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Use of Monoclonal Antibodies Against Two 75,000-Molecular-Weight Glycoproteins Specified by Herpes Simplex Virus Type 2 in Glycoprotein Identification and Gene Mapping

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We produced two monoclonal antibodies that precipitate different glycoproteins of similar apparent molecular weight (70,000 to 80,000) from extracts of cells infected with herpes simplex virus type 2. Evidence is presented that one of these glycoproteins is the previously characterized glycoprotein gE, whereas the other maps to a region of the herpes simplex virus type 2 genome collinear with the region in herpes simplex virus type 1 DNA that encodes gC.

Balachandran et al. (1) recently described two type-specific monoclonal antibodies that precipitate two different herpes simplex virus type 2 (HSV-2) glycoproteins, the multiple electrophoretic forms of which range in apparent molecular weight from 50,000 (50K) to 80K. They suggested that one of these glycoproteins was the Fc-binding glycoprotein gE, based on the reported molecular weights for HSV-1 and HSV-2 gEs (3, 10, 17); they designated the other gF. While isolating monoclonal antibodies capable of binding to the surfaces of HSV-1 or HSV-2 virions (M. F. Para, R. Sprague, A. G. Noble, K. M. Zezulak, M. L. Parish, and P. G. Spear, manuscript in preparation), we also obtained antibodies that precipitate the two HSV-2 glycoproteins just described. Here we demonstrate that one of these monoclonal antibodies reacts with a type-specific determinant on the previously mapped and characterized HSV-2 glycoprotein gE (10, 17), whereas the other antibody reacts with a glycoprotein that is encoded by a gene located between 0.58 and 0.69 map units on the HSV-2 genome. The only HSV-1 glycoprotein known to be encoded in the same region of the HSV-1 genome is gC (8a, 12, 19). Consequently, the HSV-2 glycoprotein could be related either to HSV-1 gC or to an unknown product encoded by a gene closely linked to the gC gene.

Procedures used for the production of glycoprotein-specific monoclonal antibodies will be described in detail elsewhere (Para et al., manuscript in preparation). Briefly, BALB/c mice were immunized with purified UV-inactivated virions of strain HSV-1(HFEM)syn or HSV-2(G), and their spleen cells were fused with the myeloma cell line SP2/0-Ag14 (21). Hybridomas secreting antibodies capable of binding to the surfaces of purified virions were cloned and injected into mice for the production of ascites fluids.

The hybridoma antibodies chosen for this study were an anti-gC antibody designated II73 (from a mouse immunized with HSV-1) and two HSV-2-specific antibodies designated III188 and III347. Neither of these last two antibodies has neutralizing activity, and both belong to the immunoglobulin G2a subclass of immunoglobulin. Both antibodies reacted type-specifically with HSV-2 virions in the solid-phase binding assay used for screening hybridomas. Neither antibody precipitated polypeptides from cells infected with HSV-1 or from uninfected cells (data not shown), and both precipitated multiple glycosylated polypeptides from cells infected with HSV-2 (Fig. 1). Pulse-chase experiments to be published elsewhere demonstrated that the mature forms of both glycoproteins (about 74K in apparent molecular weight) derived from 68K precursors in infected HEp-2 cells. The lower-molecular-weight forms of 63K (III188) and 58K (III347) seen in Fig. 1 are probably catabolic products of the glycoproteins. Radioimmunocompetition experiments performed with extracts from pulse-labeled infected cells (data not shown) and experiments to be described verified that antibodies III188 and III347 react with different glycoproteins.

The results presented in Fig. 1 are similar to those reported by Balachandran et al. (1) for their antibodies designated 17αA2 and 17βC2, which were shown to precipitate different glyco-

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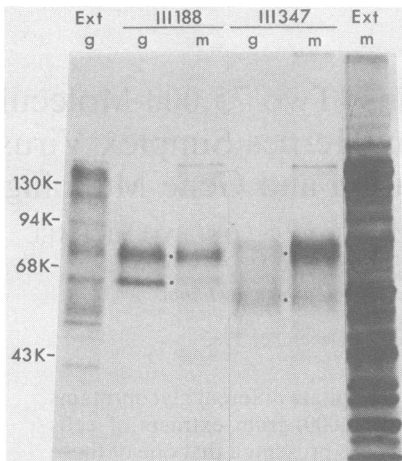

