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Characterization of a New Virus-neutralizing Epitope that Denotes a Sequential Determinant on the Rabies Virus Glycoprotein

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SUMMARY

Two new monoclonal antibodies (MAbs) derived from mice immunized with the Pitman-Moore (PM) strain of rabies virus were used to identify and characterize two unique antigenic determinants on the rabies virus glycoprotein. One of the determinants, which defined an additional antigenic site on the rabies virus glycoprotein, was delineated as a distinct epitope by the newly generated MAb, 6-15C4, in competitive binding studies and by comparative antigenic analysis of neutralization-resistant variant viruses. Both antigenic determinants were compared with the five previously described antigenic sites which bind virus-neutralizing antibodies on the challenge virus standard (CVS) and Evelyn-Rokitnicki-Abelseth (ERA) strain glycoproteins. The results presented in this communication show that the 6-15C4 epitope is the first epitope described in the rabies virus glycoprotein that does not depend on the native conformation of the glycoprotein for binding virus-neutralizing antibody. These data suggest that it may be possible to generate a synthetic peptide vaccine against rabies.

INTRODUCTION

Both humoral and cell-mediated immunity executed by antibodies directed against virus and antiviral T cell responses have been shown to contribute to the protection of animals against rabies virus infection (Wiktor *et al.*, 1974, 1977; Turner, 1985). Previous studies have demonstrated that virus-neutralizing antibodies are induced solely by the glycoprotein of rabies virus (Wiktor *et al.*, 1973, 1984; Cox *et al.*, 1977). In addition, several CNBr cleavage fragments of the rabies virus glycoprotein have been shown to be capable of inducing virus-neutralizing antibodies (Dietzschold *et al.*, 1982, 1983) and specific T cell responses (Macfarlan *et al.*, 1984). Studies of neutralization-resistant variant viruses selected in the presence of excess amounts of neutralizing monoclonal antibodies (MAbs) and competition binding assays using MAbs have provided evidence for at least three distinct antigenic sites on the glycoprotein of rabies virus in the challenge virus standard (CVS-11) strain and five in the Evelyn-Rokitnicki-Abelseth (ERA) strain (Lafon *et al.*, 1983, 1984). Many of these studies suggest that the virus-neutralizing antibodies preferentially recognize conformational epitopes derived from the secondary structure of the glycoprotein (Dietzschold *et al.*, 1982; Wunner *et al.*, 1985). In the present paper, we have further investigated the antigenic structure of the rabies virus glycoprotein, using additional MAbs recently generated in mice that were immunized with the Pitman-Moore (PM) strain of rabies virus. We present evidence for the existence of a novel conformation-independent antigenic determinant, which we have delineated as a separate antigenic site in the functional antigenic map of the glycoproteins of the CVS-11 and ERA rabies virus strains.

METHODS

Virus strains. CVS-11 and ERA strains of rabies virus and the rabies-related Duvenhage-6 (DUV-6) (Duvenhage strain of European Duvenhage bat virus) and Mokola (MOK) viruses were propagated in BHK-21 cell culture monolayers as previously described (Wiktor *et al.*, 1973). The PM strain of rabies virus was propagated in dog kidney cells. Viral antigens were prepared from concentrated, purified virus suspensions inactivated with β-propiolactone according to standard methods (Van Wezel *et al.*, 1978).

ELISA. Microtitre plates (Titertek Type III; Flow Laboratories) were coated with test material in phosphate-buffered saline (PBS) usually for 1 h at 37 °C unless specified. All further incubations were carried out in PBS containing 1% bovine serum albumin (BSA) (Organon Technika, Oss, The Netherlands) and 0·05% Tween 80. Incubations with antibodies or antigens were carried out at 37 °C for 1 h. Plates were developed for 10 min at room temperature using 100 µl/well of tetramethylbenzidine substrate solution (Bos *et al.*, 1981). The reaction was stopped with 1 M-H_2SO_4 and absorbance at 450 nm was read with a Titertek Multiskan (Flow Laboratories).

Generation of MAbs. Hybridomas were generated by fusion of immune spleen cells from BALB/c mice with P3-X63-Ag8.653 cells (Kearney *et al.*, 1979), essentially as previously described (Osterhaus *et al.*, 1982). Briefly, BALB/c mice were primed intraperitoneally with 150 µg of PM virus antigen. Rabies virus-specific antibody-secreting hybridomas were selected by ELISA using PM virus antigen-coated microtitre plates. Hybridomas were single cell-cloned and ultimately propagated as ascitic tumours in BALB/c mice. Antibodies were isolated from ascitic fluid by Protein A–Sepharose affinity chromatography (Pharmacia). Subclass determination and light chain composition of the MAbs were achieved using subclass-specific and light chain-specific goat anti-mouse antibody (Meloy Laboratories, Springfield, Va., U.S.A.) by the Ouchterlony double diffusion technique. Other MAbs used in this study constituted a panel of anti-glycoprotein antibodies generated by the Wistar Institute. These 40 MAbs, which were specific for the five distinct antigenic sites on the rabies virus glycoprotein, were derived from BALB/c mice immunized with several strains of rabies virus, including ERA and CVS-11, as previously described (Wiktor & Koprowski, 1978; Flamand *et al.*, 1980; Lafon *et al.*, 1983, 1984).

