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TYPE-COMMON AND TYPE-SPECIFIC MONOCLONAL ANTIBODIES TO HERPES SIMPLEX VIRUS TYPES 1 AND 2

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Summary. - Seventeen monoclonal antibodies (Mabs) reacting specifically with the cells infected with herpes simplex viruses type 1 (HSV-1) and type 2 (HSV-2) were characterized by a variety of immunological tests such as radioimmunoprecipitation, immunoblotting and virus-neutralization. The majority of Mabs was directed against glycoprotein B (anti-gB), six reacted with glycoprotein C (anti-gC) and one with glycoprotein G (anti-gG). Six anti-gB Mabs reacted with both types of HSV (anti-gB-1,2), two anti-gB and all the six anti-gC Mabs have been specific for HSV-1 (anti-gB-1 and anti-gC-1). The remaining two anti-gB Mabs and the anti-gG have been specific to HSV-2 (anti-gB-2 and anti-gG). Only three out of the seventeen examined Mabs neutralized the virus.

Key words: herpes simplex virus; viral glycoproteins; monoclonal antibody

Introduction

Herpes simplex virus (HSV) has two serotypes from which HSV-1 is rather associated with facial infections, whereas HSV-2 is more frequently connected with genital lesions. Both serotypes show about 70% of genomic homology, which explains the wide extent of structural similarity between antigenically related HSV-1 and HSV-2 virus-specific proteins (McGeoch, 1989).

In the course of productive infection HSV expresses at least seven glycoproteins designated as gB, gC, gD, gE, gG, gH, and gI, which are incorporated into the envelope of virions and into the membranes of infected cells (Spear, 1985). Glycoproteins gB, gD, gE, and gH are considered to be type-common, although they possess also type-specific antigen determinants (Kousoulas *et al.*, 1984; Eisenberg *et al.*, 1985; Fuller *et al.*, 1989). Glycoprotein C has proven to be predominantly, although not exclusively, type-specific (Zweig *et al.*, 1983*b*;

Zezulak and Spear, 1983), whereas glycoprotein gG first identified by monoclonal antibodies seems to be so far type-specific (Roizman *et al.*, 1984 Ackerman *et al.*, 1986).

Preparation of monoclonal antibodies (Mabs) to HSV glycoproteins has been reported by many workers (Pereira *et al.*, 1980; Showalter *et al.*, 1981; Balachandran *et al.*, 1981, 1982; Eisenberg *et al.*, 1982). They proved to be a valuable tool for differentiation between the HSV serotypes in immunodiagnostic work, for identification and characterization of viral glycoproteins, for revealing their epitope structure and in other immunological studies. We have utilized the hybridoma technology to develop a set of Mabs that would react specifically with the glycoproteins of HSV-1 or HSV-2 and could be used for specific antigen detection and HSV typization in different immunological tests.

Materials and Methods

Cell lines. VERO (green monkey kidney) and HEL (human embryonal lungs) cells were grown in BEM supplemented with 10% foetal bovine serum, antibiotics, and glutamine. Sp2/0 myeloma cells and hybridoma cells were maintained in Dulbecco's modified Eagle's minimal essential medium (DMEM) supplemented with 10–15% of foetal calf serum (FCS), gentamycine 20 μg/ml, pyruvate 50 μg/ml, and glutamine 30 μg/ml and incubated at 37 ^{0}C in 6% CO_2 humid atmosphere.

Viruses and the infected cell extract. HSV-1 strain Kupka and HSV-2 strain Praha were obtained from Dr. V. Vonka (SEVAC, Prague). Viruses were propagated in VERO or HEL cells and their titres were determined in VERO cells. For preparation of infected cell extracts, monolayers of VERO or HEL cells were infected with the virus dose of 5–10 PFU/cell. When CPE developed in about 70% of cells, the maintenance medium was discarded, the cells were washed once with PBS and then 5 ml of extraction buffer (0.5 mol/l NaCl, 0.5 % octyl-ß-D-glucopyranoside, 0.1 mmol/l phenylmethylsulphonyl fluoride) was added and allowed to act for 30 min at room temperature at occasional shaking. Thereafter the cells were scraped off and the cell debris was removed by low-speed centrifugation; the infected extract was stored at -20 ^{0}C until used. For immunization the infected cell extract was extensively dialysed against PBS at +4 ^{0}C to remove the majority of detergent.

