INFECTION AND IMMUNITY, Dec. 1981, p. 684-692
0019-9567/81/120684-09$02.00/0

Vol. 34, No. 3

Monoclonal Antibodies to Herpes Simplex Virus Type 1 Proteins, Including the Immediate-Early Protein ICP 4

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Received 9 April 1981/Accepted 10 August 1981

Monoclonal antibodies were prepared against herpes simplex virus type 1 (strain 14012) by two immunization procedures. Procedure A utilized infectious virus propagated in mouse cells, and procedure B utilized mouse cells infected with herpes simplex virus in the presence of cycloheximide and harvested 1 h after removal of the inhibitor. A total of 52 monoclonal antibodies were obtained against 10 herpes simplex virus proteins, including four glycosylated proteins (a 110,000-molecular-weight protein, gB, gC, and gD) and six nonglycosylated proteins (a 68,000-molecular-weight protein, ICP 9, ICP 8, ICP 6, ICP 5, and the immediate-early ICP 4). The antibodies were assayed against herpes simplex virus types 1 and 2 by sodium dodecyl sulfate-polyacrylamide gel electrophoresis of radioimmunoprecipitates, immunofluorescence, and neutralization. Using the reagents prepared, we concluded that the 110,000-molecular-weight protein, gD, ICP 9, ICP 9, ICP 6, and the 68,000-molecular-weight protein express both type-specific and cross-reactive antigenic determinants. In contrast, nine antibodies against gB all cross-reacted with herpes simplex virus type 2, whereas eight antibodies to gC all reacted type specifically.

Herpes simplex virus (HSV) types 1 (HSV-1) and 2 (HSV-2) each code for approximately 50 proteins (reviewed in reference 16). Although the majority of these proteins share common antigenic determinants, it has been possible, using experimentally produced monospecific antisera and sensitive radioimmunoassay techniques, to show that some HSV-1- and HSV-2-coded proteins express both cross-reactive and type-specific antigenic determinants (7). Studies to define specific antigenic sites on individual proteins have been facilitated by the development of procedures for producing monoclonal antibodies (10). Monoclonal antibodies against HSV-coded proteins have been described and have been employed for detecting cross-reactive and type-specific determinants on individual nonglycosylated (18) and glycosylated (14) polypeptides. Monoclonal antibodies have also been used to detect determinants that are shared between different classes of HSV proteins (17), indicating heretofore unrecognized relationships between distinct protein classes. Obviously, an expansion of the present collection of monoclonal antibodies against HSV would greatly facilitate the identification and characterization of additional viral proteins.

We describe here a battery of 52 monoclonal antibodies against 10 different HSV-1 proteins. Included are monoclonal antibodies against a major immediate-early protein and a variety of glycosylated and nonglycosylated proteins.

MATERIALS AND METHODS

Immunization of Mice. Two procedures were employed for immunization of mice.

(i) Procedure A. HSV-1 (strain 14012) was propagated in mouse 10E2 cells and assayed in Vero cells (6). Virus was released from infected cell pellets by resuspension in phosphate-buffered saline (PBS) and sonication. The virus suspension was clarified, and 0.5 ml (10^8 plaque-forming units per ml in Vero cells) was inoculated subcutaneously at 7-day intervals over a 14-day period in 8-week-old BALB/c mice (Microbiological Associates). Serum samples obtained by orbital bleeding were tested for antibody production by polyacrylamide gel electrophoresis of radioimmunoprecipitates (RIP-PAGE; see below). Three days after the last immunization, three to five animals showing a good antibody response were sacrificed, and their spleens were removed for hybridization.

(ii) Procedure B. Mouse 10E2 cells (6) were infected with strain 14012 at a multiplicity of infection of 20 (determined by virus titration in Vero cells) in the presence of cycloheximide (50 $\mu g\ ml^{-1}$) (3). The cells were incubated on a rocker platform at 36°C for 5 h, washed five times, refed with growth medium (Eagle minimal essential medium containing 10% heat-inactivated fetal calf serum), incubated for 1 h, dislodged by trypsin-ethylenediaminetetraacetate, pelleted, resuspended in PBS (6×10^7 cells in 1.5 ml of PBS), and sonicated. The cell suspension was clarified, and the supernatant was mixed with an equal volume of Freund complete adjuvant for intraperitoneal inoculation of BALB/c mice. The procedure was repeated after 7 days with incomplete adjuvant and subcutaneous inoculation. A final subcutaneous injection without adjuvant was given after an additional 7

days. Spleens were harvested 3 days later from antibody-producing animals.