Membrane immunofluorescence assay. To select MAbs that specifically reacted with the rabies virus glycoprotein, the hybridoma culture fluids were tested in a membrane immunofluorescence assay using unfixed virus-infected (CVS-11 strain) BHK-21 cells as previously described (Wiktor & Koprowski, 1978).

SDS–PAGE and Western blot analysis. Purified rabies virus (50 to 100 µg) was boiled for 2 min in 100 µl of 1 M-Tris–HCl pH 8·2, 1% SDS, 100 mM-dithiothreitol to reduce disulphide bonds. The cysteinyl residues were then alkylated by addition of recrystallized iodoacetamide to a final concentration of 300 mM and incubated for 1 h at 37 °C. Salts were removed by dialysis against 0·1 M-NH_4HCO_3 and the protein sample was dried under vacuum. Electrophoresis of virus proteins was carried out in a discontinuous SDS–polyacrylamide gel system described by Laemmli (1970). The electrotransfer of the separated proteins from the resolving gel to nitrocellulose membranes (0·2 µm; Schleicher & Schuell) was carried out for 2 h at 90 V and 5 °C in electrotransfer buffer containing 25 mM-Tris–HCl pH 8·3, 20% methanol and 1 mM-dithiothreitol, essentially as previously described (Towbin *et al.*, 1979). After electrotransfer, the nitrocellulose membranes were prepared for immunostaining by blocking the nitrocellulose with 2% normal horse serum as previously described (Dietzschold *et al.*, 1987*a*). The blocked membranes were incubated for 1 h with MAb diluted in 0·01 M-phosphate buffer pH 7·5 containing 0·15 M-NaCl and 0·05% Tween 20 (TPBS) at room temperature. After incubation with MAb, the membranes were blocked again with 2% normal horse serum in TPBS and then incubated for 1 h with horseradish peroxidase-conjugated goat anti-mouse IgG complex (Organon Teknika-Cappel, Malvern, Pa., U.S.A.). The membranes were then washed in 50 mM-Tris–HCl pH 6·8, and incubated in freshly prepared substrate solution [10 ml 0·3% solution of 4-chloro-1-naphthol (Sigma) in methanol, 40 ml 50 mM-Tris–HCl pH 6·8 and 50 µl 30% H_2O_2] for colour development as previously described (Dietzschold *et al.*, 1987*a*).

Rapid fluorescent focus inhibition test (*RFFIT*). Virus-neutralizing activity of MAbs was assayed in an RFFIT as previously described (Wiktor & Koprowski, 1978).

Competitive binding assay. Purified anti-glycoprotein MAbs were coupled to horseradish peroxidase (HRP; Type VI, Sigma) according to standard methods (Nakane & Kawaoi, 1974). Competitive binding experiments were carried out by ELISA. Protein A-purified MAb or ascitic fluid at various concentrations were added to PM virus antigen-coated wells (600 ng/well). After incubation and washing, 100 µl/well of anti-glycoprotein HRP-conjugated MAbs were added at dilutions that were known to give 90% of the maximum absorbance value on PM virus antigen. Inhibition values were expressed as percentages of homologous competition according to the formula $\{[A_{450}\text{ (uninhibited)} - A_{450}\text{ (test)}^{ht}]/A_{450}\text{ (uninhibited)}\} \times \{[A_{450}\text{ (uninhibited)}^{hm}]/[A_{450}\text{ (uninhibited)} - A_{450}\text{ (test)}]\} \times 100$, where ht and hm are the heterologous and homologous samples respectively.

Rabies virus variant analysis. Two anti-glycoprotein MAbs (6-15C4 and 2-22C5) were used to select neutralization-resistant variant viruses of the CVS-11 strain of rabies virus as described elsewhere (Wiktor & Koprowski, 1980). These viruses are designated CVS-11 RV 6-15C4 and CVS-11 RV 2-22C5, respectively. In addition, a panel of previously described neutralization-resistant CVS-11 and ERA virus variants (Lafon *et al.*, 1983, 1984) and a sequential (seventh generation) variant selected from the CVS-11 strain (CVS-11 RV-7)

(Dietzschold *et al.*, 1987*b*) were used in this study. The neutralizing effect of the MAbs on the antigenic variants and on the parental CVS-11 and ERA virus was evaluated by determining the virus-neutralizing index; a difference in the virus titres in the presence and absence of antibody of at least 2 $\log_{10}$ units was considered evidence for virus neutralization (Wiktor *et al.*, 1978).