Immunization of mice. Balb/c mice 6-week-old were given 300 μl extract of HSV-1 infected cells mixed with complete Freund's adjuvant by intramuscular route. Two weeks later, the same intramuscular injection was repeated. After another 3 weeks the mice received 500 μl of HSV-1 infected cell extract with incomplete Freund's adjuvant intraperitoneally and again 3 weeks later 100 μl of infected cells extract without adjuvant intravenously. Three days later the mice were sacrificed and their spleen cells removed for fusion. Balb/c mice were immunized with the HSV-2 extract using a schedule reported by Killington *et al.* (1981). Briefly, the mice received 50 μl of HSV-2 infected extract without adjuvant in the rear footpad and the injection was repeated 10 days later. After two weeks they received 300 μl of infected cell extract with incomplete Freund's adjuvant intramuscularly and two weeks later 100 μl of the infected cell extract intravenously without adjuvant. Three days later the mice were sacrificed and their spleens were used for fusion.

Generation and production of monoclonal antibodies. Spleens of immunized mice were fused with Sp2/O cells according the procedure described by Lane *et al.* (1986). Positive hybridoma cultures were cloned by two cycles of cloning in semisolid agar and the strong positive clones were expanded. Mabs were produced both *in vitro* and *in vivo*. For preparing ascitic fluids, an intraperi-

toneal injection of 5 x 10^6 hybridoma cells was given to adult Balb/c or DBA/2 mice which had been primed 10 days before with 0.5 ml paraffine oil given intraperitoneally.

Radioimmunoassay (RIA) procedures. Screening of positive hybridomas was performed by an indirect or sandwich RIA using ^{125}I-labelled affinity purified sheep antimouse IgG (^{125}I-ShAMo-IgG). As antigen in the indirect RIA we used purified HSV-1 or HSV-2 virions (300 ng protein/well) as controls served lysates of noninfected VERO or HEL cells diluted 1:10 in PBS (Bystrická *et al.*, 1985). In sandwich RIA the wells of microplates were coated with purified swine IgG (raised against purified HSV-1 virions) in a concentration of 1 μg IgG/well overnight at +4 ^{0}C in a humid chamber. After saturation with 10% FCS in PBS for 1 hr, infected cell extracts diluted 1:10 or 1:20 with 0.1% FCS in PBS were added for 2 hr. After washing, 30 μl of hybridoma fluid was given into the wells, incubated for 2 hr, washed and then the bound immunoglobulins were detected with ^{125}I-ShAMo-IgG.

Surface immunoassay. Monolayers of HSV-1, HSV-2 infected or noninfected VERO cells were washed with 0.1% FCS in PBS, scrapped off of the glass in a small volume and counted. Aliquots of 30 - 50 000 cells in 30 μl were added to the U-shaped wells of microtitre plates saturated for 1 hr with 10 % FCS in PBS. The ascitic fluids were appropriate diluted in 0.1% FCS in PBS and 30 μl of each dilution was incubated with the cells for 90 min at occasional shaking. The cells were washed 3 times with 0.1% FCS in PBS, centrifuged, and incubated with the ^{125}I-ShAMo-IgG (70 000 cpm/60 μl) again for 90 min. After washing 3 times with 0.1% FCS in PBS, the cell sediments were transferred to the vials and their radioactivity was counted.

Isotype determination. Mab isotypes were determined using RIA method with affinity purified rabbit antimouse IgM, IgGl, IgG2a, IgG2b, IgG3 antibodies. Rabbit IgG was detected with affinity purified ^{125}I-goat antirabbit IgG (Poláková and Russ, 1983). All incubations in described RIA procedures were performed at room temperature. Sandwich RIA and surface immunoassay were also used for titration of ascitic fluids. Endpoint antibody titres against HSV-1, 2 antigens were expressed as reciprocals of the highest ascites dilution at which triple levels (as compared to control) of radioactive tracer were bound.