Hybridization, selection, and propagation. The fusion procedure was a modification of that described by Nowinski et al. (13). Spleens removed from virus-immunized mice were minced, passed through sterile gauze, placed in a 50-ml conical centrifuge tube, and washed three times in cold serum-free medium by centrifugation at 200 × *g* for 5 min. The spleen cells were counted and mixed with actively growing P3-NSI/1-Ag4-1 (NSI/1) (10) cells at a 5:1 ratio. The cell mixtures were pelleted at 200 × *g* for 5 min and were gently resuspended during a 1-min period in 1 ml of 50% polyethylene glycol (PEG 1500; Fisher Scientific Co.) per 1.6×10^8 lymphocytes. After 2 min, 2 ml of RPMI-1640 containing 15% fetal calf serum was added dropwise with gentle mixing over a period of 2 min. At 5 min after the addition of the PEG 1500, 10 ml of additional medium was added rapidly, and the cells were pelleted at 200 × *g* for 5 min. The supernatant fluids were aspirated, the pellets were resuspended very gently in 22 ml of medium, and the cells were apportioned (~100 μl/well) into 96-well tissue culture plates (Costar). One day later, 100 μl of RPMI-1640 with 15% fetal calf serum containing hypoxanthine-aminopterin-thymidine was added. On days 2, 3, 4, 7, and 10 after hybridization, approximately 100 μl of medium was removed from each well and was replaced with 100 μl of medium containing hypoxanthine-aminopterin-thymidine. On day 14 and at 3- to 4-day intervals thereafter until 1 month after hybridization, the cells were refed with medium containing hypoxanthine-thymidine. Thereafter, cells were maintained in medium without hypoxanthine-thymidine. Beginning about day 10, the clarified medium from wells showing cell growth was tested by RIP-PAGE for antibody activity. The cells from positive wells were then transferred to 24-well plates and were later cloned by limiting dilution in 96-well plates with feeder layers of compatible thymocytes.

Clones producing desired antibodies were passaged in ascites form by intraperitoneal injection of 2×10^6 to 5×10^6 cells into BALB/c mice primed 2 weeks previously with 0.5 ml of pristane (2,6,10,14-tetramethyl pentadecane; Aldrich Chemical Co.) which had been injected intraperitoneally. The resulting fluids were harvested, clarified, and tested.

Virus neutralization. Ascites fluids were tested for neutralizing activity against HSV-1 (strain 14012) and HSV-2 (strain 333). The reaction mixtures containing 0.5 ml of virus (10^3 plaque-forming units) plus 0.5 ml of diluted ascites fluid plus 40 μl of fresh or heat-inactivated guinea pig complement were placed at 34°C for 30 min. Fresh medium (Eagle minimal essential medium plus 5% fetal calf serum) was added to bring the volume to 10 ml, and 1-ml aliquots were inoculated on Vero cell monolayers in 60-mm petri dishes. The infected cells were incubated for 2 h at 37°C, and the inocula were removed and replaced with medium containing methylcellulose. After 3 to 4 days, the cells were fixed and stained, and plaques were counted. The antibody titer was determined as that dilution of ascites fluid which reduced plaque numbers by 50% or more.

Immunofluorescence. Vero cells or 10E2 cells grown on cover slips were infected with either HSV-1 (strain 14012) or HSV-2 (strain 333) at a multiplicity of infection of 0.001 to 10. After 24 to 48 h, when discreet plaques were evident, the cover slips were washed in PBS, and the cells were air dried at room temperature, fixed for 3 to 4 min in acetone, and dried at room temperature. Ascites fluids diluted in PBS were added to the cells, which were then placed in a humidified chamber at room temperature for 45 min. After the cells were washed several times in PBS, fluorescein isothiocyanate-conjugated goat anti-mouse immunoglobulins (Cappel Laboratories) were added, and the cells were incubated in a humidified chamber for 45 min. The cells were washed in PBS, counterstained with Evans blue, and mounted on slides with elvanol for ultraviolet microscopy.

