JOURNAL OF VIROLOGY, Nov. 1979, p. 676-678
0022-538X/79/11-0676/03$02.00/0

Vol. 32, No. 2

Production of Monoclonal Antibodies Against Nucleocapsid Proteins of Herpes Simplex Virus Types 1 and 2

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Received for publication 26 June 1979

We prepared mouse hybrid cell lines which produced antibodies against herpes simplex virus type 1 and 2 nucleocapsids. Cell lines 1D4 and 3E1, respectively, secreted immunoglobulin G1 herpes simplex virus type 1 and immunoglobulin G1 herpes simplex virus type 2 antibodies which immunoprecipitated proteins designated p40 and p45 from homologous nucleocapsid preparations but precipitated no proteins from heterologous preparations. In contrast, guinea pig antisera prepared against either herpes simplex virus type 1 or 2 p40 precipitated p40 and p45 from both homologous and heterologous preparations. These findings suggest that p40 and p45 possess similar antigenic determinants and that the monoclonal antibodies that were tested reacted preferentially with the homologous determinants.

A polypeptide of about 40,000 molecular weight (p40) is a major component of the nucleocapsids of herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2) (2, 4). This protein is found in disulfide-linked complexes in a species of intranuclear nucleocapsids which contains viral DNA (4, 11), but is present in much smaller amounts in a species of nucleocapsids partially deficient in DNA (2, 4). Previous studies employing competition immunoassays suggested that the p40's of HSV-1 and HSV-2 are antigenically distinct, since antibodies reactive with at least two determinants (type specific and cross-reactive) are elicited after HSV-1 or HSV-2 immunization of guinea pigs or natural human infection (4). One method for directly demonstrating the existence of antibodies with distinct specificities has been made feasible recently by procedures which allow for the selection of cells producing monoclonal antibodies that react with single antigenic determinants (5, 6). The monoclonal antibodies are secreted from continuous hybrid cell lines which are prepared by fusing mouse myeloma cells with lymphocytes from immunized animals. Monoclonal antibodies prepared in this way have been used successfully to analyze many different virus-specified proteins (7, 9, 10). In this report we demonstrate the application of monoclonal antibodies to the immunological analysis of HSV p40.

To prepare hybrid cell lines for this study, HSV-1 (MAL strain) and HSV-2 (MS strain) nucleocapsids purified as described previously (4) were injected intraperitoneally three times at weekly intervals into BALB/c mice (about 200 μg of protein into each animal per injection). Complete Freund adjuvant was used in the first injection, and incomplete adjuvant was used in the two subsequent immunizations. Spleen and lymph node cells obtained 3 days after the last injection were fused to BALB/c NSI/1 myeloma cells (obtained indirectly with permission from C. Milstein), using polyethylene glycol 1500 (Fisher Scientific Co., Pittsburgh, Pa.) as described by Nowinski et al. (10). After fusion, the cell suspension was distributed into wells of MicroTest II plates (BBL Microbiology Systems, Cockeysville, Md.), and hybrid cells were selected and cloned as described previously (10). The presence of antibodies in the culture fluids was tested by the antibody-binding assay of Nowinski et al. (10). Briefly, purified nucleocapsids (2.5 μg of protein in 50 μl of 0.05 M sodium carbonate-bicarbonate buffer, pH 9.6) were adsorbed to each well of MicroTest II plates overnight at room temperature in a humidified chamber. The wells were then incubated with 5% bovine serum albumin in phosphate-buffered saline, pH 7.2, before testing for antibody. Culture fluids from cloned hybrid cell lines were then added, followed sequentially by rabbit anti-mouse immunoglobulin G, immunoglobulin A, and immunoglobulin M (Behring Diagnostics, Somerville, N.J.) and ^{125}I-labeled protein A (100,000 cpm in 50 μl of phosphate-buffered saline per well). During the intervals between

these incubations, the wells were washed with 1% bovine serum albumin in phosphate-buffered saline and finally with phosphate-buffered saline to remove unbound ^{125}I-labeled protein A. Immune reactions were detected on autoradiographs prepared by exposing the plates to Kodak NS-2T film and Kodak X-Omatic intensifying screens at −70°C for 24 h.