Virusneutralization test (VN). Ascitic fluids were inactivated for 30 min at 56 ^{0}C and their appropriate dilutions were incubated for 2 hr with 100 PFU of HSV - 1,2 in the presence or absence of complement (10-20 U in 100 μl of fresh guinea pig serum) as described (Hsiung, 1982). Endpoint antibody titres were expressed as reciprocals of the highest ascitic fluid dilution at which 90% inhibition (10 times reduction) of CPE foci was observed.

Immunofluorescence (IF) - was performed with HSV-1 and/or HSV-2 infected VERO cell monolayers fixed in acetone as described (Hsiung, 1982). Sheep anti-Mo FITC-labelled IgG (SEVAC, Prague) was used in dilution 1:10, the ascitic fluids were diluted from 1:25 to 1:6400 or higher.

Radioimmunoprecipitation assay (RIPA). ^{35}S-methionine or ^{14}C-amino acids- or ^{14}C-glucosamine labelled viral glycoproteins were prepared from HSV-1 or HSV-2 infected HEL or VERO cells. At the time of infection the maintenance medium was replaced with methionine free BEM or with BEM containing 1/10 reduced concentration of amino acids or with glucose free BEM, supplemented with 2% FCS. At 4 hr p. i. fresh medium was added to the flask together with ^{35}S-methionine (5 μCi per 1 ml medium) or ^{14}C-amino acids (1 μCi per 1 ml medium) or ^{14}C-glucosamine (5 μCi per 1 ml of medium); the cells were labelled till 16 - 18 hr p. i. The labelled cells extracts were prepared and RIPA was carried out as described by Zezulak and Spear (1984). The precipitated proteins were separated by SDS-PAGE in 8% polyacrylamide gel according to Laemmli (1970). The gels were treated with 1 mol/l sodium salicylate, dried and exposed to Kodak-X-Omat film.

Immunoblot assay. Extracts of infected VERO cells were prepared as described by Snowden *et al.* (1985). Solubilized proteins were separated by SDS-PAGE in 8% acrylamide gel and electroblotted onto nitrocellulose filter (Sleicher - Schuell) as described by Towbin *et al.* (1979). The nitrocellulose sheet was blocked 4x15 min. in Blotto solution (5% skim milk powder, 0.2% NP-40 in PBS) and then cut into strips. Different Mabs (ascitic fluids) diluted at least 1:100 in Blotto solution or undiluted culture supernatants were incubated with nitrocellulose strips for 2 hr. The strips were washed 3x15 min in Blotto solution and then incubated for 2 hr with ^{125}J-SwAMo-IgG.

The strips were washed 3x15 min in Blotto solution, then 1.5 min in PBS, dried and exposed to Kodak X-Omat film with Perlux Extra - Rapid intensification screens.

Results

Screening of the hybridomas

In the first screening attempts by indirect solid-phase RIA purified HSV-1 or HSV-2 virions were directly adsorbed to the polystyrene microtitre plate. In this case we detected many positive hybridomas, but the vast majority of them proved to be negative in a variety of other tests including VN, IF, sandwich RIA, RIPA, and immunoblotting. For example, from 500 clones tested, 320 were positive in indirect RIA but only 5 of them reacted with native viral antigens in other immunological tests.

Like other investigators (Friquet *et al.*, 1984; Smith and Wilson, 1986) we concluded that direct immobilization of purified virus on polystyrene plate exposed several nonrelevant antigenic sites which are not present on the native

