Epitopes Functional in Neutralization of Varicella-Zoster Virus

BAGHER FORGHANI,* KENT W. DUPUIS, AND NATHALIE J. SCHMIDT†

Viral and Rickettsial Disease Laboratory, Division of Laboratories, California State Department of Health Services, 2151 Berkeley Way, Berkeley, California 94704

Received 9 February 1990/Accepted 23 August 1990

By competition neutralization assay using monoclonal antibodies (MAbs) to varicella-zoster virus (VZV) glycoproteins (gps), we attempted to determine the topographical relationship of epitopes which are functional in VZV neutralization. MAbs against gpI interfered moderately to strongly with neutralization of MAbs against gpIII, and one antigenic domain with two distinct epitopes was identified on gpIII. Competition neutralization assays performed with MAbs to gpI revealed at least three distinct antigenic domains: the first contained two complement-dependent neutralizing epitopes; the second contained five complement-dependent neutralizing, overlapping epitopes and one nonneutralizing, nonoverlapping epitope; and the third contained one complement-enhanced neutralizing epitope. Competition neutralization assays performed with MAbs to gpIV showed one antigenic domain with two distinct epitopes which competed with nonneutralizing gpI MAbs. gpII did not interfere with neutralization of gpI, gpIII, or gpIV. Our data suggest that neutralizing and nonneutralizing MAbs can interfere with the action of viral neutralization either by inhibition or by enhancement. This report describes the epitope mapping of VZV gps by a functional biological assay.

Epitopes which are functional in viral neutralization have been demonstrated on three of the five glycoproteins (gps) of varicella-zoster virus (VZV) (3). The gpI protein is the most abundant of the immunogenic VZV envelope proteins and is resolved into polypeptides ranging in molecular weight (MW) from 45,000 to 98,000 by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE). Monoclonal antibodies (MAbs) which have been reported against this protein are complement-dependent neutralizing antibodies (4, 6, 7, 9, 14). The gpII protein is a dimer linked by disulfide bonds and reacts with polypeptides in two MW ranges by SDS-PAGE: 115,000 to 140,000 and 57,000 to 66,000. Both complement-independent (4, 34) and complement-dependent (4) neutralizing MAbs against gpII have been reported. The gpIII protein migrates as a single species with an MW of 105,000 to 118,000, and neutralizing MAbs reported against this protein have been complement independent (7-9, 14). Recently, MAbs against gpIV have been described (33), but no neutralizing MAbs against it have been reported.

This report describes a competition neutralization assay using MAbs to VZV gps meant to determine topographical relationships of the antigenic domains and their epitopes which are functional in neutralization. In addition, by the competition neutralization assay we showed that neutralizing and nonneutralizing MAbs to VZV gps can compete for the epitopes of neutralizing MAbs.

MATERIALS AND METHODS

Virus and cell culture. The Batson strain of VZV (passages 52 to 58), an isolate of this laboratory, was propagated in a human fetal diploid lung cell line (27).

MAbs. Production of MAbs to VZV proteins by using sucrose density gradient-purified virions as immunizing antigens has been described in detail previously (8). Briefly, the first injection was given intraperitoneally with complete Freund adjuvant, and the second was given 4 weeks later

intravenously without adjuvant. Hybridization of mouse BALB/c spleen cells with mouse myeloma cells (SP2/0) was done by standard procedures with polyethylene glycol as the fusing agent. The hybrid cells were screened and selected for secretion of MAbs by indirect immunofluorescence assay (IIF) and virus neutralization test (8).

Plaque reduction neutralization assay. Production of cell-free VZV for use in neutralization tests (28) and the procedures for performance of neutralization tests without and with guinea pig complement have been described elsewhere (27).

Isotyping. The isotype of each MAb was determined by the procedure previously described (8). Briefly, tissue culture media from VZV-positive hybridomas were concentrated 25 to 30 times by neutralized saturated ammonium sulfate and tested by agarose gel immunodiffusion against goat monospecific antisera to mouse immunoglobulin G1 (IgG1), IgG2a, IgG2b, IgG3, IgA, and IgM (Research Products International Corp., Elk Grove, Ill.).

