MONOCLONAL ANTIBODIES TO THE DISTINCT ANTIGENIC SITES ON GLYCOPROTEINS C AND B AND THEIR PROTECTIVE ABILITIES IN HERPES SIMPLEX VIRUS INFECTION

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Summary. – The relative importance of the humoral immune response to various antigenic sites on the glycoproteins C and B (gC, gB) of herpes simplex virus (HSV) was evaluated in BALB/c and DBA/2 mice passively immunized with monoclonal antibodies (MoAbs) and then challenged with lethal dose of infectious virus. Eight MoAbs to three topographically distinct antigenic sites on gC and eight MoAbs to two distinct antigenic sites on gB were selected. The results indicated that any antigenic site on gC and gB contains epitopes for the protective immunity. However, individual MoAbs to different epitopes of the same antigenic site (i.e. antigenic site III on gC, and antigenic site II on gB) varied extremely in their protective ability. The protection did not correlate with the virus neutralization *in vitro* whereas it correlated significantly with the immune cytolysis in the presence of complement. The information about protective epitopes is essential for understanding the immunology of HSV infection at molecular level and may have implications for the design of HSV vaccine.

Key words: herpes simplex virus; viral glycoprotein; monoclonal antibody; antigenic site; protective immunity

Introduction

Virus-specific glycoproteins associated with the envelope of HSV virion and plasma membranes of productively infected cells represent the major viral gene products responsible for the induction of the host humoral immune response to infection (Dowbenko and Lasky, 1984; Fuller and Spear, 1987; Pereira *et al.*, 1989; Fuller *et al.*, 1989; Kohl, 1992). Most of the HSV glycoproteins can serve as antigens for induction of a protective response against lethal challenge (Roberts *et al.*, 1985; Rajčáni *et al.*, 1995; Miriagou *et al.*, 1995). Protective immunity can be induced also by immunization with naked plasmid DNA encoding viral proteins (Manickan *et al.*, 1995a) or with recombinant viruses expressing viral antigens (Martin *et al.*, 1989; Forester *et al.*, 1991; Ghiasi *et al.*, 1994, Manickan *et al.*, 1995b; Heineman *et al.*, 1995). In addition, other investigators have documented the ability of MoAbs specific for these glycoproteins to confer a protection *in vivo* against lethal challenge or to enable a recovery (Rector *et al.*, 1982; Kino *et al.*, 1985; Metcalf *et al.*, 1988; Sanna *et al.*, 1996).

Virus-neutralization (VN), phagocytosis, complement activation with immunoregulatory and lytic effect, and antibody-dependent cell-mediated cytotoxicity (ADCC) are the mechanisms that may be involved in the antibody-mediated protection *in vivo*, although no close correlation of protection to any *in vitro* activity was documented (Balachandran *et al.*, 1982; Kumel *et al.*, 1985; Piga *et al.*, 1990;

Abbreviations: AbC = antibody-dependent complement-mediated cytolysis test; ADCC = antibody dependent cell-mediated cytotoxicity; CPE = cytopathic effect; gB, gC = glycoproteins B, C; HSV = herpes simplex virus; i.v. = intravenous(ly); i.p. = intraperitonea(ly); MoAb = monoclonal antibody; PBS = phosphate-buffered saline; p.i. = post infection; RIA = radioimmunoassay; SDS = sodium dodecyl sulphate; VN = virus neutralization

Mester and Rouse, 1991). Recently, Manickan *et al.* (1995a,b) showed a primary importance of $CD4^+$ T cells for the immunity to HSV.

So far, the protection capacity has not been studied for non-neutralizing MoAbs to distinct antigenic sites or epitopes on gC and gB. An information about such protective epitopes may be useful in designing synthetic or recombinant vaccines against herpesvirus infections. In this report, we identified antigenic sites on gC and gB with a set of previously prepared gC and gB-specific MoAbs (Bystricka *et al.*, 1991) and characterized them for their ability to confer passive protection to mice lethally challenged with HSV-1 and HSV-2. Of 16 MoAbs tested, 13 were protective, but we did not find any correlation between their protective ability *in vivo* and corresponding antigenic site or VN activity *in vitro*.