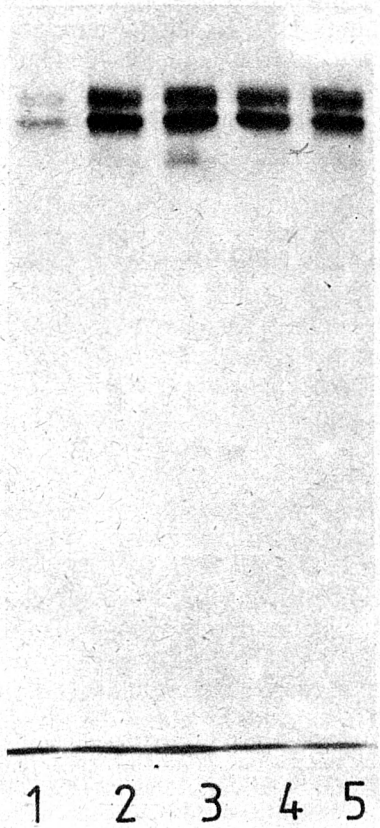

Fig. 1
Autoradiogram of electrophoretically separated polypeptides in immunoprecipitates obtained by the reaction of monoclonal antibodies (lane 1- 159, lane 2- 144, lane 3- 170, lane 4-T111, lane 5-T63) with the extract of HEL cells infected with HSV-1 and labelled with ^{35}S-methionine from 4–20 hr p.i.

virions. So, we decided to use the sandwich RIA, which presumably could be more effective in selecting Mabs that recognize native antigen. Purified IgG from polyclonal swine serum raised by immunization with HSV-1 virions was adsorbed to the polystyrene plate and the native viral protein was immobilized after being extracted from the infected cells with a nonionic detergent. Using this test, all the selected hybridomas were positive also in many other tests such as IF, VN, RIPA, immunoblotting and surface immunoassay. By sandwich RIA we selected 17 Mabs for further study. None of these reacted with the lysates of noninfected VERO or HEL cells in indirect RIA.

Identification of viral proteins detected by monoclonal antibodies

To identify the viral proteins reactive with Mabs selected by sandwich RIA, two assays have been performed: 1) RIPA with ^{35}S-methionine or ^{14}C-amino acids labelled extracts prepared from infected cells, and 2) immunoblotting. Monoclonal antibodies of group I (summarized in Table 1) – antibodies 49, 144, 159, 170, 763, T111 precipitated a protein complex of 130 kD, 120 kD from extracts of HSV-1 and HSV-2 infected HEL cells, respectively (Fig. 1 and Fig. 2, lanes 1, 7, 8). Using extracts of HSV-1 or HSV-2 infected VERO cells we detected 120, 110, 50, 40 kD proteins (Fig. 3, lane 1, Fig. 4, lane 7). This pattern of precipitation was very similar to that reported for gB by other authors (Pereira *et al.*, 1982; Zezulak and Spear, 1984) and we supposed that the precipi-

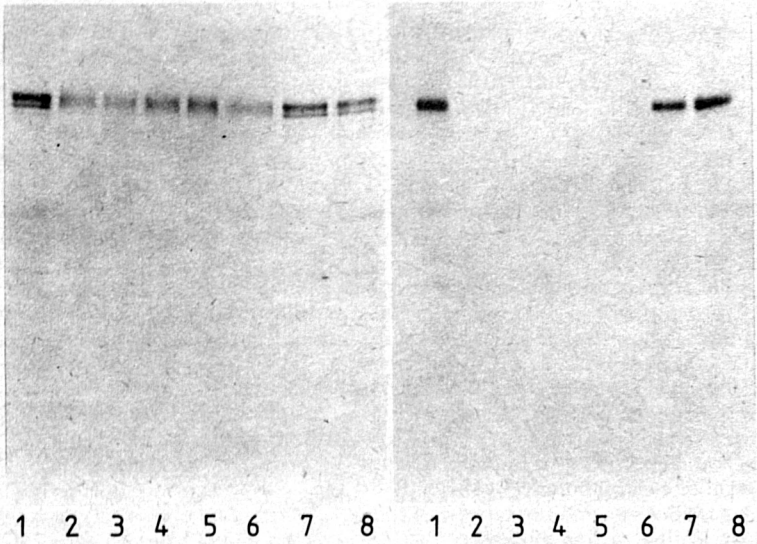

Fig. 2

Autoradiogram of electrophoretically separated polypeptides in immunoprecipitates obtained by the reaction of monoclonal antibodies (lane 1- T63, lane 2- T51, lane 3- T96, lane 4- T50, lane 5- 809, lane 6- T90, lane 7,8- T111) with extracts of HEL cells infected with HSV-1 (left panel) and HSV-2 (right panel) and labelled with ^{35}S-methionine from 4–20 hr p. i.