Radiolabeling of cell cultures and radioimmunoprecipitation assay. Virus-infected monolayers were prepared by mixing VZV-infected and uninfected human fetal diploid lung cells at a ratio of 1:3. At 24 to 48 h postinfection, depending on the extent of the cytopathic effect, the growth medium was replaced with methionine-free medium containing 20 μ Ci of [35 S]methionine per ml and labeled for 18 to 20 h. Radiolabeled viral antigen was prepared by lysing the washed labeled cells with Tris-saline buffer (0.05 and 0.15 M, respectively [pH 7.4]) containing 1% deoxycholate, 1% Nonidet P-40, and 1% aprotinin as a protease inhibitor and clarifying them at 40,000 $\times$ g for 1 h. Radioimmunoprecipitation assays were performed by incubating labeled cell lysate with tissue culture fluid of hybridoma-secreting MAbs. The immune complexes were removed with protein A-Sepharose 4CL, disrupted with SDS and 2-mercaptoethanol, subjected to 10% polyacrylamide slab gel electrophoresis (7), and autoradiographed.

Western immunoblot analysis. Denatured uninfected and VZV-infected cell lysates were separated by SDS-PAGE. Preparation of VZV-infected and uninfected cell lysates was the same as that described for radioimmunoprecipitation

* Corresponding author.

† Deceased.

assays, except that they were not radiolabeled. The PAGE-separated proteins were electroblotted onto nitrocellulose filters (1, 32). The nitrocellulose filters were reacted with MABs for 1 h at 37°C and overnight at 4°C, followed by three washings. They were then treated with horseradish peroxidase-conjugated rabbit anti-mouse IgG for 1 h at room temperature and washed three times. The enzymatic reaction was visualized by addition of H₂O₂ as the substrate and aminoethyl carbazole as the chromogen donor (6).

Competition neutralization assay. Competition neutralization assays to test for blocking of complement-independent neutralizing MABs were performed as follows. A 0.1-ml volume of a 1:50 dilution of the blocking MAB was mixed with 0.1 ml of virus diluted to contain approximately 200 PFU/ml, and the mixture was incubated at 37°C for 25 min. Limiting dilutions of the neutralizing MABs were then added in a volume of 0.2 ml, the mixtures were again incubated at 37°C for 25 min and then added in 0.1-ml volumes to duplicate 30-mm-diameter culture dishes containing human fetal diploid lung cell monolayers. After an adsorption period of 1 h at 37°C, a nutrient overlay was added and the cultures were incubated for 6 days at 37°C and then overlaid with a second layer of agar by our standard plaque reduction neutralization procedure (27). The test was read 24 h later, and 50% reduction of plaques was considered positive for the presence of neutralizing activity. When sera were examined for ability to block complement-dependent neutralizing MABs, 0.1 ml of a 1:8 dilution of guinea pig complement (approximately 10 hemolytic units) was added after the first incubation of virus and the blocking MAB, followed by 0.1-ml volumes of limiting dilutions of the complement-dependent neutralizing MAB. Controls included the test dilutions of the neutralizing MAB examined against virus preincubated with ascitic fluid or serum containing no VZV antibody, and the test virus dilution was tested against a blocking antiserum without the neutralizing MAB. Plaque counts in the presence of the blocking MAB were compared with those in controls without the blocking MAB. Each test was repeated at least once. Initially, competitive neutralization assays were conducted with mouse ascitic fluid or DEAE-purified mouse IgG in which the IgG concentration was adjusted to its original ascitic fluid volume. Preliminary experiments indicated that 1:10 and 1:100 dilutions of either immune ascitic fluid or purified IgG were equally effective as blocking MABs, and therefore a median 1:50 dilution of ascitic fluid was used for subsequent studies.

RESULTS

The MABs to VZV which were used in the competition neutralization assays are shown in Table 1.