Materials and Methods

Cell lines. Vero (African green monkey kidney) cells were grown in Eagle's Minimum Essential Medium (MEM) supplemented with 10% heat-inactivated foetal bovine serum, antibiotics, and glutamine.

Viruses. Strain SC16 of HSV-1 described by Hill *et al.* (1975), strain Kupka of HSV-1 isolated by Dr. R. Benda, Prague and strain Praha of HSV-2 kindly supplied by Dr. V. Vonka, Prague, were grown in Vero cells.

Mice. 6-8-week-old female BALB/c and DBA/2 mice (Velaz, Prague) were used in protective experiments.

Production and characterization of MoAbs as to isotype, target antigen, type-specificity and VN activity was described in detail elsewhere (Bystrická *et al.*, 1991). Briefly, splenocytes of immunized mice were fused with Sp2/0 cells according to the procedure described by Lane *et al.* (1986). Positive hybridoma cultures were cloned by two cycles of cloning in semisolid agar and the clones were expanded. Immune or control ascitic fluids were prepared from positive hybridoma cells or control Sp2/0 cells, respectively.

Purification and radiolabelling of MoAbs. MoAbs were purified from culture media according to Ey *et al.* (1978), using protein A Sepharose CL-4B. Purified MoAbs were labelled with radioactive ^{125}I (Amersham, specific activity 580 MBq/μg using the Chloramine T method (Greenwood *et al.*, 1963).

Competetive antibody binding radioimmunoassay (RIA). Extracts of Vero cells infected with HSV-1 or HSV-2 prepared according to Bystrická *et al.* (1991) were adsorbed on wells of 96-well plates at a concentration corresponding to 50 % of maximal binding of ^{125}I-labelled MoAb. After 24 hrs of adsorption at 4°C in humid chamber, the plates were washed and saturated for 1 hr with 10% foetal calf serum in phosphate-buffered saline (PBS). After washing, serial tenfold dilutions of ascitic fluid (10^{-1} to 10^{-11}) in 30 μl and a constant amount of ^{125}I-labelled MoAb in 30 μl were added and incubated for 3 hrs at room temperature. The plates were washed 3 times and the radioactivity bound was eluted with hot 2 mol/l NaOH and counted. The RIA titers of all scitic fluids used in this test were higher than 5×10^5.

Antibody-dependent complement-mediated cytolysis test (AbC) was done according to McClung *et al.* (1976). Monolayers of Vero cells were infected with HSV-1 strain SC16 or HSV-2 strain Praha at a multiplicity of infection of 3 – 5 PFU/cell. Viable cells (3×10^6) in 300 μl were labelled with 150 μCi of ^{51}Cr (sodium chromate, NEN, Germany) for 90 mins at 37°C under moderate shaking. Three times washed cells were resuspended in MEM supplemented with 10% foetal calf serum and added to the 96- well plates (10^4 cells in 50 μl per well), followed by 50 μl of diluted (1:20 to 1:5120) immune or control ascitic fluid (inactivated at 56°C for 30 mins) and 50 μl of guinea pig complement (10 U). After 2 hrs of incubation at 37°C the cells were pelleted at 300 x g for 5 mins and the radioactivity of the supernatant (75 μl) was counted. The percentage of specific release of ^{51}Cr was calculated by the formula:

$$\text{spec.}^{51}\text{Cr rel.} = \frac{(\text{exp.}^{51}\text{Cr rel.}) - (\text{spont.}^{51}\text{Cr rel.})}{(\text{max.}^{51}\text{Cr rel.}) - (\text{spont.}^{51}\text{Cr rel.})} \times 100$$

(where spec. = specific, rel. = release, spont. = spontaneous and max. = maximum). For the maximum release determination, 2.5% sodium dodecyl sulphate (SDS) was used for solubilization of cells. The spontaneous release (infected cells incubated in the presence of growth medium and complement) did not reach values higher than 15% of the maximum release. The AbC titer was expressed as a reciprocal value of the immune ascites dilution causing ^{51}Cr release higher than the the average value of the control ascitic fluid plus the two-fold of the standard deviation. In control experiments, non-infected Vero cells were used as a target.