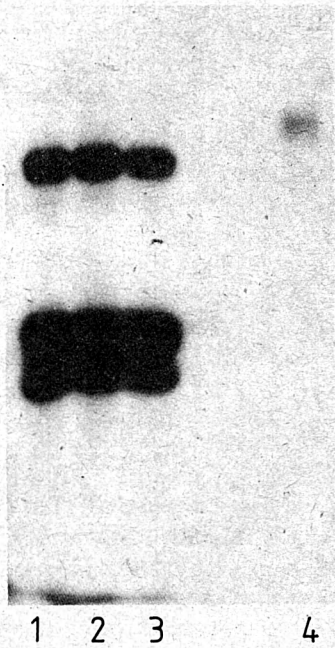

Fig. 3
Autoradiogram of electrophoretically separated polypeptides in immunoprecipitates obtained by the reaction of monoclonal antibodies (lane 1- 144, lane 2- 499, lane 3- 201, lane 4- 303) with extracts of VERO cells infected with HSV-2 and labelled with ^{35}S-methionine from 4 – 20 hr p. i.

tated protein complex was gB. However, with Mabs 733, 740 we detected the gB glycoprotein complex only from HSV-1 infected HEL or VERO cells (results not shown). On the contrary, Mabs 201, 499 precipitated very strongly the gB complex only from extracts of HSV-2 infected VERO or HEL cells (Fig. 3, lanes 2, 3).

This group of Mabs was further characterized with respect to their reactivity with denatured HSV-1 or HSV-2 infected VERO cells polypeptides in immunoblotting. Antibody 170 reacted with 100, 55, and 45 kD proteins from HSV-1 infected VERO cells corresponding to mature form and degradation product of gB-1 (Fig. 5, strip 1). In addition to antibody 170, also 201 and 499 antibodies reacted only with the 45–50 kD polypeptides from HSV-2 infected VERO cells corresponding probably to a degradation product of gB-2 (Fig. 5, strips 7, 8, 9). However, the majority of Mabs of this group were negative in immunoblotting.

Mabs of group II (Table 1), i. e. antibodies 809, T50, T51, T90, T96 precipitated a protein of apparent M_r 125 kD from extracts of HSV-1 infected HEL or VERO cells, which is typical of the mature form of glycoprotein gC. These Mabs failed to precipitate any other protein from the extracts of HSV-2 infected HEL cells (Fig. 2, lanes 2, 3, 4, 5, 6). Occasionaly we detected a precursor form of this glycoprotein at about 95 kD and a presumably related product at 65 kD (Fig. 4, lanes 3, 5, 9). In immunoblotting analysis Mabs T50, T51, T60, and T96 reacted with denatured HSV-1 infected VERO cell polypeptides and we detected broad zones of 120–110 kD, 80 and 60 kD, corresponding probably to

Table 1. Characterictics of monoclonal antibodies to glycoproteins of HSV-1 and 2