In the experiment for the isolation of cells producing anti-HSV-1 antibodies, only 1 out of 96 growing hybrid cell cultures produced such antibodies. In the HSV-2 experiment, 6 out of 117 growing cultures produced antiviral antibodies. The antibody-producing cultures were cloned, and immunodiffusion analysis revealed that one cell line produced immunoglobulin G1 HSV-1 antibodies and two cell lines synthesized immunoglobulin G1 HSV-2 antibodies. Two of these clones secreted antibodies which immunoprecipitated nucleocapsid polypeptides; therefore, these cultures were propagated further, and their antibodies were used in additional studies (see below).

The nucleocapsids of HSV-1 and HSV-2 contain five major and six or seven minor protein components (Fig. 1) (2, 4). The protein patterns of both types of nucleocapsids, demonstrated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis, are very similar. However, HSV-2 p40 and p45 (a minor protein of about 45,000 molecular weight) are slightly larger than their HSV-1 counterparts. The antibodies synthesized by clones 1D4 (anti-HSV-1) and 3E1 (anti-HSV-2) precipitated p40 and p45 from homologous antigen preparations, but precipitated no proteins from heterologous preparations (Fig. 1). This indicates that antibodies produced by each clone react with a type-specific antigenic determinant present on both p40 and p45. The type specificity of the monoclonal antibodies

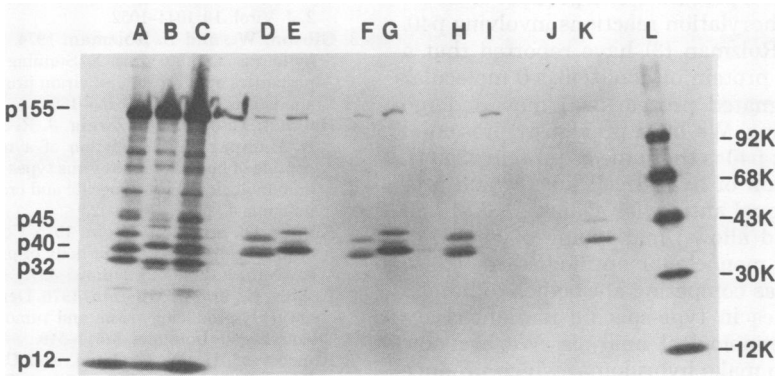

FIG. 1. *Sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis of nucleocapsid proteins immunoprecipitated by guinea pig antisera and mouse monoclonal antibodies. To analyze nucleocapsid polypeptides, we heated [^{35}S]methionine-labeled nucleocapsids prepared as described previously (11) at 100°C for 5 min in 1% sodium dodecyl sulfate–10% glycerol–2.5% β-mercaptoethanol–0.0625 M Tris-hydrochloride (pH 6.8)–0.002% bromophenol blue (4, 8). Immunoprecipitated proteins were analyzed by suspending [^{35}S]-methionine-labeled nucleocapsids in 0.5% sodium dodecyl sulfate–2.5% β-mercaptoethanol–0.05 M Tris-hydrochloride (pH 8) and heating them at 100°C for 5 min. The sample was diluted 10-fold with buffer A (0.1 M Tris-hydrochloride [pH 8], 10% glycerol, 0.5% Nonidet P-40, 0.5% sodium deoxycholate, 5 mM dithiothreitol, 0.2 mM phenylmethylsulfonyl fluoride), and portions (0.5 ml containing 150,000 cpm) were mixed with guinea pig antiserum (10 μl) (4) or culture fluid (25 μl) from a hybrid cell line. After incubation for 3 h at 4°C, 0.12 ml of a 33% (vol/vol) suspension of protein A–Sepharose CL-4B (Pharmacia Fine Chemicals, Inc., Piscataway, N.J.) in buffer A was added to each sample, and the suspension was gently mixed for 1 h at 4°C. The protein A-Sepharose beads were washed six times by centrifugation and suspension in 0.5 M LiCl–0.1 M Tris-hydrochloride (pH 8)–1% β-mercaptoethanol and, finally, in an equal volume of 2% sodium dodecyl sulfate–20% glycerol–5% β-mercaptoethanol–0.125 M Tris-hydrochloride (pH 6.8)–0.004% bromophenol blue. The preparation was heated at 100°C for 5 min, and the polypeptides were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis on a 5 to 20% polyacrylamide gel gradient (4, 8). The bands are shown on a fluorograph made on Kodak SB-5 X-ray film (1). Proteins of: (A) HSV-1 nucleocapsids; (B) HSV-2 nucleocapsids; and (C) a mixture of HSV-1 and HSV-2 nucleocapsids. Proteins precipitated by: (D, E) guinea pig anti-HSV-1 p40 serum; (F, G) guinea pig anti-HSV-2 p40 serum; (H, I) culture fluid from cell line 1D4; and (J, K) culture fluid from cell line 3E1. The antibodies were incubated with (D, F, H, J) HSV-1 and (E, G, I, K) HSV-2 nucleocapsid proteins. (L) methyl-^{14}C-labeled polypeptides (New England Nuclear Corp., Boston, Mass.) serving as molecular weight standards: phosphorylase B (92,000); bovine serum albumin (68,000); ovalbumin (43,000); carbonic anhydrase (30,000); cytochrome c (12,000). The presence of p155 in the immunoprecipitates was due to its insolubility, since it was also present when no immune serum was used (data not shown).*