Passive immunization studies. Groups of 7-10 mice were injected intravenously (i.v.) with 100 μl of an immune inactivated (56°C, 30 mins) ascitic fluid containing the respective MoAb of a RIA titer higher than 5×10^5. Control groups of mice were injected with control ascitic fluid (prepared with myeloma Sp2/0 cells) or with PBS. Two hrs later, all groups of mice were challenged by intraperitoneal (i.p.) inoculation of 200 LD_{50} of HSV-1 strain SC16 or HSV-2 strain Praha in 100 μl. The second dose of the same amount of immune or control ascitic fluid or PBS was given i.v. 24 hrs later. Survival rates were assessed on day 30 p.i. when the mortality reached a final value. The survival significance of immune ascites-treated mice versus control ascites-treated mice was determined by the Fisher-Yates test (Mayr *et al.*, 1982) at 10% level of significance ($P = 0.1$).

Results

Characteristics of MoAbs specific for gC and gB

MoAbs specific for gC and gB have been previously produced and characterized as to their type-specificity, target glycoprotein, isotype, and ability to neutralize virus in the presence or absence of complement (Bystrická *et al.*, 1991). These data and the ability of the MoAbs to lyse infected cells in the presence of complement including their reactivity with the respective antigenic site are summarized in Table 1.

Table 1. Characteristics of MoAbs specific for gC and gB

MoAb antigen	Isotype site	Target	Antigenic	VN[a]				AbC[b]	
				HSV-1		HSV-2		HSV-1	HSV-2
				+C	−C	+C	−C		
T90	IgG2a	gC-1	I	<20	<20	<20	<20	<20	<20
T50	IgG1	gC-1	II	<20	<20	<20	<20	<20	<20
740	IgG1	gC-1	II	<20	<20	<20	<20	<20	<20
T96	IgG2b	gC-1	III	<20	<20	<20	<20	<20	<20
733	IgG2a	gC-1	III	<20	<20	<20	<20	2560	<20
T51	IgG2b	gC-1	III	<20	<20	<20	<20	5120	<20
T60	IgG1	gC-1	III	<20	<20	<20	<20	<20	<20
809	IgG2b	gC-1	III	<20	<20	<20	<20	1280	<20
499	IgG1	gB-2	I	<20	<20	200	200	<20	<20
201	IgG2b	gB-2	I	<20	<20	200	200	<20	<20
170	IgG2a	gB-1,2	I	<20	<20	<20	<20	<20	<20
T111	IgG2b	gB-1,2	II	<20	<20	<20	<20	<20	<20
T63	IgG2b	gB-1,2	II	<20	<20	<20	<20	<20	<20
159	IgG1	gB-1,2	nt	200	200	200	200	<20	<20
49	IgG2b	gB-1,2	nt	<20	<20	<20	<20	nt	nt

[a]VN titers in the presence (+C) or absence (−C) of complement are expressed as reciprocals of the highest dilutions of ascitic fluids at which 90% inhibition of foci of cytopathic effect (CPE) was observed.
[b]AbC titers are expressed as reciprocals of dilutions of ascitic fluids causing ^{51}Cr-release higher than the average value for control Sp2/0 ascitic fluid plus the two-fold of standard deviation.
nt = not tested.