FIG. 1. Polypeptides precipitated by ascites fluids containing monoclonal antibodies III188 or III347 from extracts of HEp-2 cells infected with HSV-2(G) at 10 PFU per cell. The infected cells were maintained in medium 199 supplemented with 1% calf serum and were labeled by the addition at 2 h after infection of [^{14}C]glucosamine (g) to 5 μCi/ml or [^{35}S]methionine (m) to 5 μCi/ml (in medium containing 1/10 the usual concentration of methionine). At 20 h after infection, extracts were prepared from the infected cells for immunoprecipitation with the monoclonal antibodies (11), and the immunoprecipitates were analyzed by polyacrylamide gel electrophoresis (9). Polypeptides present in samples of the extracts (Ext) are shown for comparison along with the precipitated polypeptides identified by dots. Note that the polypeptides precipitated by antibody III188 were more heavily labeled with [^{14}C]glucosamine than with [^{35}S]methionine, whereas the converse is true for the polypeptides precipitated by III347. The molecular weight markers used were β-galactosidase (130K), phosphorylase b (94K), bovine serum albumin (68K), and ovalbumin (43K).

proteins by peptide mapping. As mentioned in a later publication by Balachandran et al. (2), we used monoclonal antibodies supplied by these authors in radioimmunocompetition experiments to show that the polypeptides precipitated by antibodies III188 and III347 were the same as those precipitated by 17αA2 and 17βC2, respectively (data not shown).

Evidence that the glycoprotein precipitated by antibody III347 is HSV-2 glycoprotein gE emerged from two kinds of experiments. First, pulse-labeled polypeptides precipitated by III347 and by an anti-gE rabbit serum were compared by Cleveland analysis (4). The rabbit antiserum was prepared against purified HSV-1 gE and has been shown previously to precipitate HSV-1 gE and to cross-react preferentially with newly synthesized forms of HSV-2 gE (16, 17). The peptides generated by proteolysis of the polypeptides precipitated by antibody III347 and the anti-gE serum were indistinguishable by electrophoretic analysis (Fig. 2). Second, analyses of the HSV-1 × HSV-2 recombinant viruses listed in Fig. 3 revealed that the only HSV-2 DNA sequence shared by all of the viruses which expressed the III347 determinant was that in the region of the S component known to encode gE (10, 11, 17). The use of III347 to detect a type-specific determinant in HSV-2 gE confirms previous results which suggested that HSV-1 and HSV-2 gEs were related but not identical in structure (10, 17).

Analyses of the recombinant viruses listed in Fig. 3 also permitted mapping of the HSV-2 gene responsible for expression of the III188 determi-

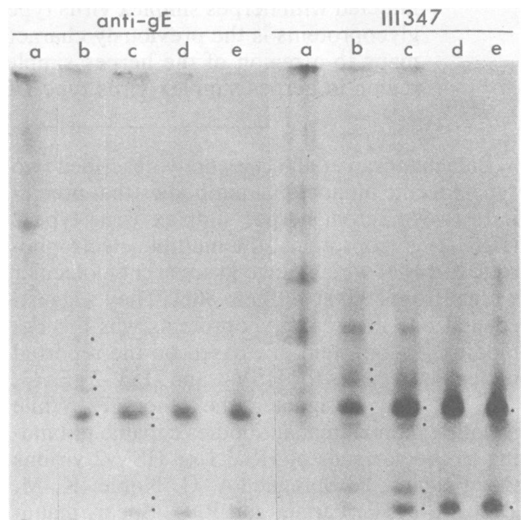

FIG. 2. Comparison of peptides obtained after partial proteolysis of the pulse-labeled polypeptides precipitated by anti-gE serum and by ascites fluid containing the III347 monoclonal antibody. HEp-2 cells infected with HSV-2(G) were pulse-labeled with [^{35}S]methionine (20 μCi/ml in methionine-free medium 199) for 7 min at 4 h after infection, and extracts were prepared immediately after the pulse. After preparative polyacrylamide gel electrophoresis of immunoprecipitates obtained from the extract, replicate gel slices containing the appropriate polypeptide were placed in the wells of an analytical 15% polyacrylamide gel, along with 0.8 (b), 4 (c), 20 (d), or 100 (e) μg of V8 protease from *Staphylococcus aureus* or no protease (a), for partial proteolysis and fractionation of the resulting peptides by the procedure of Cleveland et al. (4). The photograph is a composite prepared from two different autoradiograms of a single gel slab, the left half from an autoradiogram exposed for 1 month and the right half from one exposed for 2 weeks. The polypeptide precipitated by antibody III347 was partially proteolyzed even in the well to which no protease was added (a) because of leakage of protease from the adjacent well on the left. The dots identify peptides that appear to be characteristic degradation products of both polypeptides.