RIP-PAGE. 10E2, Vero, or HEp-2 cells were infected with HSV-1 (strain 14012, Miyama, MP, or MAL) or HSV-2 (strain 333, MS, or Savage) at a multiplicity of infection of 5. After incubation of the cells for 1 h at 37°C, the inocula were removed, and the cells were radiolabeled for 8 h with either 100 μCi of [^{35}S]methionine (800 to 1,200 Ci/mmol; Amersham Corp.) per ml in methionine-free Eagle minimal essential medium containing 5% dialyzed, heat-inactivated fetal calf serum or 50 Ci of D-[2-^{3}H]mannose (16 Ci/mmol; Amersham) per ml in Eagle minimal essential medium containing 20% the normal concentration of glucose and 5% heat-inactivated fetal calf serum. The cell monolayers were washed three times with cold tris(hydroxymethyl)aminomethane-buffered saline (pH 7.2) and were then mixed for 1 h at 4°C in extraction buffer [0.1 M tris(hydroxymethyl)aminomethane-hydrochloride (pH 8.0), 10% (vol/vol) glycerol, 0.5% Nonidet P-40, 0.5% sodium deoxycholate, 0.2 mM phenylmethylsulfonyl fluoride]. After clarification by centrifugation at 60,000 × *g* for 1 h, the extracts were incubated with ascites fluids and subsequently with protein A-Sepharose CL-4B (Pharmacia Fine Chemicals, Inc.) as previously described (17, 18). The immunoprecipitated proteins were separated by electrophoresis on a 5 to 20% polyacrylamide gradient containing sodium dodecyl sulfate (7, 11), and autoradiographs or fluorographs were prepared on Kodak SB-5 X-ray film as described previously (18).

To test antibody activity against immediate-early proteins, cells were infected at a multiplicity of infection of 20 in the presence of 50 μg of cycloheximide per ml. After incubation for 5 h, the cells were washed five times, refed with medium, incubated for 1 h, and labeled as above for 1 h.

Determination of immunoglobulin class. The immunoglobulin class and subclass were determined by double diffusion using agar gel plates (Hyland Diagnostics) and rabbit antisera to specific mouse immunoglobulins (Miles Laboratories). Test samples (8 μl) and typing reagents (8 μl) were added to adjacent wells and allowed to diffuse overnight at room temperature in a humidified chamber.

RESULTS

After cell fusion and seeding of microtiter plates, the fluids from wells showing viable hybrids of spleen and NSI/1 cells were harvested

and tested for antibody activity by immunoprecipitation of [^{35}S]methionine-labeled, HSV-1-infected cell extracts and RIP-PAGE. Hybrids showing positive antibody activity were expanded, cloned, and retested. One positive clone from each group was then passaged in mice in an ascites form. The final results were limited to hybrids which had been serially passaged in mice as ascites tumors with continuing production of antibody. A summary of the monoclonal antibodies so obtained with immunization procedures A and B described above is shown in Table 1. The tentative designation of the proteins precipitated by each antibody is given according to the classifications proposed by Spear (15) and Honess and Roizman (9) and is based on the apparent molecular weights we observed by RIP-PAGE. Antibodies which precipitated proteins which could be labeled with either [^{35}S]methionine or [^{3}H]mannose (data not shown) were classified as being directed against glycosylated proteins, whereas those which precipitated proteins which could only be labeled with [^{35}S]methionine were classified as being directed against nonglycosylated proteins. Antibodies were classified as cross-reactive or type specific based on their ability to immunoprecipitate proteins from four HSV-1 strains and three HSV-2 strains. Antibodies designated 1S-45S were isolated by immunization procedure A, whereas those designated 46S-67S were isolated by procedure B.

TABLE 1. *Monoclonal antibodies isolated with different immunization procedures*[a]

Protein MW	Glycosylated	Tentative designation[b]	No. of antibodies isolated by procedure:	
			A	B
59,000	+	gD	6	4
68,000	−	?	1	4
110,000	+	?	1	3
120,000	+	gB	8	1
125,000	−	ICP 9	7	1
130,000	+	gC	8	0
132,000	−	ICP 8	1	0
140,000	−	ICP 6	1	4
155,000	−	ICP 5	0	1
175,000	−	ICP 4	0	1

[a] Procedures are described in the text.
[b] Designation according to the classification in Spear (15) or in Honess and Roizman (9).

Properties of monoclonal antibodies against glycosylated proteins. The following glycosylated proteins with indicated molecular weights (MW) were precipitated by monoclonal antibodies.

(i) 59,000 MW (gD). Ten monoclonal antibodies were obtained against a 59,000-MW glycoprotein whose designation as gD was confirmed by G. Cohen (personal communication) (Table 2). The electrophoretic pattern of the immunoprecipitated proteins (Fig. 1, lane C) consisted of a broad major band (gD) at an apparent MW of 58,000 and a sharp minor band at an apparent MW of 49,000 (1, 5, 15). Based on RIP-PAGE, fluorescent antibody (FA; cytoplasmic staining), and neutralization data, the 10 antibodies fell into five groups depending on their reactivity with HSV-1 and -2. Group 1 (4S, 50S) precipitated proteins from both HSV-1- and HSV-2 (data not shown)-infected cell extracts, reacted by FA with HSV-1- and HSV-2-infected cells, and neutralized both viruses in the presence or absence of complement. Group 2 (12S) showed cross-reactivity by RIP-PAGE and FA, but only neutralized HSV-1. Group 3 (11S) showed cross-reactivity by RIP-PAGE