Eight MoAbs directed to gC-1 were type-1-specific. All of them were negative in VN test in the presence or absence of complement. This finding is rather surprising since most MoAbs to gC-1 prepared by other authors displayed VN activity (Pereira *et al.*, 1980; Holland *et al.*, 1983; Marlin *et al.*, 1985). However, the ability to mediate lysis of HSV-1-infected cells in the presence of complement was detected by three of these MoAbs (733, 809, and T51). In the group of eight MoAbs to gB tested, six (170, 144, T111, T63, 159, and 49) appeared to be cross-reactive with HSV-1 and HSV-2. Only one of them (159) was able to neutralize virus in the presence or absence of complement. On the other hand, two remaining MoAbs were specific for gB-2 (499 and 201) and had a complement-independent VN activity. This apparent involvement of type-specific MoAbs in VN could be due to the fact that primary type-specific epitopes of gB are exposed at the surface of both virions and infected cells (Eberle and Courtney, 1989; Kousoulas *et al.*, 1988, 1989). None of the MoAbs specific for gB lysed HSV-1- or HSV-2-infected cells in the presence of complement.

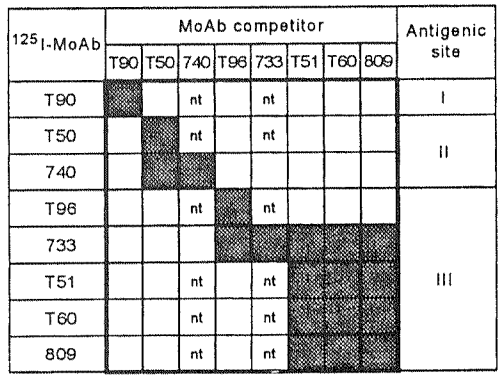

Fig. 1
Antigenic sites on gC-1
Topographical analysis of antigenic sites on gC-1 was done by competitive binding RIA with a set of eight MoAbs to gC-1 reacting with HSV-1 antigen immobilized on solid phase. Black squares indicate a competition higher than 50%. Empty squares indicate a competition lower than 50%. nt = not tested.

Delineation of antigenic sites on gC

For topographical analysis of epitopes on gC we used competitive antibody-binding RIA. MoAbs to gC were purified using protein A-Sepharose labelled with ^{125}I and evaluated in competitive binding studies. Unlabelled MoAbs as ascitic fluids in appropriate dilutions were tested for their ability to compete with a radiolabelled MoAb for binding to a limited amount of HSV-1 antigen in solid phase. We arbitrarily set an inhibition of binding higher than 50% as an indication that competing antibodies bind to the same antigenic site. The inhibition patterns of eight MoAbs identified three distinct antigenic sites on gC-1 (Fig. 1). With MoAbs reactive with the antigenic domain III we observed a non-reciprocal inhibition. These patterns might indicate the presence of a complex antigenic site composed of a minimum of three

a

^{125}I-MoAb	MoAb competitor				Antigenic site
	170	144	T111	T63	
170	■				I
144		■	■	■	II
T111			■	■	
T63			■	■	

b

^{125}I-MoAb	MoAb competitor						Antigenic site
	499	201	170	144	T111	T63	
499	■	■	■				I
201	■	■	■				
170	■	■	■				
144				■	■	■	II
T111					■	■	
T63					■	■	

Fig. 2
Antigenic sites on gB-1 and gB-2
Topographical analysis of antigenic sites on gB-1 (a) and gB-2 (b) was done by competitive binding RIA with four MoAbs to gB-1 and six MoAbs to gB-2 reacting with HSV-1 or HSV-2 antigen immobilized on solid phase. Black squares indicate a competition higher than 50%. Empty squares indicate a competition lower than 50%.

Table 2. Protective effect of MoAbs specific for gC-1

MoAb antigen	Target site	Antigenic	Survival rate in %[a]			
			HSV-1		HSV-2	
			BALB/c	DBA/2	BALB/c	DBA/2
T90	gC-1	I	86*	0	nt	14
T50	gC-1	II	90*	86*	nt	0
740	gC-1	II	86*	14	nt	0
T96	gC-1	III	80*	57*	nt	0
733	gC-1	III	71	43	nt	0
T51	gC-1	III	86*	14	nt	0
T60	gC-1	III	14	29	nt	0
809	gC-1	III	90*	86*	nt	0
Control ascitic fluid	–	–	5-27	0-9	nt	0-12
PBS	–	–	0	0	nt	0

[a]Survival rate was evaluated on day 30 after lethal challenge and statistically analyzed by Fisher-Yates test on the 10% level of significance (P = 0.1). MoAbs showing P <0.1 were considered protective and are labelled with asterisk.
nt = not tested.

epitopes. This antigenic site seems to be identical with the complex antigenic site II consisting of three distinguishable subregions as defined by Marlin *et al.* (1985).