Our collection of MABs to VZV gps included the following: (i) 7 complement-dependent MABs, 1 complement-independent MAB which showed enhanced neutralizing activity in the presence of complement, and 4 nonneutralizing MABs in gpI; (ii) one nonneutralizing MAB in gpII; (iii) 2 complement-independent MABs in gpIII; (iv) 2 complement-dependent and 2 nonneutralizing MABs in gpIV; (v) 6 nonneutralizing MABs in viral protein Vp(98,36-43); (vi) one nonneutralizing MAB in Vp(30); and (vii) 10 nonprecipitable, nonneutralizing MABs. The nonprecipitation may result from nonsolubility of viral antigen by the cell lysis, or the antigen may be labile, and thus denatured, and the specific epitope may be damaged beyond antibody recognition. Since all 10 MABs stained the cytoplasm of infected cells by IIF and were nonneutralizing MABs, we decided to use groups

TABLE 1. Neutralizing activities of MABs to VZV gps

Target protein(s)	Clone	IgG sub-class	Neutralizing MAB titer ^a		IIF titer (log dilutions)	Western blot result
			Complement independent	Complement dependent		
gpI	3G8	2b	N	5.0	6.0	+
	4E6	1	N	5.0	6.0	+
	6G9	1	2.0	3.0	6.0	+
	3XF10	1	N	2.0	6.0	+
	2XD1	1	N	3.0	5.0	+
	39F11	1	N	3.0	5.0	+
	84G9	2a	N	2.5	5.0	+
	4G6	1	N	1.5	6.0	+
	53H8	1	N	N	5.0	+
	2C11	1	N	N	5.0	+
	54A8	1	N	N	5.0	+
	54F3	1	N	N	5.0	+
gpII	3C7	1	N	N	5.0	—
gpIII	4B7	1	6.5	6.5	6.0	—
	8G9	1	6.5	6.5	6.0	—
gpIV	1B7	2b	N	1.5	5.0	—
	1F12	2a	N	1.5	5.0	—
	3B8	1	N	N	5.0	—
	50E12	1	N	N	5.0	—
Vp(98,36-43)	50B11	1	N	N	5.0	+
	3XA10	1	N	N	5.0	+
	51A6	1	N	N	5.0	+
	55E5	1	N	N	5.0	+
	56A1	1	N	N	5.0	+
	59B7	1	N	N	5.0	+
Vp(30)	4G8	2a	N	N	6.0	—
Nonprecipitants	10 clones	1	N	N	5.0–6.0	—

^a Negative log₁₀ of the 50% plaque reduction endpoint with immune ascitic fluids. N, A titer of <1:10⁻¹ was considered negative.

v, vi, and vii as controls in this competition assay. All MABs had high IIF titers, regardless of their neutralization titers and were isotyped by monospecific antisera to mouse immunoglobulin subclasses. The results are summarized in Table 1.

To determine the specificity and purity of each MAB, [³⁵S]methionine-labeled cell lysates were reacted with MABs in each group and subjected to SDS-PAGE. The results are shown in Fig. 1, in which each lane represents the polypeptides precipitated by MABs of each group. In terms of MW and glycosylation, these precipitated polypeptides fully agree with previously published results reported by us and other investigators (4, 7, 9, 14, 25).

Experiments were conducted to react the cell culture media of antibody-producing hybridomas and ascitic fluids of the individual hybridomas with denatured VZV proteins

FIG. 1. Analysis of VZV glycoproteins by SDS-PAGE. VZV-infected human fetal diploid lung cells were labeled with [35 S] methionine for radioimmunoprecipitation assay. The labeled cell extracts were reacted with several MABs which had specificity to VZV proteins and then separated on a 10% polyacrylamide gel. Each lane represents an individual group of VZV glycoproteins, and the arrows refer to the individual glycopeptides precipitated in each group. MWM, MW markers of 14 C-labeled proteins in thousands (myosin, phosphorylase *b*, bovine serum albumin, the IgG heavy chain, ovalbumin, carbonic anhydrase, and the IgG light chain).

which were separated by SDS-PAGE and electrophoretically transferred onto nitrocellulose membranes. Only the cell culture media and ascitic fluids of gpI and Vp(98,36-43) were reactive; the others were nonreactive. This is illustrated in Fig. 2, and the results are summarized in Table 1.

To delineate the interference of MABs among the various VZV gps, we used a competition neutralization assay which showed MAB specificity to each gp. An example of one such an experiment is illustrated in Fig. 3. MABs to gpII, gpIV, and normal mouse ascitic fluid showed no interference on the percent reduction of virus neutralization of gpIII (4B7) compared with the control cell culture media. In contrast, gpI (3G8) did interfere with the neutralization capacity of gpIII by 2 log dilutions. Such specific interference was determined for each MAB included in this study.

