Most Virus-specific Polypeptides in Cells Productively Infected with Marek's Disease Virus or Herpesvirus of Turkeys Possess Cross-reactive Determinants

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SUMMARY

Most virus-specific polypeptides in cells productively infected with Marek's disease virus (MDV) or herpesvirus of turkeys (HVT) possess cross-reactive antigenic determinants, although the two viruses have very little DNA homology. The cross-reactivity appeared to be more evident when [35S]methionine-labelled polypeptides were immunoprecipitated than when the [3H]glucosamine-labelled polypeptides were immunoprecipitated, suggesting that the glycoproteins of MDV and HVT may be less related in structure than other proteins. The major cross-reactive glycoproteins excreted into the culture mediums of MDV- and HVT-infected cells had molecular weights of 64000 (gp64) and 56000 (gp56) respectively.

Marek's disease virus (MDV) is the aetiological agent of Marek's disease (MD), a highly contagious malignant lymphoma of chickens. A herpesvirus of turkeys (HVT) has been successfully used as MD vaccine. All strains of MDV and HVT are antigenically closely related (Chubb & Churchill, 1968; Purchase, 1969; Witter et al., 1970). The 'A' antigen, detected by immunodiffusion analysis of both cell extracts and culture fluids of MDV-infected cells (Churchill et al., 1969), has also been found in cells infected with HVT (Purchase et al., 1971) and identified as a glycoprotein (Ross et al., 1973). Serological cross-reaction between virus-specific membrane antigens of cells infected with MDV and HVT has been shown by an immunofluorescence test (Ishikawa et al., 1972; Nazerian, 1973). In spite of these antigenic similarities, previous studies on the extent of DNA homology between MDV and HVT have revealed that the two viruses have very little DNA homology, probably less than 5% (Hirai et al., 1979; Kaschka-Dierich et al., 1979; Lee et al., 1979). Therefore, further studies on the extent of antigenic cross-reactivity between MDV and HVT are required for a clear understanding of these unexpected results. Recently, we have identified more than 40 distinct MDV-specific polypeptides in cells infected with MDV using antibodies purified by affinity chromatography (Ikuta et al., 1981). This method has allowed us to analyse systematically the extent of cross-reactivity of proteins and glycoproteins of MDV and HVT. The results are reported in this paper.

The BC-1 and JM strains of MDV and the 01 strain of HVT were propagated in primary chick embryo fibroblasts (CEF) prepared from embryos of specific pathogen-free chickens as described previously (Ikuta et al., 1981). The BC-1 strain was used at the 22nd (BC/LP) and 66th (BC/HP) passages. The JM strain was used at the 14th (JM/LP) and 58th (JM/HP) passages. At the lower passages, both MDV strains have the ability to form typical MD lymphoma, whereas at the higher passages both MDV strains have lost oncogenicity. The 01 strain of HVT was used at the 28th (01/LP) and the 85th (01/HP) passages. HVT 01/LP has the ability to protect chickens from MD while 01/HP has lost this ability. We confirmed by DNA–DNA reassociation kinetics that the MDV and HVT used here did not contain any detectable amounts
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Fig. 1. Fluorograms of polypeptides immunoprecipitated from MDV- or HVT-infected cells labelled with \(^{35}\)S)methionine and separated by SDS–PAGE. Infected or mock-infected CEF in 60-mm diam. dishes were labelled with 1 ml minimum essential medium (MEM) containing 1/10th the normal concentration of methionine, 2% dialysed calf serum, and 50 μCi \(^{35}\)S)methionine (1250 Ci/mmol) from 24 to 48 h after infection. The cell extracts were immunoprecipitated with purified antibodies by affinity chromatography and also with preimmune duck IgG and then with rabbit anti-duck IgG serum as described previously (Ikuta et al., 1981). The immunoprecipitates were analysed by SDS–PAGE (10% polyacrylamide gel) as described previously (Ikuta et al., 1981). (a) MDV BC/LP antibody; (b) HVT 01/LP antibody; (c) preimmune duck IgG. The molecular weights of virus-specific polypeptides were calculated by comparison of their mobilities with those of marker proteins in a calibration kit for molecular weight determination (Pharmacia). Numbers on the left of (a) and on the right of (b) indicate molecular weights \((\times 10^{-3})\) of major polypeptides specific to MDV and HVT respectively. The other virus-specific polypeptide bands easily detected are indicated by horizontal lines without a number.

of HVT and MDV respectively. Antisera to MDV or HVT were obtained from ducks immunized with virus-infected duck embryo fibroblasts (DEF). Virus-specific antibodies were purified from these antisera by affinity chromatography conjugated with the lysates of homologous virus-infected CEF as described previously (Ikuta et al., 1981). The IgG fraction purified from preimmune duck serum by ammonium sulphate precipitation was used as a control. The antibodies obtained were absorbed with uninfected CEF and DEF fixed with 0.5% glutaraldehyde. The immunofluorescence titres of the BC/LP, BC/HP and 01/LP antibodies used here were 1:2048. The titres per μg of IgG protein increased about ten times after affinity chromatography. Techniques used for immunoprecipitation of virus-specific antigens from cell extracts and culture fluids with homologous and heterologous antibodies and SDS–
Fig. 2. Fluorograms of polypeptides immunoprecipitated from MDV- or HVT-infected cells and their culture fluids labelled with [3H]glucosamine and separated by SDS–PAGE. Infected CEF in 60-mm diam. dishes were labelled with 1 ml MEM containing 2% calf serum and 100 μCi [3H]glucosamine (30.3 Ci/mmol) from 24 to 48 h after infection. (a) Cell extracts and (b) culture fluids were immunoprecipitated with purified MDV BC/LP and HVT 01/LP antibodies for (a), and HVT 01/LP antibody for (b), and then with rabbit anti-duck IgG serum. Numbers on the left and right indicate molecular weights (× 10^-3) of polypeptides specific to MDV and HVT respectively.

polyacrylamide gel electrophoresis (SDS–PAGE) were as described previously (Ikuta et al., 1981).