TABLE 2. *Properties of monoclonal antibodies to the 59,000-MW glycoprotein (gD)*

Group	Monoclonal antibody	Immunoglobulin type	RIP-PAGE (HSV-1/HSV-2)	FA (acetone fixed)		Neutralization			
						No complement		Complement	
				HSV-1	HSV-2	HSV-1	HSV-2	HSV-1	HSV-2
1	4S	G2a	+/+	5,120	2,560	25,600	25,600	25,600	25,600
	50S	G2a	+/+	1,280	40	1,600	100	3,200	200
2	12S	G1	+/+	5,120	5,120	3,200	<25	6,400	<25
3	11S	G2a	+/+	640	640	<25	<25	800	100
4	1S	G2a	+/−	2,560	<20	800	<25	1,600	<25
	41S	G2a	+/−	2,560	<20	1,600	<25	3,200	<25
	45S	G2a	+/−	>1,280	<20	25	<25	200	<25
5	47S	G1	+/−	>1,280	<20	<25	<25	<25	<25
	55S	G2a	+/−	>5,120	<20	<25	<25	<25	<25
	57S	G2a	+/−	>2,560	<20	<25	<25	<25	<25

and FA, but neutralization with both viruses was observed only in the presence of complement. Group 4 (1S, 41S, 45S) reacted type specifically by RIP-PAGE, FA, and neutralization. Group 5 (47S, 55S, 57S) reacted type specifically by RIP-PAGE and FA, but failed to neutralize either virus in the presence or absence of complement.

(ii) 110,000 MW. Four monoclonal antibodies were obtained against a 110,000-MW glycoprotein which remains unclassified (Table 3) (15). These antibodies precipitated proteins from infected cell extracts which migrated in sodium dodecyl sulfate gels as a closely spaced band doublet (Fig. 1, lane D). The antibodies fell into two groups: group 1 (53S) showed cross-reactivity between HSV-1 and -2 by RIP-PAGE and FA (cytoplasmic staining) and type specificity by neutralization; group 2 (37S, 46S, 52S) showed type specificity by RIP-PAGE, FA, and neutralization.

(iii) 120,000 MW (gB). Nine monoclonal antibodies were obtained against a 120,000-MW glycoprotein, whose designation as gB (15) was confirmed by G. Cohen (personal communication) (Table 4). With labeled 10E2 or Vero cells, these antibodies precipitated several protein bands in addition to the major bands at 120,000 MW (data not shown). These additional bands, which were identified as degradation products (manuscript in preparation), were not seen in precipitates from labeled HEp-2-infected cell extracts (Fig. 1, lane A). The antibodies reacting with gB fell into three groups: group 1 (3S) showed cross-reactivity by RIP-PAGE, FA (cytoplasmic staining), and neutralization; group 2 (33S, 40S, 61S) showed cross-reactivity by RIP-PAGE and FA, with neutralizing activity at low levels only being observed against HSV-1 in the presence of complement; group 3 (21S, 24S, 25S, 30S, 35S) showed cross-reactivity by RIP-PAGE and FA, but no neutralizing activity.

(iv) 130,000 MW (gC). Eight monoclonal

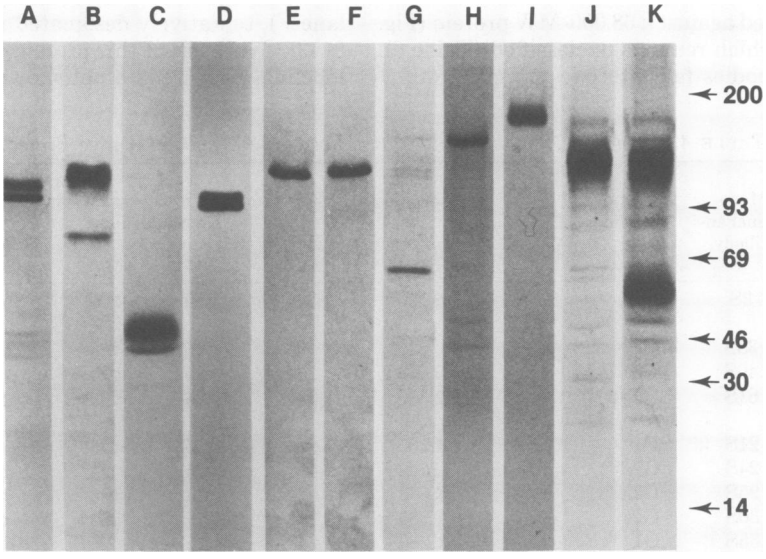

FIG. 1. *RIP-PAGE of HSV-1-infected cell extracts labeled with [^{35}S]methionine and reacted with monoclonal antibodies. The procedures were as described in the text with either mouse 10E2 cells (lanes B through K) or HEp-2 cells (lane A). Lanes: A, gB; B, gC; C, gD; D, 110,000 MW protein; E, ICP 9; F, ICP 8; G, 68,000-MW protein; H, ICP 5; I, ICP 4 (cyclohexmide-treated cells); J and K, ICP 6.*