Competitive inhibition with gpIII. In competition neutralization assays between gpI and gpIII, the following results were obtained and are summarized in Table 2 and illustrated in Fig. 4a and b. MABs 3G8, 3XF10, 39F11, and 53H8, which are directed against gpI, showed marked competition for MABs 4B7 and 8G9 directed against gpIII; MABs 84G9, 4G6, 2C11, and 54A8 competed only against 4B7; and 54F3 did not compete with either of the gpIII MABs. In contrast, MAB 4E6 had no effect on 8G9 but enhanced by more than 60%

FIG. 2. Analysis of VZV glycoproteins by Western blotting. Denatured uninfected and VZV-infected cell lysates were separated by SDS-PAGE, transferred electrophoretically onto a nitrocellulose membrane, and reacted with different MABs by using horseradish peroxidase-conjugated rabbit anti-mouse IgG as the detector signal. Lanes: 1, 3, 5, 7, 9, and 11, uninfected cell lysate; 2, 4, 6, 8, 10, and 12, VZV-infected cell lysate. The MWs of marker proteins (in thousands) are shown at the left.

the neutralizing titer of 4B7. We tentatively designated the antigenic domain (D) of gpIII, which competed with gpI, gpIII-D1 and the two epitopes gpIII-D1a and gpIII-D1b.

It appears likely that MABs 4B7 and 8G9 are directed against an antigenic domain of gpIII and that their epitopes are very closely situated. With the exception of four MABs that showed a low level of inhibition for 4B7 but none for 8G9, the other competing MABs showed similar inhibitory activities for both antibodies.

It is interesting that MABs 3G8 and 4E6 both possess equally high-titer complement-dependent neutralizing activities but one inhibits and the other enhances the neutralizing activity of the MAB against gpIII. The results of competition neutralization assays (Table 2) suggest that either their epitopes are spatially separate or they are represented by two distinct antigenic domains. The designated epitope of gpI which competes with gpIII is epitope gpI-D1a, and the other, which does not compete with gpIII or enhances, is epitope gpI-D1b (Fig. 4a). This antigenic domain of gpI appears to be distinct, since no MABs have been shown to compete with it (see Table 3).

None of the MABs against gpII, gpIV, Vp(98,36-43), Vp(30), and 10 nonprecipitant proteins competed with complement-independent neutralizing MABs directed against gpIII, although all had high IIF titers, indicating the presence of a large amount of antibody (Table 1).

Competitive inhibition with gpI. To determine whether there were additional functional epitopes on gpI, seven complement-dependent MABs (3G8, 4E6, 3XF10, 2XD1, 39F11, 84G9, and 4G6) and one complement-enhanced MAB (6G9) were used in competition inhibition assays versus several groups of MABs which did not exhibit virus neutral-

FIG. 3. Competition neutralization assay among VZV glycoproteins. Constant dilutions of competitive MABs (gpI, gpII, and gpIV) were reacted with cell-free virus, and then serial dilutions of neutralizing MABs (gpIII) were added to the mixture; after proper incubation, the virus mixture was used to infect cell monolayers. The percentage of plaque reduction on each dilution is considered to be virus neutralized as compared with normal mouse serum and cell culture media.

ization. As shown in Table 3 and illustrated in Fig. 4c, nonneutralizing MABs 2C11 and 54A8 inhibited the neutralizing activity of 84G9 by 100 and 65%, respectively, but had no effect on the other MABs of this group. This suggests that the epitopes of these two MABs are either the same or very closely situated. We tentatively designated the epitopes of these two MABs gpI-D2a and gpI-D2b. However, MAB 53H8 achieved low-to-moderate inhibition with every MAB in its group, while 54A8 had no effect on any other MAB. It appears likely that three additional overlapping epitopes, which we designated gpI-D2c, gpI-D2d, and gpI-D2e, and one nonoverlapping epitope, gpI-D2f, exist on this domain.

The epitope on which complement-enhanced MAB 6G9 directed against gpI was situated on the VZV virion was clearly distinct from the other two neutralizing domains. None of the gpI MABs which competed for antigenic domains of gpIII competed with this MAB. We designated this nonoverlapping antigenic domain of gpI antigenic domain 3 and the epitope gpI-D3a. MABs against gpII, gpIV, and other groups did not interfere with neutralization of gpI (Table 3).