As shown in Fig. 1, at least 20 distinct polypeptides labelled with [35S]methionine were identified as MDV- and HVT-specific polypeptides by SDS–PAGE of immunoprecipitates of MDV- and HVT-infected CEF with their homologous antibodies. These polypeptide bands were not detected in immunoprecipitates of mock-infected CEF with BC/LP or 01/LP antibodies. The other control assay showed that a polypeptide was detected as a non-specific 92K band in the immunoprecipitates of virus-infected CEF with preimmune duck IgG (Fig. 1 c). Therefore, the polypeptide bands shown here represent MDV- or HVT-specific polypeptides. No obvious differences were detected between the immunoprecipitates from BC-1 strain- and JM strain-infected cells and also between the immunoprecipitates from BC/LP- and BC/HP-infected cells or from JM/LP- and JM/HP-infected cells, with BC/LP antibody (Fig. 1 a). These patterns were not significantly different from those of the MDV-specific polypeptides immunoprecipitated with BC/HP antibody (data not shown). The virus-specific polypeptides of HVT 01/LP and 01/HP immunoprecipitated by HVT 01/LP antibody showed clear resemblance in both number and size distribution to the virus-specific polypeptides (Fig. 1 b). Surprisingly, when MDV- and HVT-specific polypeptides were immunoprecipitated with heterologous HVT and MDV antibodies respectively, almost all of the MDV- and HVT-specific
polypeptides contained cross-reactive antigenic determinants, except for small MDV- or HVT-specific polypeptides of less than 28K (Fig. 1a, b). Judging from the densities of the fluorograms of these immunoprecipitates in Fig. 1, the virus-specific polypeptides seemed to be immunoprecipitated less with heterologous antibodies than with homologous antibodies. This could be due to the presence of non-cross-reactive MDV- or HVT-specific polypeptides in the virus-infected cells. However, the exact reason for the poor immunoprecipitation of virus-specific polypeptides with heterologous antibodies is not known at present.

Next, we focused our attention on the virus-specific glycoproteins of MDV and HVT that appear to be serologically cross-reactive. Since little is known about the biochemical nature of MDV- and HVT-specific glycoproteins, we identified the glycoproteins by labelling MDV- and HVT-infected cells with $[^3H]$glucosamine as described in the legend to Fig. 2. As shown in Fig. 2(a), at least seven distinct glycoprotein bands were obtained by SDS-PAGE of the immunoprecipitates of MDV BC/LP-infected cells with BC/LP antibody. These glycoproteins were tentatively designated gp110, gp92, gp75, gp64, gp53, gp50 and gp42 according to their molecular weights. At least six distinct glycoprotein bands, designated gp115, gp75, gp68, gp56, gp50 and gp42, were identified as HVT-specific glycoproteins in immunoprecipitates of HVT 01/LP-infected cells with 01/LP antibody (Fig. 2a). None of the virus-specific glycoproteins was detected in immunoprecipitates of mock-infected CEF with BC/LP or 01/LP antibody (data not shown). Most of the MDV- and HVT-specific glycoproteins appeared to cross-react with their heterologous HVT 01/LP and MDV BC/LP antibodies respectively. However, several HVT-specific glycoproteins, such as gp75 and gp68 in 01/LP-infected cells, did not cross-react with BC/LP antibody. It is noteworthy that the cross-reactivities seem more evident when $[^35S]$methionine-labelled polypeptides were immunoprecipitated than when the $[^3H]$glucosamine-labelled polypeptides were immunoprecipitated, suggesting that the glycoproteins of MDV and HVT may be less related in structure than other proteins. Among these cross-reactive glycoproteins, MDV gp64 and HVT gp56 were clearly detectable in immunoprecipitates of culture fluids of BC/LP- and 01/LP-infected cells respectively (Fig. 2b). These glycoproteins may correspond to the 'A' antigen described previously (Churchill et al., 1969).

Thus, most virus-specific polypeptides in cells productively infected with MDV or HVT possess cross-reactive antigenic determinants, although the two viruses have very little DNA homology (Hirai et al., 1979; Kaschka-Dierich et al., 1979; Lee et al., 1979). This discrepancy could be explained by supposing that only a small portion of the amino acid sequences responsible for the antigenicity of the cross-reactive determinants is common in MDV and HVT cross-reactive polypeptides, or that, although the hybridization study in stringent conditions shows very little homology between DNAs of the two viruses, the amino acid sequences derived from the DNA sequences coding for MDV and HVT cross-reactive polypeptides show a high degree of homology between the polypeptides of the two viruses as found in the genomes of SV40, polyoma virus and BK virus (Soeda et al., 1980). These possibilities could be tested by comparison of the nucleotide sequences of MDV and HVT DNAs. Studies on the cross-reactivities of the specific polypeptides of MDV and HVT with monoclonal antibodies are now in progress.

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REFERENCES


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