Delineation of antigenic sites on gB

To obtain information about topographical arrangement of epitopes on gB, the same procedure as for gC was used. Competitive antibody binding studies using MoAbs to gB were limited by a failure of two of them (49 and 159) to retain specific binding activity after radioactive labelling. Our competitive binding study of four type-common and two type 2-specific MoAbs revealed two independent antigenic sites on gB-1 (Fig. 2a) and gB-2 (Fig. 2b). A reciprocal inhibition of the binding occurred among MoAbs corresponding to antigenic site I of gB-2. Epitopes at this site appeared to be in close proximity or only in partial overlap. Similarly, a reciprocal competition was observed with MoAbs T111 and T63 reactive with antigenic site II for both gB-1 and gB-2. Non-reciprocal inhibition detected by MoAb 144 reactive with the same antigenic site might reflect a lower affinity of this MoAb.

With regard to the type-specificity of individual antigenic sites on gB, it seems that the antigenic site I on gB-2 contained predominantly type-specific epitopes while the antigenic site II appeared to be type-common, as it was recognized by the same set of MoAbs to gB-1 and gB-2.

Passive immunization with MoAbs defining three distinct antigenic sites on gC

Further we examined the ability of the MoAbs to gC to confer passive protection to mice against lethal challenge with HSV-1 or HSV-2. In this series of experiments, we used besides commonly used BALB/c strain also DBA/2 strain of mice that is deficient in C5 component of the complement cascade (Berden *et al.*, 1981). This experimental arrangement could provide us with some information about the role of complement in passive protection *in vivo*.

Eight MoAbs as undiluted ascitic fluids were administered i.v. 2 hrs before and 24 hrs after the lethal virus challenge. Control mice were injected with Sp2/0 ascites or PBS, respectively. Survival rates of immunized and control mice after lethal challenge were calculated from 2 independent observations. The results of these protection experiments are summarized in Table 2. Six MoAbs (T90, T50, 740, T96, T51, and 809) directed to three distinct antigenic sites on gC were able to protect the mice from lethal infection. These results indicated that any antigenic site on gC defined with these MoAbs can provide epitopes for the protective immunity. A comparison of protective abilities of five MoAbs defining the antigenic site III (T96, 733, T51, T60, and 809) revealed that no correlation could be made between the protective capacity of MoAbs T96, T51, 809 and partially also 733, and the reactivity with individual epitopes forming this antigenic site. The protective capacity of some MoAbs correlated more significantly with IgG2a,b isotype (MoAbs T96, T51, 809 and

733) and activity in AbC test (MoAbs T51, 733 and 809). MoAbs with IgG2a,b isotype are considered more effective than those with IgG1 in protecting mice against viral challenge (Ishizaka *et al.*, 1995).

Protection experiments carried out with DBA/2 mice showed that MoAbs T50, 809 and T96 protected DBA/2 and BALB/c mice with similar efficiency. This finding suggests a complement-independent mode of protection with these MoAbs. On the contrary, MoAbs T90, 740, and T51 did not protect the challenged DBA/2 mice from infection. Of these MoAbs, only T51 showed a high titer of complement-dependent cytolytic activity *in vitro* what suggests an apparent role of the complement system in the protection of mice with this MoAb.

The survival rate of mice in control groups injected with Sp2/0 ascites and virus ranged from 0 to 27%. These values may indicate that partial protection of animals can be also mediated by other factors than MoAbs present in ascitic fluids. However, control mice injected with PBS and virus died within 10 days.