Competitive inhibition with gpIV. We also attempted to map neutralizing epitopes of gpIV. The neutralizing MABs in this group, which were complement dependent, were inhibited by nonneutralizing MABs in the gpIV group (3B8 and 50E12) but not by nonneutralizing MABs directed against other VZV gps, except for two MABs from gpI (53H8 and 50F3) (Table 4; Fig. 4d). Conversely, the MABs in this group failed to compete with neutralizing MABs to other VZV gps (Tables 2 and 3). These results suggest the existence of at least one antigenic domain with two distinct epitopes, which we designated gpIV-1a and gpIV-1b.

Other workers have reported complement-independent (4, 34) or complement-dependent (5) neutralizing MABs directed against gpII. However, our MAB 3C7 to this protein had no demonstrable neutralizing activity and did not compete with neutralizing MABs to gpI, gpIII, or gpIV.

TABLE 2. Competitive inhibition with gpIII-neutralizing antibodies

Competing MAB group	Neutralization ^a	Clone	% Inhibition of neutralization ^b by:		Antigenic domain	Epi-tope(s)
			4B7	8G9		
gpI	C	3XF10	100	70	gpIII-D1	1a, 1b
		3G8	98	60		1a, 1b
		2XD1	70	60		1a, 1b
		39F11	33	50		1a, 1b
		84G9	33	0		1a
		4G6	25	0		1a
		4E6	60 ^c	0		1a
	N	53H8	40	40		1a, 1b
		2C11	33	0		1a
		54A8	25	0		1a
gpII	N	3C7	0	0		
gpIV	C	2 clones ^d	0	0		
	N	2 clones ^d	0	0		
Vp(98,36-43)	N	6 clones ^d	0	0		
Vp(30)	N	4G8	0	0		
Nonprecipitants		10 clones	0	0		

^a C, Complement-dependent neutralizing MAB; N, no neutralizing MAB.

^b Neutralization without complement.

^c Enhanced.

^d See Table 1.

FIG. 4. Computer graphics-generated diagram of competition neutralization assay of VZV glycoproteins. Panels: a and b, competition between gpIII and gpI; c, competition within gpI; d, competition between gpIV and gpI. Large circles indicate the individual domains of the glycoproteins; small circles represent locations of individual epitopes that bind to specific MABs.

TABLE 4. Competitive inhibition with gpIV-neutralizing antibodies

Competing MAb group ^a	Clone	% Inhibition of neutralization by:		Antigenic domain	Epitope(s)
		1B7	1F12		
gpI	53H8	100	60	gpIV-D1	1a, 1b
	54F3	60	0		1a
	2C11	0	0		
	54A8	0	0		
gpII	3C7	0	0		
gpIV	3B8	60	90	gpIV-D1	1a, 1b
	50E12	70	70		1a, 1b
Vp(98,36-43)	6 clones ^b	0	0		
Vp(30)	4GB	0	0		
Nonprecipitants	10 clones ^b	0	0		

^a No neutralizing MABs were used.

^b See Table 1.

DISCUSSION

Most epitope mapping of viral proteins has been performed by competitive-binding assays. Usually, a purified virion or viral antigen is fixed onto a solid matrix and reacted with the first MAB, and then the second enzyme- or radio-labeled MAB is applied (11, 13, 15–17, 19, 20, 29–31). More recently, a liquid-phase enzyme immunoassay has been described for epitope mapping (21, 22). The degree of binding inhibition or enhancement in the assay determines the relatedness of the epitopes. Several factors may influence the outcome; e.g., less-avid MABs may not bind

TABLE 3. Competitive inhibition with gpI-neutralizing MABs

Competing MAb group	Neutralization ^a	Clone	% Inhibition of neutralization ^b by:					Antigenic domain(s)	Epitope(s)
			84G9	39F11	2XD1	3XF10	4G6		
gpI	C	7 clones ^c						gpI-D1, gpI-D3	
	N	2C11	100	0	0	0	0	gpI-D2	2a
	N	53H8	30	75	50	47	66		2a, 2b, 2c
	N	54F3	0	65	0	0	0		2d, 2e
	N	54A8	0	0	0	0	0		2b
gpII	N	3C7	0	0	0	0	0		2f
gpIV	N	2 clones ^c	0	0	0	0	0		
	C	2 clones							
Vp(98,36-43)	N	6 clones ^c	0	0	0	0	0		
Vp(30)		4GB	0	0	0	0	0		
Nonprecipitants	N	10 clones ^c	0	0	0	0	0		

^a C, Complement-dependent neutralizing MAB; N, no neutralizing MAB.