Group	Monoclonal anibody	Glycoprotein	Type specificity	Isotype	RIPA		BLOTTING		IMMUNO-FLUORE-SCENCE [a]		SANDWICH RIA [a]		SURFACE IMMUNO-ASSAY [a]		VIRUS NEUTRALIZA-TION [a]			
															HSV-1		HSV-2	
					HSV-1	HSV-2	HSV-1	HSV-2	HSV-1	HSV-2	HSV-1	HSV-2	HSV-1	HSV-2	+C	−C	+C	−C
	49	gB-1,2	TC	G2b	+	+	−	−	1600	1600	10 000	10 000	10 000	10 000	−	−	−	−
	144	gB-1,2	TC	G2b	+	+	−	−	3200	3200	200 000	200 000	100 000	10 000	−	−	−	−
	159	gB-1,2	TC	G1	+	+	−	−	1600	1600	100 000	100 000	10 000	10 000	200	200	200	200
	170	gB-1,2	TC	G2a	+	+	+	+	3200	3200	100 000	10 000	100 000	ND	−	−	−	−
I.	T63	gB-1,2	TC	G2b	+	+	−	−	640	1200	ND	ND	100 000	100 000	−	−	−	−
	T111	gB-1,2	TC	G2b	+	+	−	−	1200	1600	1 000 000	1 000 000	1 000 000	10 000	−	−	−	−
	201	gB-2	TS-2	G2b	−	+	−	+	10	640	200	20 000	20	20 000	−	−	200	200
	499	gB-2	TS-2	G1	−	+	−	+	10	640	20	200 000	20	20 000	−	−	200	200
	733	gB-1	TS-1	G2a	+	−	−	−	1600	10	1 000 000	100	100 000	10	−	−	−	−
	740	gB-1	TS-1	G1	+	−	−	−	1800	<40	1 000 000	100	10 000	10	−	−	−	−
	809	gC-1	TS-1	G2b	+	−	−	−	1600	10	100 000	100	100 000	10	−	−	−	−
	T50	gC-1	TS-1	G1	+	−	+	−	1600	ND	1 000 000	100	100 000	10	−	−	−	−
	T51	gC-1	TS-1	G2b	+	−	+	−	1600	100	100 000	10	100 000	10	−	−	−	−
II.	T60	gC-1	TS-1	G1	−	−	+	−	1600	40	1 000 000	10	100 000	10	−	−	−	−
	T90	gC-1	TS-1	G2a	+	−	−	−	1600	ND	100 000	100	100 000	100	−	−	−	−
	T96	gC-1	TS-1	G2b	+	−	+	−	1600	100	1 000 000	100	1 000 000	10	−	−	−	−
III.	303	gG-2	TS-2	G2a M	−	+	−	+	10	320	100	100 000	20	20 000	−	−	−	−

[a]) reciprocal value of endpoint titre

mature, precursor form and related product of gC-1 respectively (Fig. 5, strips 3, 4, 5, 6). None of Mabs of this group reacted with denatured HSV-2 infected VERO cell polypeptides what confirmed our presumption that these Mabs are type 1 specific.

Monoclonal antibody 303 (group III - Table 1) specifically precipitated a 120–125 kD protein from the extracts of HSV-2 infected VERO or HEL cells (Fig. 3, lane 4). We were not able to detect any protein precipitated by this Mab from extracts of HSV-1 infected cells. The apparent M_r corresponding to the protein precipitated with this Mab is close to that of gG-2 glycoprotein, so we supposed that the Mab 303 reacted with this glycoprotein. Type 2 specificity of this Mab was confirmed by immunoblotting, where we detected broad zones of M_r about 120–110 kD, 60–50 kD, and faint zone at 75 kD with the HSV-2 infected cell extracts only (Fig. 5, strip 10).

For glycoprotein labelling we performed an additional RIPA with extracts of HSV-1 and HSV-2 infected cells labelled with ^{14}C-amino acids or ^{14}C-glucosamine. Representative Mabs of group I (170, T 111), group II (T51, T96, 809), and of group III (303) precipitated proteins, which were heavily labelled with ^{14}C-glucosamine (Fig. 5). The results of these studies confirmed our presumption, that all proteins precipitated with prepared Mabs are glycoproteins.

