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AN ANALYSIS OF STRUCTURE PROTEIN OF TURKEY HERPESVIRUS BY  
MONOCLONAL ANTIBODIES PREPARED AGAINST THE VIRUS

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Herpesvirus of turkeys (HVT), which is antigenically closely related to Marek's disease virus (MDV), is used widely to make Marek's disease (MD) vaccine. Since the virus is highly cell-associated in nature, it is difficult to obtain cell-free virus from the culture medium. Recently, we established a variant type of HVT (HVT/VT) which releases a large amount of cell-free HVT into the culture medium. The purpose of the present study is to analyse the structural protein of HVT/VT by monoclonal antibodies (HV. 8, HV. 24, HV. 12 and HV. 13) prepared against particles of virus and those (G4, B2, and C12) against homogenates of the cultured cells infected with a wild type of HVT (HVT/WT).

These antibodies reacted specifically with cells infected with serotype 3 virus (HVT/VT, HVT/WT and Type 2-PPA) by the immunofluorescent test and enzyme-linked immunosorbent assay (Cell-ELISA), and recognized mainly the virus-specific antigens appeared in the late stage of infection. Furthermore, HV. 8 and HV. 24 antibodies reacted with membrane antigens of the cells. HV. 12 and HV. 13 antibodies were positive for serotype 3 virus in the neutralization test and for HVT/VT-infected cells in the complement-dependent antibody cytotoxicity test. The usefulness of Cell-ELISA using the cell antigens fixed on plates and stored at  $-20^{\circ}\text{C}$  was confirmed.

By SDS-PAGE analysis of the HVT/VT particles purified with sucrose cushion and the HVT/VT-infected cells treated with sonication, 6 bands (74 kilodaltons (K), 64.5K, 52K, 46K, 33K and 14K) detected in the particles were not present in the cells. By immunoprecipitation and analysis of SDS-PAGE, G4 antibody precipitated the 34K, 30K and 24K polypeptides, and HV. 8 and HV. 24 precipitated the 23.5K in HVT/VT-infected cells. C12 antibody slightly precipitated the 70K, 45K and 24K polypeptides, and HV. 12 and HV. 13 antibodies slightly precipitated the 70K polypeptide in the infected cells. HV. 8 antibody immunoprecipitated the 74K, 60K, 54/46K, 39K, 30K and 23.5K polypeptides in the virus particles.

On the other hand, G4, C12, HV.8 and HV.13 antibodies reacted with the 39K and 30K polypeptides in HVT/VT-infected cells by SDS-PAGE and Western blotting analyses, while G4 and C12 antibodies reacted with the 39K, HV. 13 antibody did with the 115K, 74K, 64.5K and 39K, and HV.8 did with the 74K, 64.5K and 39K in the virus particles. Since the 39K reacted with an unrelated monoclonal antibody used as negative control, the polypeptide seems to be detected as a nonspecific reaction.