^b There was no inhibition by 3G8, 4E6, and 6G9.

^c See Table 1.

efficiently or may elute readily because of excessive washing steps required by the assays. In most instances, the viral antigens are presented in modified form (inactivated or denatured), the detector signals are modified by labeling of the MAb, and little is known about the protein-plastic interaction.

However, the competition neutralization assay we used in our studies is a functional biologic assay; neither the virus nor the MAb is modified. The virus is biologically active (infectious), the neutralizing and competing MAbs are present in the solution simultaneously, and living cells are used as the detector signal. Our results clearly indicate that the assay is not influenced by the murine immunoglobulin subclass or the MAb titer, as determined by various virological assays (Table 1).

Most of the MAbs against the gpIII protein of VZV that have been described have been neutralizing MAbs which are complement independent. However, Montalvo and Grose (23) reported on a nonneutralizing MAb against this protein which was noncompetitive with neutralizing MAbs. We have now shown that complement-dependent and nonneutralizing MAbs to gpI interfere with the epitopes of gpIII, and one possible antigenic domain with two distinct epitopes which are functional in viral neutralization was identified. These results were reproducible in four different runs. The cell line producing these MAbs was recloned four times to ensure the purity of the MAbs (7), and thus, contamination with MAbs to gpIII cannot account for the blocking activity of gpI. We and others previously had determined the MWs of gpI and gpIII by SDS-PAGE; each protein migrates in different regions of the gel (7, 9, 14).

The possibility that MAbs 3G8 and 4E6, with high-titered neutralizing activities, are directed against different epitopes on gpI is suggested by their opposite effects in competition assays with MAbs 4B7 and 8G9 of gpIII. MAb 3G8 showed moderate-to-strong inhibition of neutralization of these MAbs, while MAb 4E6 enhanced the neutralizing activity of 4B7 by 60% but had no effect on neutralization by 8G9. MAbs which enhance infectivity or neutralization have been reported for several viruses (2, 16, 26).

We also used our panel of MAbs to gpI to map additional functional antigenic domains for gpI. The data in Table 3 and the schematic depiction in Fig. 4c indicate that gpI contains at least three distinct antigenic domains. Domain 1 may contain two complement-dependent nonoverlapping epitopes, domain 2 may contain at least five overlapping epitopes and one nonoverlapping epitope, and domain 3 contains at least one nonoverlapping, complement-enhanced neutralizing epitope.

The MAbs against VZV previously described appear to be either totally complement independent or totally complement dependent. One of our MAbs to gpI, 6G9, is interesting in that it has neutralizing activity which is enhanced by, but not dependent upon, complement. Competition assays showed that it is directed against an epitope distinct from those of the other neutralizing MAbs to gpI, and we considered this a third antigenic domain, since none of the MAbs in this or other major groups interfered with neutralization of 6G9.

We also attempted to determine which epitopes are functional in virus neutralization within the gpIV group. The results presented in Table 4 and the schematic depiction in Fig. 4d indicate the existence of at least one antigenic domain with two epitopes. It was also surprising that the nonneutralizing MAbs of gpI also competed strongly with gpIV MAbs. We postulated that the interference between

gpI and gpIV is probably similar to that between gpI and gpIII.

The interaction of the MAbs described in this report depicts their possible topographic relationship with the gps on the surface of the VZV virion. The antigenic domains that interact with gpI and gpIII may be spatially clustered (24). This was shown by competitive reactions between other MAbs and gpI and gpIII, as well as by interaction among the MAbs themselves. No competition between gpII and other gps has been shown, and they are presumed to be spatially separated from gpI, gpIII, gpIV, and each other. We did not have MAbs to gpV available for this study. To locate their relative positions with other gps will require preparation of additional MAbs.

Two alternative mechanisms have been proposed to explain interference between MAbs to a given protein in a competitive-binding assay: steric hindrance and conformational changes (10, 12, 18–20, 26, 31). The interference we have described is between antibodies directed against different gps and most likely is the result of one of these mechanisms. On whatever basis, the functional significance of these findings for vaccine development is clear and points out the desirability of using functional assays in addition to standard binding assays in assessing epitope relationships. This might be applicable to any viruses, whether the specific amino acid sequences that make up the antigenic domain are known or not.