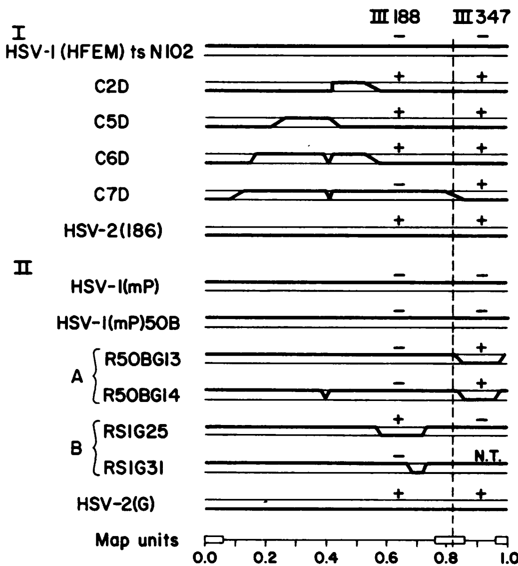

FIG. 3. Analysis of HSV-1 × HSV-2 recombinant viruses and parental strains for expression of polypeptides precipitable by the monoclonal antibodies III188 and III347. The recombinants in set I were isolated from cells mixedly infected with the parental strains indicated, and their genome structures were analyzed by Morse et al. (14). The recombinants in set IIA, isolated and analyzed with respect to genome structure by Tognon et al. (23), were obtained by marker rescue from cells transfected with mixtures of HSV-2(G) DNA fragments and intact DNA from the cold-sensitive mutant HSV-1(mP)50B. The recombinants in set IIB, isolated and analyzed with respect to genome structure by A. J. Conley and B. Roizman (manuscript in preparation), were obtained by marker rescue from cells transfected with mixtures of HSV-2(G) DNA fragments and intact DNA from the temperature-sensitive mutant HSV-1(mP)*ts*SB1. In recombinant RS1G25 the maximum extent of replacement of HSV-1 sequences by HSV-2 sequences is defined by the presence of the HSV-1 *Bgl*II-D to G site on the left and the absence of the HSV-2 *Bgl*II-I to H site on the right. The minimum left border is defined by the presence of the HSV-2 *Bgl*II-C to N site, and the minimum right border is defined by the presence of the HSV-2 *Eco*RI-L to H cleavage site. The maximum left border for RS1G31 is defined by the presence of the HSV-1 *Kpn*I-A to Y′ site, and the minimum left border is defined by the absence of the HSV-1 *Bgl*II-G to F site. The maximum right border is defined by the absence of the HSV-2 *Bgl*II-I to H cleavage site, and the minimum right border is defined by the presence of the HSV-2 *Eco*RI-L to H cleavage site. The upper and lower lines of each doublet in the figure represent HSV-1 and HSV-2 DNA sequences present in each recombinant virus as determined by the presence or absence of characteristic restriction endonuclease sites; the diagonal regions span the boundaries of the crossover sites. The vertical dashed line marks the boundary between the L and S components of the HSV genome, and the boxes on the map unit line represent the reiterated sequences of the L and S components. The + and − symbols summarize the results of immunoprecipitation experiments performed with each of the virus strains listed and indicate the presence or absence, respectively, of polypeptides precipitable by the monoclonal antibodies III188 and III347. Each column of symbols is positioned to indicate the location of DNA sequences which determine the expression of the appropriate antigenic determinant.

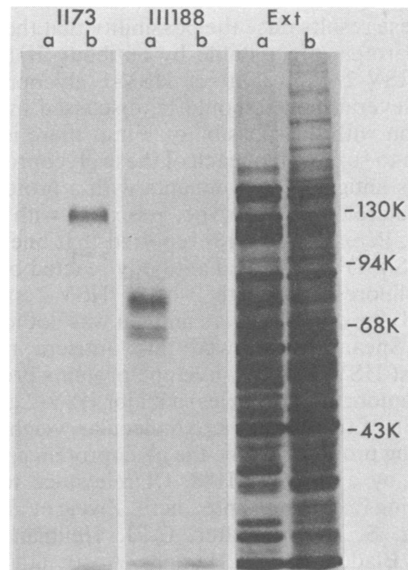

FIG. 4. Polypeptides precipitated by antibodies II73 (anti-HSV-1 gC) and III188 from extracts (Ext) of HEp-2 cells infected with the recombinant viruses RS1G25 (a) or RS1G31 (b). The extracts were prepared from cells infected and labeled as described in the legend to Fig. 1, except that the labeling medium contained [^{35}S]methionine at 25 μCi/ml and was present from 4 to 24 h after infection.

nant, to the region of the genome from 0.58 to 0.69 map units. Marsden et al. (13) previously mapped a 63K HSV-2 glycoprotein to the same region of the genome. This glycoprotein could be the same as the one precipitable by antibody III188.

