NOTES

Identification with Monoclonal Antibodies of Virus-Specific DNA-Binding Proteins in the Nuclei of Cells Infected with Three Serotypes of Marek’s Disease Virus-Related Viruses

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Two groups of virus-specific polypeptides were identified in the nuclei of infected cells by cross-reacting monoclonal antibodies with three serotypes of Marek’s disease virus. Of these, a 135,000-molecular-weight polypeptide common to all three serotypes was found to bind to both double-stranded and single-stranded DNAs.

Marek’s disease virus (MDV) is the etiological agent of Marek’s disease, a highly contagious malignant T-cell lymphoma in chickens. The various pathological features of the disease provide an experimental model system in medical oncology, as well as in other areas of medical research. An avian herpesvirus serologically related to MDV has been subdivided into three serotypes (1). Serotype 1 includes MDV strains with various pathogenicities to chickens and also includes their attenuated, nonpathogenic strains. Serotype 2 is a naturally occurring nonpathogenic chicken herpesvirus, whereas serotype 3 is a nonpathogenic herpesvirus of turkeys that has been used as a vaccine against Marek’s disease.

Isolation of mouse monoclonal antibodies specific to and cross-reactive with MDV-related viruses (4, 11) led to the identification of several virus-specific polypeptides and to confirmation of the validity of the classification of the three serotypes (5, 6, 8, 9, 15; K. Hirai, K. Nakajima, K. Ikuta, R. Kirisawa, Y. Kawakami, T. Mikami, and S. Kato, Arch. Virol., in press). However, little is known about the virus-specific nuclear antigens of MDV-related viruses. Identification of virus-specific proteins, such as those binding DNA, in the nuclei of infected cells is essential because of the possible regulatory roles of these proteins in viral and cellular gene expression and in replication of viral DNA. In the present study virus-specific polypeptides in the nuclei of cells infected with the three serotypes were identified by radioimmunoprecipitation with monoclonal antibodies.

Virus strains used were as follows: the oncogenic BC-1 strain of serotype 1 at low passages of 16 to 23 (BC-1-LP) and its nononcogenic variant at higher passages of 40 to 48 (BC-1-HP), nononcogenic strain C2 of serotype 1, strain HPR24 of serotype 2, and vaccine strain 01 of serotype 3. These viruses were propagated in primary chicken embryo fibroblasts (CEF) as described previously (3). Mock-infected and infected cells were labeled 24 to 48 h after infection with 50 μCi of L-[35S]methionine (1,250 Ci/mmol; Amersham Corp., Buckinghamshire, United Kingdom) or 500 μCi of H32PO4 (Japan Radioisotope Association, Tokyo, Japan), per ml as described previously (5). The methods used for production and culture of hybridomas and screening for virus-specific antibodies are described in detail elsewhere (4). The techniques for immunoprecipitation of virus-specific polypeptides from lysates of infected CEF were as described previously (7). The immunoprecipitates were dissolved in sample buffer containing 2% sodium dodecyl sulfate (SDS), 5% 2-mercaptoethanol, 10% glycerol, 0.001% bromophenol blue, and 62.5 mM Tris hydrochloride and subjected to one-dimensional SDS-polyacrylamide gel electrophoresis (PAGE) (10% polyacrylamide gel) as described by Laemmli (10). For two-dimensional (2D) gel electrophoresis, the immunoprecipitates were subjected to isoelectric focusing essentially as described by Ikuta et al. (8), but the SDS-PAGE was carried out under the same conditions as described above for one-dimensional SDS-PAGE. For the study of DNA-binding activities with virus-specific polypeptides, labeled cells were extracted with a high concentration of salt, as described by Shiraiki et al. (14). After dialysis against 50 mM NaCl–20 mM Tris hydrochloride (pH 8.2)–1 mM EDTA–1 mM 2-mercaptoethanol–10% glycerol (DB buffer), the cell extract was subjected to chromatography on double- or single-stranded calf thymus DNA cellulose (Wako Pure Chemical Industries, Osaka, Japan). The column was washed with DB buffer, and then DNA-binding polypeptides were eluted by the stepwise addition of 0.15, 0.3, and 2 M NaCl to DB buffer. Each fraction was then mixed with an equal volume of buffer consisting of 1% Nonidet P-40, 1 M NaCl, 2 mM phenylmethylsulfonyl fluoride, and 100 mM Tris hydrochloride (pH 8.0) and subjected to immunoprecipitation with monoclonal antibodies and then SDS-PAGE.