TABLE 3. *Properties of monoclonal antibodies to the 110,000-MW glycoprotein*

Group	Monoclonal antibody	Immunoglobulin type	RIP-PAGE (HSV-1/HSV-2)	FA (acetone fixed)		Neutralization			
						No complement		Complement	
				HSV-1	HSV-2	HSV-1	HSV-2	HSV-1	HSV-2
1	53S	G2a	+/+	5,120	2,560	200	<25	200	<25
	37S	G2a	+/−	160	<20	25	<25	25	<25
	46S	G2a	+/−	640	<20	50	<25	50	<25
2	52S	G2a	+/−	640	<20	800	<25	800	<25

antibodies were obtained against a 130,000-MW glycoprotein which we have classified as gC (Table 5). Two proteins were precipitated by these antibodies (Fig. 1, lane B); one which produced a band (gC) at approximately 130,000 MW and one which produced a sharp band at about 90,000 MW. As reported by others (15), gC was not produced in cells infected with the MP strain of HSV-1 (data not shown). The antibodies to gC fell into one group showing type specificity by RIP-PAGE and FA (cytoplasmic staining). Neutralizing activity at relatively high titers was observed against HSV-1 in the presence of complement, but little or no activity was observed in the absence of complement.

Properties of monoclonal antibodies against nonglycosylated proteins. The following nonglycosylated proteins with indicated MW were precipitated by monoclonal antibodies. None of these antibodies neutralized HSV-1 or -2 either in the presence or absence of complement.

(i) 68,000 MW. Five monoclonal antibodies were obtained against a 68,000-MW protein (Fig. 1, lane G) which remains unclassified (Table 6). These antibodies fell into two groups: group 1 (49S, 62S) showed cross-reactivity by RIP-PAGE and FA (cytoplasmic staining); group 2 (43S, 54S, 67S) was type specific.

(ii) 125,000 MW (ICP 9). Eight monoclonal antibodies were obtained against a 125,000-MW protein (Fig. 1, lane E) tentatively classified as ICP 9 (Table 7) (9). Pulse-labeling studies in HSV-infected Vero and 10E2 cells (data not shown) indicated that this protein was first synthesized at 3 to 4 h postinfection, and its synthesis continued throughout the infectious cycle. On this basis, the protein was classified as a late (γ) protein (9). To insure that these antibodies were precipitating the same protein, peptide maps were prepared after partial digestion with V8 protease (data not shown). The peptide maps were identical in each case. The antibodies to ICP 9 fell into two groups: group 1 (65S) showed cross-reactivity by RIP-PAGE and FA (cytoplasmic staining); group 2 showed type specificity.

(iii) 132,000 MW (ICP 8). One antibody was obtained against a 132,000-MW protein (Fig. 1, lane F), tentatively designated as ICP 8 (Table 8) (9). Synthesis of this protein, based on pulse-labeling studies (data not shown), was initiated

TABLE 4. *Properties of monoclonal antibodies to the 120,000-MW glycoprotein (gB)*

Group	Monoclonal antibody	Immunoglobulin type	RIP-PAGE (HSV-1/HSV-2)	FA (acetone fixed)		Neutralization			
						No complement		Complement	
				HSV-1	HSV-2	HSV-1	HSV-2	HSV-1	HSV2
1	3S	G2a	+/+	5,120	1,280	800	200	3,200	1,600
2	33S	G1	+/+	640	160	<25	<25	50	<25
	40S	G1	+/+	2,560	160	<25	<25	25	<25
	61S	G2b	+/+	2,560	1,280	<25	<25	100	25
3	21S	G1	+/+	1,280	1,280	<25	<25	<25	<25
	24S	G2a	+/+	2,560	2,560	<25	<25	<25	<25
	25S	G2a	+/+	2,560	2,560	<25	<25	<25	<25
	30S	G2a	+/+	1,280	1,280	<25	<25	<25	<25
	35S	G1	+/+	1,280	1,280	<25	<25	<25	<25

