molecules, the mechanism of neutralization of viral infectivity by monoclonal antibodies (MAbs) to operationally defined epitopes on the HA molecule was analyzed. Epitopes were mapped on the HA of A/seal/Massachusetts/1/80 (H7N7) influenza virus by genetic analysis of variants selected with MAbs. Electron microscopic studies demonstrated that the sites and the directions to which hemagglutination-inhibiting (HI) and non-HI MAbs bound were different on the HA molecule. The results suggest that whether hemagglutination of the virus is inhibited by antibodies depends on the location of the site where antibodies bind on the HA molecule. Morphological analyses together with a fluorescence dequenching assay revealed that, after attachment to the host cells, viruses were internalized by endocytosis into intracellular vacuoles, and then the fusion of the viral envelope with the vacuolar membrane occurred. HI MAbs blocked attachment of the virus to the host cells, while non-HI MAbs did not. Although the latter MAb-bound virus particles were then found in the intracellular vacuoles, the fusion was not observed. It was thus shown that non-HI neutralizing MAbs did not inhibit attachment of the virus to the host cell receptor, but inhibited the fusion step in intracellular vacuoles. The present results revealed a novel mechanism of neutralization of viral infectivity by antibodies, that is inhibition of fusion of the viral envelope with the intracellular membranes of the host cells, in addition to blocking attachment of the virus to susceptible cells.

To provide information on the origin of the highly pathogenic H5N1 influenza viruses that infected chickens and humans in Hong Kong in 1997, the HA of H5 influenza virus strains isolated from ducks in Asia were analyzed their antigenicity, phylogeny, and pathogenicity potential. Antigenic analysis using a panel of MAbs reveale that the HA of H5 influenza viruses isolated from ducks are antigenically closely related to each other. Phylogenetic analysis indicates that the isolates from ducks in Hokkaido were derived from an ancestor common with the pathogenic isolates in Hong Kong. The results indicate that the pathogenic H5N1 influenza viruses which infected chickens and humans in Hong Kong in 1997 is originated from influenza viruses maintainted in ducks nesting in Siberia. To prepare for the emergence of next pandemic influenza, it is important to expand surveillance study on avian influenza.

Original papers of this thesis appeared in *"Virus Research"* Vol. 53, 129–139 (1998), and *"Japanese Journal of Veterinary Research"* Vol. 46, 171–177 (1999).