We prepared a series of hybridoma clones producing antibodies against specific and cross-reactive antigens of serotypes 1 (strain BC-1-LP) and 3 (strain 01) (4). Of these, seven hybridoma clones were found to produce antibodies that reacted with the nuclei of CEF infected with the three serotypes in immunofluorescence (IF) tests. The isotypes of the monoclonal antibodies produced by these antibodies were as follows: M12, immunoglobulin G1 (IgG1); M29, IgG3; M37, IgG2a; M38, IgG2a; M40, IgG2b; M55, IgG2a; and M57, IgG2b. Antibodies produced by M37 and M38

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reacted with nuclei infected with either serotype 1 (BC-1-LP) or serotype 2 (HPRS24), whereas those produced by the five other clones cross-reacted with cells infected with one of the three serotypes. Examples of IF detected with M29 antibody are shown in Fig. 1. Virus-specific nuclear antigens of the three serotypes gave fine granular IF patterns. The other antibodies to virus-specific nuclear antigens also gave similar IF patterns (data not shown).

The one-dimensional SDS-PAGE profiles of \([^{35}S]\) methionine-labeled immunoprecipitates with M29 and M57 antibodies from cells infected with the three serotypes are shown in Fig. 2. The monoclonal antibodies were subdivided

\[\text{Antibody} \quad \text{Infected cells} \quad \text{M29} \quad \text{M57} \]

\[\begin{array}{c|c|c}
\text{A} & \text{M29} & \text{M57} \\
\text{B} & \text{M29} & \text{M57} \\
\end{array}\]

\[\begin{array}{c|c|c}
\text{Antibody} & \text{Infected cells} & \text{M29} & \text{M57} \\
\text{A} & \text{M29} & \text{M57} \\
\text{B} & \text{M29} & \text{M57} \\
\end{array}\]

\[\text{Numbers on the right indicate the molecular weights of virus-specific nuclear polypeptides specific to the three serotypes.}\]
into two groups based on the sizes of reactive polypeptides: group A included M29, M37, and M38 and group B included M12, M40, M55, and M57. The antibodies of groups A and B showed SDS-PAGE profiles that were almost identical to those of M29 and M57 antibodies, respectively, except for their varied cross-reactivities with the three serotypes. Therefore, the results obtained with the other antibodies are not shown in Fig. 2. Group A antibody M29 reacted with virus-specific polypeptides of 135,000 molecular weight (135K polypeptides) in cells infected with any one of the three serotypes (Fig. 2A). However, the 135K polypeptide of strain 01 was precipitated less with M29 antibody than those of the BC-1-LP and HPRS24 strains were. Because the faint band at 135K of strain 01 was seen only with group A antibodies, but not with any other virus-specific mouse monoclonal antibodies available in our laboratories (data not shown), the 135K polypeptide was virus specific. Group B antibody M57 reacted with a 145K polypeptide of strain BC-1-LP, a 145K polypeptide of strain HPRS24, and a 135K polypeptide of strain 01 (Fig. 2A). Therefore, the polypeptides that reacted with two other groups of antibodies contained an epitope common to all the serotypes. The data in Fig. 2B show that there were no significant differences in the sizes and quantities of virus-specific nuclear polypeptides of the oncogenic BC-1-LP and those of its nononcogenic variant BC-1-HP and strain C2 of serotype 1. In this respect, the virus-specific nuclear polypeptides differed from serotype 1 glycoprotein A, which is lost after attenuation of serotype 1 viruses (8). However, small structural changes could occur upon attenuation of serotype 1 viruses without affecting the sizes of the virus-specific nuclear polypeptides. CEF infected with the three serotypes were labeled with [35S]methionine or H3.35PO4 and used for immunoprecipitation of cell extracts with antibodies of group A or B followed by SDS-PAGE. No 35P radioactivity was detected that comigrated with [35S]methionine-labeled, virus-specific polypeptides reactive with the antibodies of two groups (data not shown), suggesting that the virus-specific nuclear polypeptides were not phosphorylated.