TABLE 5. *Properties of monoclonal antibodies to the 130,000-MW glycoprotein (gC)*

Monoclonal antibody	Immunoglobulin type	RIP-PAGE (HSV-1/HSV-2)	FA (acetone fixed)		Neutralization			
					No complement		Complement	
			HSV-1	HSV-2	HSV-1	HSV-2	HSV-1	HSV-2
5S	G2a	+/−	5,120	<20	<25	<25	800	<25
17S	G1	+/−	10,240	<20	<25	<25	1,600	<25
18S	G1	+/−	1,280	<20	<25	<25	800	<25
19S	G2a	+/−	10,240	<20	25	<25	1,600	<25
26S	G2a	+/−	10,240	<20	<25	<25	800	<25
27S	G2a	+/−	10,240	<20	25	<25	800	<25
29S	G2a	+/−	10,240	<20	<25	<25	800	<25
31S	G2a	+/−	5,120	<20	<25	<25	800	<25

TABLE 6. *Properties of monoclonal antibodies to the 68,000-MW protein*

Group	Monoclonal antibody	Immunoglobulin type	RIP-PAGE (HSV-1/HSV-2)	FA (acetone fixed)		Neutralization			
						No complement		Complement	
				HSV-1	HSV-2	HSV-1	HSV-2	HSV-1	HSV-2
1	49S	G1	+/+	640	40	<25	<25	<25	<25
	62S	G1	+/+	160	20	<25	<25	<25	<25
2	43S	G1	+/−	160	<20	<25	<25	<25	<25
	54S	G1	+/−	160	<20	<25	<25	<25	<25
	67S	G1	+/−	80	<20	<25	<25	<25	<25

TABLE 7. *Properties of monoclonal antibodies to the 125,000-MW protein (ICP 9)*

Group	Monoclonal antibody	Immunoglobulin type	RIP-PAGE (HSV-1/HSV-2)	FA (acetone fixed)		Neutralization			
						No complement		Complement	
				HSV-1	HSV-2	HSV-1	HSV-2	HSV-1	HSV-2
1	65S	G1	+/+	320	320	<25	<25	<25	<25
2	6S,	G2a	+/−	1,280	<20	<25	<25	<25	<25
	13S,	G1	+/−	1,280	<20	<25	<25	<25	<25
	15S,	G2a	+/−	1,280	<20	<25	<25	<25	<25
	20S,	G2a	+/−	2,560	<20	<25	<25	<25	<25
	22S,	G2a	+/−	2,560	<20	<25	<25	<25	<25
	23S,	G2a	+/−	640	<20	<25	<25	<25	<25
	36S	G2a	+/−	640	<20	<25	<25	<25	<25

TABLE 8. *Properties of monoclonal antibodies to 132,000-MW (ICP 8), 140,000-MW (ICP 6), 155,000-MW (ICP 5), and 175,000-MW (ICP 4) proteins*

MW	Group	Monoclonal antibody	Immunoglobulin type	RIP-PAGE (HSV-1/HSV-2)	FA (acetone fixed)		Neutralization			
							No complement		Complement	
					HSV-1	HSV-2	HSV-1	HSV-2	HSV-1	HSV-2
132,000		39S	G2a	+/−	640	<20	<25	<25	<25	<25
140,000	1	48S,	G2a	+/+	320	320	<25	<25	<25	<25
		51S	G2b	+/+	640	640	<25	<25	<25	<25
	2	2S	G2a	+/−	2,560	<20	<25	<25	<25	<25
		59S	G2a	+/−	>1,280	<20	<25	<25	<25	<25
		66S	G3	+/−	5,120	<20	<25	<25	<25	<25
155,000		56S	G1	+/+	640	640	<25	<25	<25	<25
175,000		58S	G2a	+/−	320	<20	<25	<25	<25	<25

at 2 h postinfection, peaked between 4 and 5 h postinfection, and then declined. This is characteristic of an early (β) protein (9). This antibody reacted type specifically by RIP-PAGE and FA (nuclear staining).

(iv) 140,000 MW (ICP 6). Six antibodies were obtained against a 140,000-MW protein (Fig. 1, lanes J and K) tenatively designated ICP 6 (Table 8) (9). Pulse-labeling studies (data not shown) indicated that this was an early (β) protein (9). Additional studies with ^{32}P indicated that this protein was phosphorylated (data not shown). The broadness of this protein band in sodium dodecyl sulfate gels may be due to heterogeneity in the degree of phosphorylation. These antibodies fell into two groups: group 1 (48S, 51S) showed cross-reactivity by RIP-PAGE and FA (cytoplasmic staining); group 2 (2S, 59S, 66S) showed type specificity. One antibody (66S) and all of its subclones precipitated a 65,000-MW protein in addition to the 140,000-MW protein (Fig. 1, lane K). The remaining antibodies in groups 1 and 2 only showed precipitation of the 140,000-MW protein (Fig. 1, lane J). Peptide mapping studies showed that these antibodies all precipitated the same 140,000-MW

protein (data not shown).