ACKNOWLEDGMENTS

This study was supported by Public Health Service grant AI-15990 from the National Institute of Allergy and Infectious Diseases. We acknowledge the excellent computer graphics contributed by Joyce Meissinger.

LITERATURE CITED

1. Burnette, W. N. 1981. "Western blotting": electrophoretic transfer of proteins from sodium dodecyl sulfate-polyacrylamide gels to unmodified nitrocellulose and radiographic detection with antibody and radioiodinated protein A. *Anal. Biochem.* 112:195–203.
2. Chanas, A. C., E. A. Gould, J. C. S. Clegg, and M. G. R. Varma. 1982. Monoclonal antibodies to Sindbis virus glycoprotein E1 can neutralize, enhance infectivity and independently inhibit haemagglutination or haemolysis. *J. Gen. Virol.* 58:37–46.
3. Davison, A. J., C. M. Edson, R. W. Ellis, B. Forghani, D. Gilden, C. Grose, P. M. Keller, A. Vafai, Z. Wroblewska, and K. Yamanishi. 1986. New common nomenclature for glycoprotein genes of varicella-zoster virus and their glycosylated products. *J. Virol.* 57:1195–1197.
4. Edson, C. M., B. A. Hosler, C. A. Poodry, R. T. Schooley, D. J. Waters, and D. A. Thorley-Lawson. 1985. Varicella-zoster virus envelope glycoproteins: biochemical characterization and identification in clinical material. *Virology* 145:62–71.
5. Edson, C. M., B. A. Hosler, R. A. Respass, D. J. Waters, and D. A. Thorley-Lawson. 1985. Cross-reactivity between herpes simplex virus glycoprotein B and a 63,000-dalton varicella-zoster virus envelope glycoprotein. *J. Virol.* 56:333–336.
6. Forghani, B., and J. Dennis. 1989. Immunoenzymatic staining, p. 135–156. *In* N. J. Schmidt and R. W. Emmons (ed.), *Diagnostic procedures for viral, rickettsial and chlamydial infections*, 6th ed. American Public Health Association, Washington, D.C.
7. Forghani, B., K. W. Dupuis, and N. J. Schmidt. 1984. Varicella-zoster viral glycoproteins analyzed with monoclonal antibodies. *J. Virol.* 52:55–62.
8. Forghani, B., N. J. Schmidt, C. K. Myoraku, and D. Gallo. 1982. Serological reactivity of some monoclonal antibodies to varicella-zoster virus. *Arch. Virol.* 73:311–317.
9. Grose, C., D. P. Edwards, W. E. Friedrichs, K. A. Weigle, and