Characterization of monoclonal antibodies in other immunological tests

Ascitic fluids for each of the hybridomas were tested for reactivity with

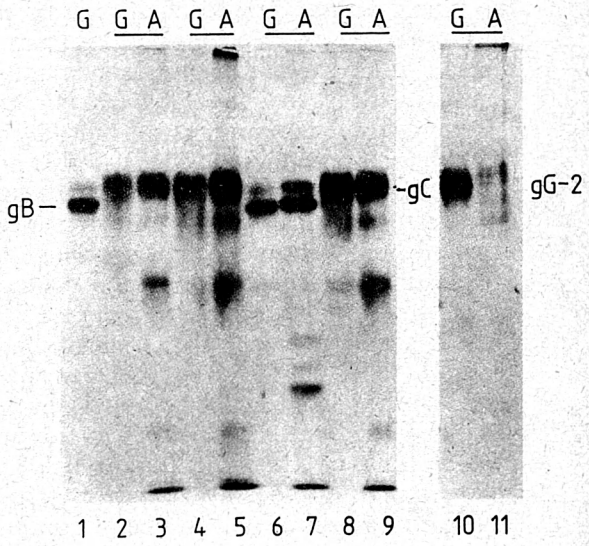

Fig. 4
Autoradiogram of electrophoretically separated polypeptides in immunoprecipitates obtained by reaction of monoclonal antibodies (lane 1- 170, lane 2,3- T96, lane 4,5- T51, lane 6,7-T111, lane 8,9- 809, lane 10,11- 303) with extracts of VERO cells infected with HSV-1 (left panel) or HSV-2 (right panel) and labelled with ^{14}C-glucosamine (lanes marked with G) or ^{14}C-amino acids (lanes marked with A)

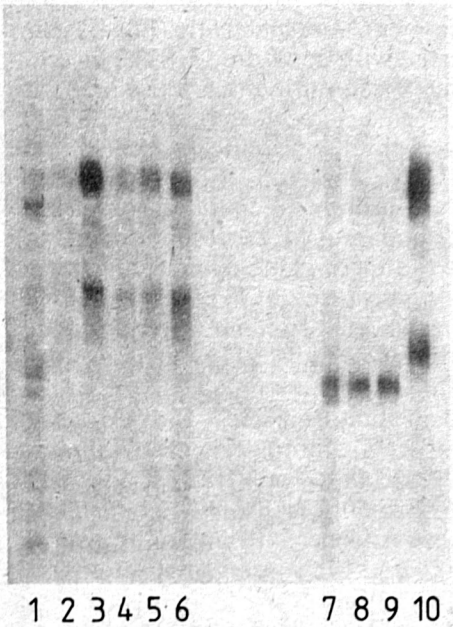

Fig. 5
Autoradiogram of immunoblotting analysis of HSV-1 (strips 1-6) and HSV-2 (strips 7-10) infected VERO cell polypeptides reactive with monoclonal antibodies (strips 1,7- 170, strip 2- T111, strip 3-750, strip 4- T51, strip 5- T96, strip 6- T60, strip 8- 201, strip 9- 499, strip 10- 303)

HSV-1 or HSV-2 infected cells by IF, VN, sandwich RIA and surface immunoassay (Table 1).

We observed that six Mabs belonging to group I (49, 144, 159, 170, T63, T111) originally produced by HSV-1 fusion showed strong cross-reactivity with the type 2 virus. They reached high titres in IF, sandwich RIA, and surface immunoassay, i. e. dilutions ranging from 1:1600 to 1:1 000 000. Only one Mab reacted in VN test both in presence and/or absence of complement. Presumably the type 1 specific Mabs 733, 740 were also type-specific in IF, sandwich RIA, and surface immunoassay, but they were negative in VN test. Mabs 201, 499 originally produced by HSV-2 fusion were type 2 specific showing high titres in IF, sandwich RIA and surface immunoassay. They reacted with the type 2 virus also in VN test in the presence or absence of complement. On the basis of these results we consider these Mabs as type 2 specific (Table 1).

Summing up, the whole group of Mabs against gB can be divided into 3 subgroups with respect to their type-specificity. Six antibodies proved to be type-common, two were type 1-specific and other two type 2-specific. Mabs of group II (809, T50, T51, T60, T90, T96) previously evaluated as anti gC-1 were type 1 specific in all performed tests, reaching high titres up to 1:1 000 000. All were negative in VN test either in the presence or absence of complement. Finally, the type 2 specificity of Mab 303 was confirmed in IF, sandwich RIA and surface immunoassay. However, this monoclonal antibody was negative in VN test in presence or absence of complement (Table 1).

Discussion

We report production and characterization of 17 Mabs to HSV-1 and HSV-2, ten of which react with glycoproteins gB-1 and gB-2, another six with glycoprotein gC-1 and one being directed against glycoprotein gG-2. The Mabs were characterized with respect to their reactivity and type specificity in immunological assays such as RIPA, immunoblotting, IF, VN, sandwich RIA, and surface immunoassay.