(v) 155,000 MW (ICP 5). One antibody was obtained against the 155,000-MW major capsid protein (Fig. 1, lane H) designated ICP 5 (Table 8) (9). This antibody showed cross-reactivity by RIP-PAGE and FA (nuclear staining).

(vi) 175,000 MW (ICP 4). One antibody was obtained against the 175,000-MW immediate-early protein (Fig. 1, lane I) designated ICP 4 (Table 8) (3, 9). This antibody showed type specificity by RIP-PAGE and FA (nuclear staining). The 175,000-MW protein, in contrast to all of the other proteins to which monoclonal antibodies were obtained, showed enhanced synthesis in cells labeled after treatment with cycloheximide.

DISCUSSION

Monoclonal antibodies were prepared against four glycosylated and six nonglycosylated proteins of HSV-1, including the 175,000-MW immediate-early protein ICP 4 (Table 1). Antibodies to three of the four glycosylated proteins were identified as reacting with gD, gC, and gB, whereas the remaining antibodies reacted with an unclassified 110,000-MW glycoprotein. gC was the only glycosylated protein that was not detected in cells infected with the MP strain of HSV-1 by RIP-PAGE or FA (8, 15). Using these monoclonal reagents, we concluded that gD (Table 2), gA (Table 3), an unclassified 68,000-MW protein (Table 6), ICP 9 (Table 7), and ICP 6 (Table 8) express both type-specific and cross-reactive antigenic determinants. In contrast, the nine antibodies against gB (Table 4) all showed cross-reactivity between HSV-1 and -2, whereas the eight antibodies against gC (Table 5) all reacted type specifically. These findings are suggestive, but not conclusive, that gB expresses only cross-reactive determinants and gC expresses only type-specific determinants (discussed in reference 12).

The monoclonal antibodies against individual proteins were divided into groups based on their reactivity by RIP-PAGE, FA, and neutralization with HSV-1 and -2. Since the tests employed measured only a limited number of properties, it is likely that additional groups will be identified as new tests are applied. For example, the 4S and 50S antibodies to gD (Table 2) were classified in one group. The fact that the 50S antibodies showed much less cross-reactivity than did the 4S antibody, however, suggests that these two antibodies react with different antigenic sites. Similarly, the 66S antibody to ICP 6 (Table 8) was classified in the same group as the 2S and 59S antibodies based on its type-specific reactivity by RIP-PAGE and FA. The fact that the 66S antibody precipitated a 65,000-MW protein in addition to the 140,000-MW ICP 6 (Fig. 1, lane K), whereas the 2S and 59S antibodies precipitated only the ICP 6 (Fig. 1, lane J), suggests that the 66S antibody reacts with different antigenic sites than the 2S and 59S antibodies.

Two procedures were employed for immunizing animals, neither of which used denatured antigens. Procedure A employed intact virus, whereas procedure B employed cycloheximide-treated cells. In several cases, monoclonal antibodies to the same protein were obtained with either procedure. The exceptions were antibodies to gC which only were isolated with procedure A, and antibodies to ICP 5 and ICP 4 which only were isolated with procedure B. The fact that antibodies to several late proteins were isolated by procedure B does not necessarily imply that these proteins were synthesized de novo during the 1-h interval after removal of the cycloheximide. It is more likely that many of the antigens which elicited antibodies with procedure B were remnants of residual virions or antigenic material adsorbed to the cell surface.

Certain properties of monoclonal antibodies against individual proteins are worthy of emphasis.

59,000 MW (gD). Five antibody groups were identified to gD (Table 2). Group 1 (4S, 50S) showed cross-reactivity between HSV-1 and -2 and was similar to monoclonal antibodies HD1 and HD2 described by Pereira et al. (14). Group 2 (12S), which was similar to monoclonal antibody HD3 described by Pereira et al. (14), showed cross-reactivity by RIP-PAGE and FA and type specificity by neutralization. The remaining three groups expressed properties not previously described. Consequently, using this battery of monoclonal antibodies, we could distinguish at least five distinct antigenic sites on gD.