- W. L. McGuire. 1983. Monoclonal antibodies against three major glycoproteins of varicella-zoster virus. *Infect. Immun.* **40**:381-388.
10. Heinz, F. X. 1986. Epitope mapping of flavivirus glycoproteins. *Adv. Virus Res.* **31**:103-168.
11. Heinz, F. X., R. Berger, W. Tuma, and C. Kunz. 1983. A topological and functional model of epitopes on the structural glycoprotein of tick-borne encephalitis virus defined by monoclonal antibodies. *Virology* **126**:525-537.
12. Heinz, F. X., R. Berger, W. Tuma, and C. Kunz. 1983. Location of immunodominant antigenic determinants on fragments of tick-borne encephalitis virus glycoprotein: evidence for two different mechanisms by which antibodies mediate neutralization and hemagglutination inhibition. *Virology* **130**:485-501.
13. Henchal, E. A., L. S. Henchal, and B. K. Thaisomboonsuk. 1987. Topological mapping of unique epitopes on the dengue-2 virus NS1 protein using monoclonal antibodies. *J. Gen. Virol.* **68**:845-851.
14. Keller, P. M., B. J. Neff, and R. W. Ellis. 1984. Three major glycoprotein genes of varicella-zoster virus whose products have neutralization epitopes. *J. Virol.* **52**:293-297.
15. Kimura-Kuroda, J., and K. Yasui. 1983. Topographical analysis of antigenic determinants on envelope glycoprotein V3 (E) of Japanese encephalitis virus, using monoclonal antibodies. *J. Virol.* **45**:124-132.
16. Kingsford, L. 1984. Enhanced neutralization of La Crosse virus by the binding of specific pairs of monoclonal antibodies to the G1 protein. *Virology* **136**:265-273.
17. Kingsford, L., L. D. Ishizawa, and D. W. Hill. 1983. Biological activities of monoclonal antibodies reactive with antigenic sites mapped on the G1 glycoprotein of La Crosse virus. *Virology* **129**:443-455.
18. Lubeck, M., and W. Gerhard. 1982. Conformational changes at topologically distinct antigenic sites on the influenza A/PR/8/34 virus HA molecule are induced by the binding of monoclonal antibodies. *Virology* **118**:1-7.
19. Massey, R. J., and G. Schochetman. 1981. Viral epitopes and monoclonal antibodies: isolation of blocking antibodies that inhibit virus neutralization. *Science* **213**:447-449.
20. Massey, R. J., and G. Schochetman. 1981. Topographical analysis of viral epitopes using monoclonal antibody mechanism of virus neutralization. *Virology* **115**:20-32.
21. McCullough, K. C., J. R. Crowther, and R. N. Butcher. 1985. A liquid-phase ELISA and its use in the identification of epitopes on foot-and-mouth disease virus antigens. *J. Virol. Methods* **11**:329-338.
22. McCullough, K. C., J. R. Crowther, W. C. Carpenter, E. Brocchi, L. Capucci, F. De Simone, Q. Xie, and D. McCahon. 1987. Epitopes on foot-and-mouth disease virus particles. I. Topology. *Virology* **157**:516-525.
23. Montalvo, E. A., and C. Grose. 1986. Neutralization epitopes of varicella-zoster virus on native viral glycoprotein gp118 (VZV glycoprotein gpIII). *Virology* **149**:230-241.
24. Najjar, J. A., J. R. Gentsch, N. Nathanson, and F. Gonzalez-Scarano. 1985. Epitopes of the G1 glycoprotein of La Crosse virus form overlapping clusters within a single antigenic site. *Virology* **144**:426-432.
25. Okuno, T., K. Yamanishi, K. Shiraki, and M. Takahashi. 1983. Synthesis and processing of glycoproteins of varicella-zoster virus (VZV) as studied with monoclonal antibodies to VZV antigens. *Virology* **129**:357-368.
26. Parekh, B. S., and M. J. Buchheimer. 1986. Proteins of lymphocytic choriomeningitis virus: antigenic topography of the viral glycoproteins. *Virology* **153**:168-178.
27. Schmidt, N. J., and E. H. Lennette. 1975. Neutralizing antibody responses to varicella-zoster virus. *Infect. Immun.* **12**:606-613.
28. Schmidt, N. J., and E. H. Lennette. 1976. Improved yields of cell-free varicella-zoster virus. *Infect. Immun.* **14**:709-715.
29. Shaw, R. D., P. T. Vo, P. A. Offit, B. S. Coulson, and H. B. Greenberg. 1986. Antigenic mapping of the surface proteins of rhesus rotavirus. *Virology* **155**:434-451.
30. Spriggs, D. R., K. Kaye, and B. N. Fields. 1983. Topological analysis of the reovirus type 3 hemagglutinin. *Virology* **127**:220-224.
31. Stone, M. R., and R. C. Nowinski. 1980. Topological mapping of murine leukemia virus proteins by competition binding assays with monoclonal antibodies. *Virology* **100**:370-381.
32. Towbin, H., T. Staehelin, and J. Gordon. 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc. Natl. Acad. Sci. USA* **76**:4350-4354.
33. Vafai, A., Z. Wroblewska, R. Mahalingam, G. Cabirac, M. Wellish, M. Cisco, and D. Gilden. 1988. Recognition of similar epitopes on varicella-zoster virus gpI and gpIV by monoclonal antibodies. *J. Virol.* **62**:2544-2551.
34. Vafai, A., Z. Wroblewska, M. Wellish, M. Green, and D. Gilden. 1984. Analysis of three late varicella-zoster virus proteins, a 125,000-molecular-weight protein and gp1 and gp3. *J. Virol.* **52**:953-959.