Mabs to gB-1, 2 (group I) reacted with corresponding extracts of infected HEL cells in RIPA and precipitated protein bands of M_r 130 and 120 kD, presumably mature and precursor forms of glycoprotein gB. However, in RIPA with extracts of infected VERO cells the M_r of these proteins was shifted down and, in addition, we detected two proteins of lower M_r of about 50 and 40 kD, probably degradation products of gB. Proteolytic cleavage of gB in VERO cells and formation of degradation products are well known (Pereira *et al.*, 1982; Zezulak and Spear, 1984). On the basis of characteristic migration pattern of gB made in HEL and VERO cells and heavy labelling of high M_r species with ^{14}C-glucosamine, we identified these proteins as members of the gB complex. Only small proportion (3 out 10) of these antibodies were reactive in immunoblotting, which is in accordance with the results of Kousoulas *et al.* (1984) who found only one antibody of 16 reactive with denatured polypeptides transferred to nitrocellulose. We also believe that the conformation of the gB molecule is critical for reaction with the majority of Mabs. In addition to high titres of Mabs in IF and sandwich RIA, we also found good reactivity in surface immunoassay using viable (nonfixed) infected cells which suggest the presence of reactive epitopes on the gB domain exposed to the extracellular space. Surprisingly, the major part of these Mabs failed to neutralize HSV-1 or HSV-2 in presence or absence of complement. We found only 3 Mabs reactive in VN test. It is noteworthy that much more Mabs against gB were found reactive in VN test by other authors (Kousoulas *et al.*, 1984; Kino *et al.*, 1985; Kumel *et al.*, 1985).

All but one Mabs against gC-1 (group II) were reactive in RIPA. They precipitated a polypeptide band of M_r 125 kD from extracts of HSV-1 infected cells heavily labelled with ^{14}C-glucosamine indicating that it was a glycoprotein. In addition, these Mabs precipitated no labelled proteins from the extracts of HSV-2 infected HEL or VERO cells. Four Mabs of this group reacted in immunoblotting with denatured polypeptides of HSV-1 infected VERO cells transferred to nitrocellulose. A M_r protein band (120 - 110 kD) and probably its precursor form 80 kD and a related product (60 kD) were detected. Again, as in previous test, negative results were obtained in reaction with the denatured polypeptides of HSV-2 infected cell extracts. Comparing our results with those of Zweig *et al.* (1983*a*; 1984) we suggest that the detected glycoprotein could be gC-1.

Mabs of group II in IF, sandwich RIA, and surface immunoassay reacted exclusively with HSV type 1. None of these antibodies had VN activity either in the presence or in the absence of complement. This contrasted with the results of Showalter *et al.* (1981) and Marlin *et al.* (1985), who found all their Mabs against gC-1 reactive in VN test in the presence of complement. Owing to type 1 specificity of this group of Mabs in all performed tests, we suppose that they may provide excellent type 1 specific reagent for potential diagnostic use.

Antibody against gG-2 (group III) showed type 2 specificity in all performed tests (Table 1). In RIPA the reactive protein formed a broad band about 125–120 kD which was labelled with ^{14}C-glucosamine confirming that it was a glycoprotein. In immunoblotting with denatured polypeptides of HSV-2 infected VERO cells we detected a broad band at approximately 120–110 kD and a lower molecular weight product at 60–50 kD. Considering the high susceptibility of gG-2 polypeptide to proteolytic cleavage (Dall'Olio *et al.*, 1987), we suppose that the 60–50 kD protein may be a degradation product rather than a precursor form. Roizman *et al.* (1984) also described the cleavage of gG-2 by VERO cell proteases in immunoblotting analysis, but their degradation products had lower M_r. This may be due to different experimental conditions.

Preliminary ELISA typing of clinical isolates showed, that our type-specific Mabs could differentiate HSV-1 and HSV-2 strains. Testing of further clinical isolates is in progress in order to select the most suitable Mabs for diagnostic purposes.

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