Passive immunization with MoAbs defining two distinct antigenic sites on gB

Protection experiments with MoAbs specific for gB were done essentially as described above for MoAbs to gC. Both strains of mice (BALB/c and DBA/2) were injected with 2 doses of MoAbs and lethally challenged with HSV-1 or HSV-2. In these experiments we used a set of six MoAbs to gB characterized as to their corresponding antigenic site, and two additional MoAbs (49 and 159) without defined epitope specificity. Average survival rates in two independent protection experiments are summarized in Table 3. MoAbs corresponding to the antigenic site I (170, 201 and 499) or II on gB-2 (144, T63 and T111) were equally effective in conferring passive immunity to mice *in vivo*. A comparison of protective ability of MoAbs reactive with individual epitopes of the same antigenic site I or II of both types of HSV showed considerable differences. Taken together, as with the MoAbs to gC-1, the ability of the MoAbs to gB to protect both strains of mice from lethal infection was not related to any individual epitope or antigenic site.

Cross-reactive MoAbs (170, 144, T111, T63, 159, and 49) significantly protected BALB/c or DBA/2 mice from lethal HSV-1 infection in spite of the fact that only one of them (159) had complement-independent VN activity *in vitro*. Three of them (170, 144, and 159) were also protective for mice challenged with HSV-2. Of the two gB-2-specific MoAbs (201 and 499) having complement-independent VN activity and reacting with the same antigenic site, only one (201) of IgG2b isotype gave a significant protection *in vivo*. However, the non-protective MoAb 499 showed IgG1 isotype that is considered less effective in conferring a passive protection (Ishizaka *et al.*, 1995). In conclusion, no correlation was found between the VN activity and the protective ability of MoAbs to gB *in vivo*.

Table 3. Protective effect of MoAbs specific for gB

MoAb antigen	Target site	Antigenic	Survival rate in %[a]			
			HSV-1		HSV-2	
			BALB/c	DBA/2	BALB/c	DBA/2
499	gB-2	I	nt	29	14	43
201	gB-2	I	nt	0	40*	57
170	gB-1,2	I	80*	86*	30	100*
144	gB-1,2	II	86*	14	100*	100*
T111	gB-1,2	II	30	100*	20	14
T63	gB-1,2	II	0	57*	29	0
159	gB-1,2	nt	nt	80*	nt	100*
49	gB-1,2	nt	nt	86*	nt	50
Control ascitic fluid	–	–	5-27	0-8	0-17	0-12
PBS	–	–	0	0	0	0

[a]Survival rate was evaluated on day 30 after lethal challenge and statistically analyzed by Fisher-Yates test on 10% level of significance ($P = 0.1$). MoAbs showing $P < 0.1$ were considered protective and labelled with asterisk.
nt = not tested.

In the course of protective experiments we noticed that some cross-reactive MoAbs (i.e. 144 and 170) appeared as type-specific depending on the mouse strain used. E.g., BALB/c mice infected with HSV-1 were protected with both MoAbs whereas DBA/2 mice were protected only with MoAb 170. In contrast, BALB/c mice challenged with HSV-2 were protected only with MoAb 144, whereas DBA/2 mice were protected with both MoAbs. Apparently, MoAb 144 behaved as cross-reactive when tested with BALB/c mice and as HSV-2-specific in DBA/2 mice. On the contrary, MoAb 170 behaved as cross-reactive when tested in DBA/2 mice and as HSV-1-specific in BALB/c mice. It remains to be resolved why some MoAbs appear as type-specific or cross-reactive in their protective ability depending on a mouse strain.