The only HSV-1 glycoprotein known to be encoded between 0.58 and 0.69 map units on the HSV-1 genome is gC (19), the structural gene of which is located between 0.62 and 0.64 map units (8a, 12). Expression of HSV-1 gC by the recombinants shown in Fig. 3 is as predicted from these mapping results, except that none of the recombinants in Fig. 3, set I, make HSV-1 gC (19), probably because the HSV-1 parental strain is itself gC$^-$ (19). In particular, the recombinant RS1G25, which expressed the HSV-2 glycoprotein precipitable by antibody III188, failed to express HSV-1 gC, whereas recombinant RS1G31 produced HSV-1 gC but not the glycoprotein precipitable by III188 (Fig. 4).

These results raise the possibility that the 75K glycoprotein precipitable by antibody III188 is the HSV-2 equivalent of HSV-1 glycoprotein gC. Several points should be discussed in connection with this possibility. First, there is evidence to suggest that each of these glycoproteins shares antigenic determinants with a protein of the heterologous serotype, probably with each other. Pereira et al. (18) reported that one anti-gC(HSV-1) monoclonal antibody reacted by immunofluorescence with 2 of 67 HSV-2 strains tested; the cross-reactive antigen was not identified. Spear (22) showed that antisera raised against HSV-1 virion envelope proteins precipitate (among other species) a major HSV-2 glycoprotein of the appropriate molecular weight and labeling properties to be the glycoprotein precipitable by antibody III188. Of relevance to the mapping results presented here, Zweig et al. (M. Zweig, S. D. Showalter, C. J. Heilman, Jr., S. V. Bladen, and B. Hampar, Abstr. 6th Cold Spring Harbor Meet. on Herpesviruses, p. 176) recently reported the isolation of a monoclonal antibody that precipitates both a 75K HSV-2 glycoprotein and HSV-1 glycoprotein gC.

Second, although HSV-1 glycoprotein gC and the HSV-2 glycoprotein precipitable by antibody III188 may be genetically and antigenically related, they differ considerably in structure (and perhaps also in function), based on differences in their apparent sizes (Fig. 4) and antigenic determinants. It has been shown that type-specific antisera can be raised against purified HSV-1 gC (6, 7). In addition, monoclonal antibodies directed against either glycoprotein appear to be almost uniformly type-specific by at least some criteria (1, 2, 18, 20), with exceptions already mentioned.

Third, HSV-2 specifies another glycoprotein that was previously designated gC based on the similarity of its apparent molecular weight to that of HSV-1 gC (~130K); this HSV-2 glycoprotein was mapped to the region of the genome from 0.645 to 0.69 map units (19). It is of interest that the HSV-1 equivalent of this HSV-2 glycoprotein, should it exist, has never been identified and that HSV-1 gC differs so markedly from the HSV-2 glycoprotein precipitable by antibody III188 (the best candidate for the genetically related HSV-2 counterpart of HSV-1 gC). These observations suggest considerable genetic divergence between the genomes of HSV-1 and HSV-2 in the region encoding these glycoproteins, more than is evident from comparisons of other glycoproteins specified by HSV-1 and HSV-2 (2, 8, 10, 15, 17, 18, 20).

Finally, we suggest that the HSV-2 glycoproteins previously designated gC (19) and gF (1, 2) be considered unnamed until the related HSV-1 counterparts are identified or until it is established that none of the known HSV-1 glycoproteins is related. This suggestion is in accordance with conventions agreed upon by participants of Cold Spring Harbor Workshops and of the International Conference on Human Herpesviruses (5).

We thank B. Roizman for making available to us HSV-1 × HSV-2 recombinant viruses prior to publication of their isolation and properties, S. Bacchetti and W. Rawls for samples of the 17αA2 and 17βC2 monoclonal antibodies, E. K. Wagner for transmitting results prior to their publication, and M. Zweig for permission to cite the abstract of results presented at a recent meeting.

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