Next, we analyzed the nuclear polypeptides of three serotypes recognized by group A antibodies by 2D gel electrophoresis to test whether the 135K polypeptides were structurally identical. The results presented in Fig. 3 show that the 135K polypeptides of strain 01 were the most acidic, with isoelectric points (pl) of 6.2 to 6.4; that of strain HPRS24 was the most basic, with pl values of 6.5 to 6.7; and that of strain BC-1 held an intermediate position. Therefore, the 135K polypeptides of three serotypes that reacted with group A antibodies differ from each other with respect to their electric charges, suggesting that they are structurally different.

To determine whether the virus-specific nuclear polypeptides of the three serotypes were DNA-binding, we applied extracts from [35S]methionine-labeled cells infected with the three serotypes to double- and single-stranded DNA cellulose columns (Fig. 4). The 135K polypeptides that reacted with group A antibody M29 were mainly eluted with 0.3 M NaCl from both double- and single-stranded DNA cellulose columns (Fig. 4A and C). However, considerable amounts of 135K polypeptides from cells infected with strain BC-1-LP or HPRS24 were also eluted in the unbound fraction, even when the DNA cellulose column was large. When the unbound fraction of BC-1-LP was applied to another double-stranded DNA cellulose column, the 135K polypeptide was recovered in both unbound and bound fractions (data not shown). Therefore, the unbound 135K polypeptide could mostly consist of DNA-binding polypeptides with low DNA-binding activity. One of the proteins, ICP4, of herpes simplex virus type 1 was shown to bind DNA only via a component of uninfected cells (2). Furthermore, phosphorylation of herpes simplex virus-specific DNA-binding proteins was reported to modify their binding ability for DNA (16). Therefore, the low binding activity of the 135K polypeptide could be due to loss of a component responsible for the DNA binding or to dephosphorylation during the extraction process. In contrast to the polypeptides that reacted with group A antibody, none of the polypeptides of three serotypes that reacted with group B antibody M57 bound to either double- or single-stranded DNA cellulose (Fig. 4B and D; data not shown for strains HPRS24 and 01).

Thus, two groups of cross-reactive virus-specific polypeptides were identified in the nuclei of cells infected with the three serotypes of MDV-related viruses. It is interesting that these nuclear polypeptides contain epitopes with a common serotype. In particular, the DNA-binding polypeptides of the three serotypes were identical in size but not in electric charge. Therefore, their primary amino acid sequences may not be the same despite antigenic similarities. We are now investigating the possibility by peptide mappings of the two nuclear polypeptides of the three serotypes. Preliminary results indicate that they have different amino acid sequences.

The DNA-binding proteins of HSV are well characterized. The major DNA-binding protein ICP8 of herpes simplex virus type 1, with a molecular weight of about 130,000, is known to be an early viral gene product required for viral DNA replication (13). The DNA-binding polypeptide of nonpathogenic turkey herpesvirus (serotype 3) also appears to be an early gene product of the viral genome, because phosphonoacetic acid, an inhibitor of viral DNA replication of herpesvirus, did not block the appearance of virus-specific antigens that reacted with group A and B antibodies in the nuclei of cells infected with the cell-free virus of strain 01.
FIG. 4. Binding to DNA cellulose of virus-specific nuclear polypeptides of the three serotypes. CEF infected with strain BC-1-LP (BL), HPRS24 (HS), or 01 were labeled with [35S]methionine. Cell extracts were applied to a double- (A and B) or single-stranded (C and D) DNA cellulose column. The polypeptides that were eluted with DB buffer only (lane 1), 0.15 M NaCl (lane 2), 0.3 M NaCl (lane 3), or 2.0 M NaCl (lane 4) were immunoprecipitated with M29 or M57 antibody and analyzed by SDS-PAGE.

(data not shown). The DNA-binding proteins of lymphotropic oncogenic herpesviruses, such as Epstein-Barr virus (12), are present as transformation-specific antigens in the nuclei of cells transformed by them. However, we could not detect any virus-specific nuclear antigens except virus-specific cytoplasmic antigens related to phosphorylated proteins in nonvirus-producing lymphoblastoid cell lines established from chickens with Marek's disease (5). At present, little is known about the functions of the nuclear antigens of the three serotypes. Further studies are in progress on the possible functions of DNA-binding polypeptides in viral DNA replication and in viral and cellular gene expression.

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cipitation of Marek's disease virus-specific polypeptides with chicken antibodies purified by affinity chromatography. Virol-