Discussion

Investigation of the immune response to HSV has progressed rapidly, mainly through the use of mouse model, MoAbs and recombinant DNA technology. Recent research of antibody-mediated protection had been focused on defining the role of individual viral envelope glycoproteins, with the ultimate goal of identifying the minimum effective immunization unit. Using lethal challenge models, several investigators have demonstrated the ability of MoAbs spe-

cific for gB,gC,gD,gE and gH to protect mice from lethal infection. In a number of these studies, no significant correlation has been found between protection and neutralizing activity, and Fc-dependent functions, mainly ADCC (Rector *et al.*, 1982; Balachandran *et al.*, 1982; Kino *et al.*, 1985; Kumel *et al.*, 1985; Mester *et al.*, 1991). In agreement with these studies, protective ability of our MoAbs did not correlate with their capacity to neutralize virus. Only two (159 and 201) of 13 MoAbs which significantly protected mice from lethal infection neutralized virus *in vitro*. This finding suggests that the neutralization of an extracellular virus *in vivo* is not a significant mechanism of protection. This conclusion is further supported by the finding that MoAb 499 with VN activity but with IgG1 isotype showed no protection. Recently, Ishizaka *et al.* (1995) assessed the effect of MoAb subclass on the protective efficacy *in vivo* and found IgG2a or IgG2b superior to IgG1. In agreement with their findings, 77% of our protective MoAbs were of IgG2a or IgG2b isotype.

On the other hand, good correlation was found between titers of MoAbs in complement-dependent cytolysis and their protective ability. All MoAbs with cytolytic activity (733, T51 and 809) conferred very good (T51 and 809) to moderate (733) protection. A comparison of protective ability of MoAbs studied in BALB/c and C5-deficient DBA/2 mouse strains showed that 70% of protective MoAbs protected BALB/c as well as DBA/2 mice from lethal infection. This finding indicated that complement was not critical for the protective ability of these MoAbs *in vivo*. The remaining 30% of protective MoAbs conferred protection only to BALB/c mice suggesting that the C5 component of complement was needed for their protective ability. However, out of three MoAbs with cytolytic activity, only T51 showed significant lack of protection in C5-deficient DBA/2 mice. This MoAb protected BALB/c mice very likely through complement-mediated mechanisms.

Protective studies with some MoAbs specific for gB (144 and 170) that were recognized as cross-reactive in a number of tests *in vitro*, revealed very unusual patterns in their protective ability *in vivo*. These MoAbs appeared either cross-reactive or type-specific depending on the type of challenge virus (HSV-1 or HSV-2) and the mouse strain (BALB/c or DBA/2) used in protective experiments. MoAb 144 behaved as type 2-specific when tested in DBA/2 mice and MoAb 170 behaved as type 1-specific in BALB/c mice. At present, we are not able to explain this peculiar phenomenon. Obviously, the type-specificity data about the anti-gB MoAbs derived from *in vitro* studies should determine their type-specific behavior *in vivo*. Nevertheless, it is conceivable that gB in addition to its multifunctional and complex character possesses also a high degree of variability in expression of type-common and type-specific epitopes depending on the challenged organism.

Recently, several different antibody-binding sites have been defined on gC and gB of HSV, particularly using neutralizing MoAbs and MoAb-resistant virus mutants (Pereira *et al.*, 1989; Highlander *et al.*, 1989; Kousoulas *et al.*, 1988; Kumel *et al.*, 1985; Marlin *et al.*, 1985). Also, a correlation between the reactivity patterns of MoAbs with distinct antigenic sites on haemagglutinin-neuraminidase glycoprotein and their protective abilities was studied in Sendai virus infection (Piga *et al.*, 1990). In these studies, no critical antigenic site was found for immune recognition by protective antibodies.

The protective experiments described here were mostly done with non-neutralizing MoAbs directed to distinct antigenic sites of gC and gB as determined by competitive antibody-binding RIA tests. The results indicated that each of the antigenic sites on gB and gC can provide epitopes for the protective immunity. However, individual MoAbs directed to distinct epitopes of the same antigenic site differed extremely in their protective ability. We believe that any region on the surface of an antigen can induce and be recognized by antibodies, and many of these antibodies probably may protect an organism (e.g. mouse) from lethal infection. Further experiments should be done to explain what else, if not the antigenic site, determines the protective capacity of particular antibody against lethal infection *in vivo*.

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