was confirmed by reacting them with antigen preparations from three different HSV-1 strains (MAL, 14-012, and Miyama) and three different HSV-2 strains (MS, 333, and Savage) (data not shown). In contrast, guinea pig antisera prepared by immunizing animals with either HSV-1 or HSV-2 p40 (4) precipitated p40 and p45 from both homologous and heterologous nucleocapsid preparations, confirming that these sera contain antibodies which react with cross-reactive antigenic determinants. Control nonimmune guinea pig serum did not precipitate any nucleocapsid proteins (data not shown).

The results shown in Fig. 1 indicate that p40 and p45 of HSV-1 and HSV-2 possess similar antigenic determinants. The partial peptide maps of p40 and p45 are also very similar (Zweig et al., manuscript in preparation), which is consistent with the immunological data. The p45 protein may be an altered form of p40; i.e., it may represent a precursor or a product of cleavage or phosphorylation reactions involving p40. Gibson and Roizman (3) have reported that a nucleocapsid protein of about 40,000 molecular weight (designated protein 22a) may undergo such processing. We have previously presented evidence that p40 is the same as protein 22a (4).

The isolation of hybrid cell lines which produce monoclonal antibodies against HSV-1 and HSV-2 should allow rapid typing of virus isolates. These monoclonal antibodies may also prove useful as competing antibodies to human serum samples in type-specific immunoassays for seroepidemiological analysis. We are now attempting to make hybridomas which produce antibodies to the cross-reactive determinants of p40 and p45. Since guinea pig antisera against HSV-1 and HSV-2 p40 also react with SA8 virus (4) and bovine mammillitis virus antigens (Zweig et al., manuscript in preparation), monoclonal cross-reacting antibodies may be extremely useful in grouping herpesviruses into families and in mapping evolutionary conserved regions of p40 and other herpesvirus-specified polypeptides.

We are grateful to R. C. Nowinski and his associates of the Fred Hutchinson Cancer Research Center, Seattle, Wash., for demonstrating procedures for the isolation of antibody-producing hybrid cell lines. We thank M. Chakrabarty, L. Newman, and S. Showalter for their expert assistance.

This research was supported by Public Health Service contract N01-CO-75380 with the National Cancer Institute.

ADDENDUM IN PROOF

Ascites tumors in mice have been raised with clones 1D4 (anti-HSV-1) and 3E1 (anti-HSV-2), which secreted antibodies of titers higher than those found in culture fluids. The 1D4 fluids immunoprecipitated only HSV-1 p40 and p45 (titer, 1:64), whereas the 3E1 fluids titrated 1:256 against HSV-2 p40 and p45 and 1:4 against HSV-1 p40 and p45.

A recent report by Howes et al. (J. Gen. Virol. **44**: 81–87, 1979) confirms the feasibility of producing monoclonal antibodies against HSV antigens.

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