110,000 MW and 120,000 MW (gB). Some uncertainty exists as to whether gA and gB are distinct proteins or closely related proteins (4). Antisera prepared by conventional immunization procedures with the gA-gB complex or with gA and gB alone react with both proteins (4, 12). If the 110,000-MW glycoprotein that was immunoprecipitated by the monoclonal antibodies 53S, 37S, 46S, and 52S (Table 3) was gA, our results suggest that gA and gB are distinct proteins, each of which migrates in sodium dodecyl sulfate gels as a closely spaced band doublet. It is possible, however, that the glycoproteins designated by Spear (15) as gA and gB are equivalent to the doublet of bands precipitated by our anti-gB antibodies (Fig. 1, lane A). If this were

the case, gA and gB could be immunologically related, and our antibodies against the 110,000-MW protein unclassified could be precipitating a previously unclassified glycoprotein. Preliminary analysis indicated that the peptide maps of immunoprecipitated 110,000-MW protein and gB are also distinct, and that neither protein can remove antibody activity to the other (manuscript in preparation). It is of interest, although possibly fortuitous, that three of the four antibodies to the 110,000-MW protein were isolated with procedure B, whereas eight of the nine antibodies to gB were isolated with procedure A.

Another distinction between these two glycosylated proteins is that gB showed evidence of partial degradation in mouse and Vero cells, but not in HEp-2 cells. On the other hand, the band patterns in the sodium dodecyl sulfate gels for the 110,000-MW protein were identical in these three cell lines. The phenomenon of degradation of a specific protein occurring in one cell but not in another is not restricted to gB (unpublished observations) and is readily amenable to study with monoclonal antibodies.

125,000 MW (gC). The eight monoclonal antibodies to gC (Table 5) were all isolated with immunization procedure A, and all were type specific. Interestingly, significant neutralization of HSV-1 by the eight antibodies was seen only when complement was employed. Pereira et al. (14) described two monoclonal antibodies to gC (HC1 and HC2) which neutralized HSV-1 (except strain MP) in the absence of complement. Whether the specificities of any of our antibodies are equivalent to HC1 or HC2 has not been determined.

Nonglycosylated proteins. Monoclonal antibodies were isolated to six nonglycosylated proteins. Antibodies to three proteins (ICP 4, 5, and 8) showed only nuclear staining by FA, whereas the remaining three showed predominantly cytoplasmic staining. The monoclonal antibody to ICP 4 was of particular interest, since ICP 4 is an immediate-early protein whose synthesis is enhanced in cells pretreated with cycloheximide (3). This reagent should prove particularly useful for studying early infection with HSV-1.

As expected, none of the monoclonal antibodies to nonglycosylated proteins showed neutralizing activity against either HSV-1 or -2. Many of the antibodies against these proteins reacted type specifically. However, two of five antibodies to a 68,000-MW protein (Table 6), one of eight antibodies to ICP 9 (Table 7), two of four antibodies to ICP 6 (Table 8), and one of one antibody to ICP 5 (Table 8) showed cross-reactivity. Additional monoclonal antibodies would have to be isolated and tested before conclusions could be drawn concerning the absence of cross-reactive antigenic determinants on nonglycosylated proteins ICP 4 and ICP 8.

One of the antibodies which precipitated ICP 6 (66S in Table 2) also precipitated a 65,000-MW protein by RIP-PAGE (Fig. 1, lane K). The fact that antibodies produced by all subclones of 66S precipitated the same two proteins indicated that this cell population did not contain a mixture of cells secreting antibodies of different specificities. Studies are presently underway to determine whether (i) the 65,000-MW protein is a degradation product of the 140,000-MW protein; (ii) the 65,000- and 140,000-MW proteins share common determinants reactive with the 66S antibodies, but not with the other antibodies to ICP 6; (iii) the 65,000-MW protein is complexed to the 140,000-MW protein, and the complex is precipitable by 66S antibodies but not by the other antibodies to ICP 6 which react at antigenic sites which preclude complexing; or (iv) the 65,000-MW protein is a cell-coded protein.

ACKNOWLEDGMENTS

We thank G. Koval, D. Simms, and M. Chakrabarty for their excellent technical assistance. We also thank G. H. Cohen and R. J. Eisenberg for confirming the specificity of monoclonal antibodies to some of the glycoproteins.

ADDENDUM IN PROOF

Preliminary studies in collaboration with P. Spear suggest that the monoclonal antibodies tentatively classified here as reacting against gB (Table 4) are directed against the gA-gB complex, while the monoclonal antibodies reacting against the 110,000-MW glycoprotein (Table 3) are directed against an unclassified glycoprotein. Until this situation is resolved, we will continue to classify our monoclonal antibodies listed in Table 4 as being directed against gB, and hold in abeyance the designation of the glycoprotein against which our monoclonal antibodies listed in Table 3 are directed.

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