RESULTS

Specificities of newly generated anti-rabies virus MAbs

From a total of six fusions, 46 stable hybridomas were established which produced anti-rabies virus MAbs as detected by ELISA (data not shown). Of these, five hybridomas [MAb 1-10B8 (IgG1, κ), MAb 3-7B6 (IgG1, κ), MAb 1-11D6 (IgG1, κ), MAb 2-22C5 (IgG1, κ), and MAb 6-15C4 (IgG2b, κ)] reacted with unfixed rabies virus-infected cells in the membrane immunofluorescence assay (data not shown) suggesting that they recognize the rabies virus glycoprotein. All five of the putative anti-glycoprotein MAbs neutralized the CVS-11 and ERA strains of rabies virus, confirming that these MAbs reacted with the surface glycoprotein of the virus as well as the glycoprotein on the surface membrane of virus-infected cells. Table 1 shows the results of CVS-11 and ERA virus neutralization by two of the MAbs, 2-22C5 and 6-15C4. These antibodies failed to neutralize the rabies-related DUV-6 virus in the RFFIT, while only MAb 2-22C5 neutralized (at a low titre) the rabies-related MOK virus.

The specificity of MAbs 2-22C5 and 6-15C4 was analysed by direct binding to proteins of the CVS-11 rabies virus variants, RV 2-22C5 and RV 6-15C4 (see below), in a Western blot assay (Fig. 1). MAb 6-15C4 showed reactivity with the two glycoprotein forms (G1 and G2) of CVS-11 RV 2-22C5, but not the glycoprotein molecules of CVS-11 RV 6-15C4. In contrast, MAb 2-22C5 showed reactivity with both forms of the glycoprotein (G1 and G2) of CVS-11 RV 6-15C4 and with the same proteins of CVS-11 RV 2-22C5. Since precautions were taken to prevent renaturation of the viral proteins by reducing the glycoprotein with dithiothreitol and alkylating with iodoacetamide prior to electrophoresis, the data suggest that these two MAbs recognize conformation-independent epitopes.

Comparison of neutralization-resistant variants CVS-11 RV 2-22C5 and CVS-11 RV 6-15C4 with previously characterized rabies variant viruses

Virus-neutralizing MAbs 6-15C4 and 2-22C5, which were used to select the neutralization-resistant viruses, CVS-11 RV 6-15C4 and CVS-11 RV 2-22C5, were tested against a panel of previously characterized neutralization-resistant antigenic variants selected with MAbs that delineated numerous epitopes in antigenic sites I to IV of the rabies virus glycoprotein. Both MAbs, 6-15C4 and 2-22C5, neutralized all of the antigenic variants tested in which a single epitope was affected, and a multiple variant virus of CVS-11 strain, CVS-11 RV V7, in which seven epitopes were affected (Table 2). Only the homologous antibodies used for selection of the rabies antigenic variants failed to neutralize their respectively selected variants, while none of the antibodies representing antigenic sites I to III of the CVS-11 strain or sites I to V of the ERA strain were able to neutralize CVS-11 RV V7. This indicates that the epitopes that bind MAbs 6-15C4 and 2-22C5 are different from epitopes previously identified on the rabies virus glycoprotein. The reactivities of the neutralization-resistant variant viruses, CVS-11 RV 6-15C4 and CVS-11 RV 2-22C5, against the panel of anti-glycoprotein MAbs were also determined by neutralization tests (Table 3). The CVS-11 RV 6-15C4 variant was neutralized by all of the MAbs that also neutralized the CVS-11 parent strain, except the selecting MAb 6-15C4, confirming that the corresponding epitope is in an antigenic site that is different from those previously described. In contrast, variant CVS-11 RV 2-22C5 not only resisted neutralization by the MAb 2-22C5 which was used for selection of RV 2-22C5, but it also could not be effectively neutralized (>2 $\log_{10}$ reduction) by MAbs 509-6 (site I), 220-8 (site II), 240-3, 718-4, 904-4 and 1108-1 (site III) and 1120-1 (site V) which neutralized the parent virus by more than 2 $\log_{10}$ units, indicating that, unlike the other variants, the altered antigenicity of the glycoprotein of this variant virus affects more than a single epitope.

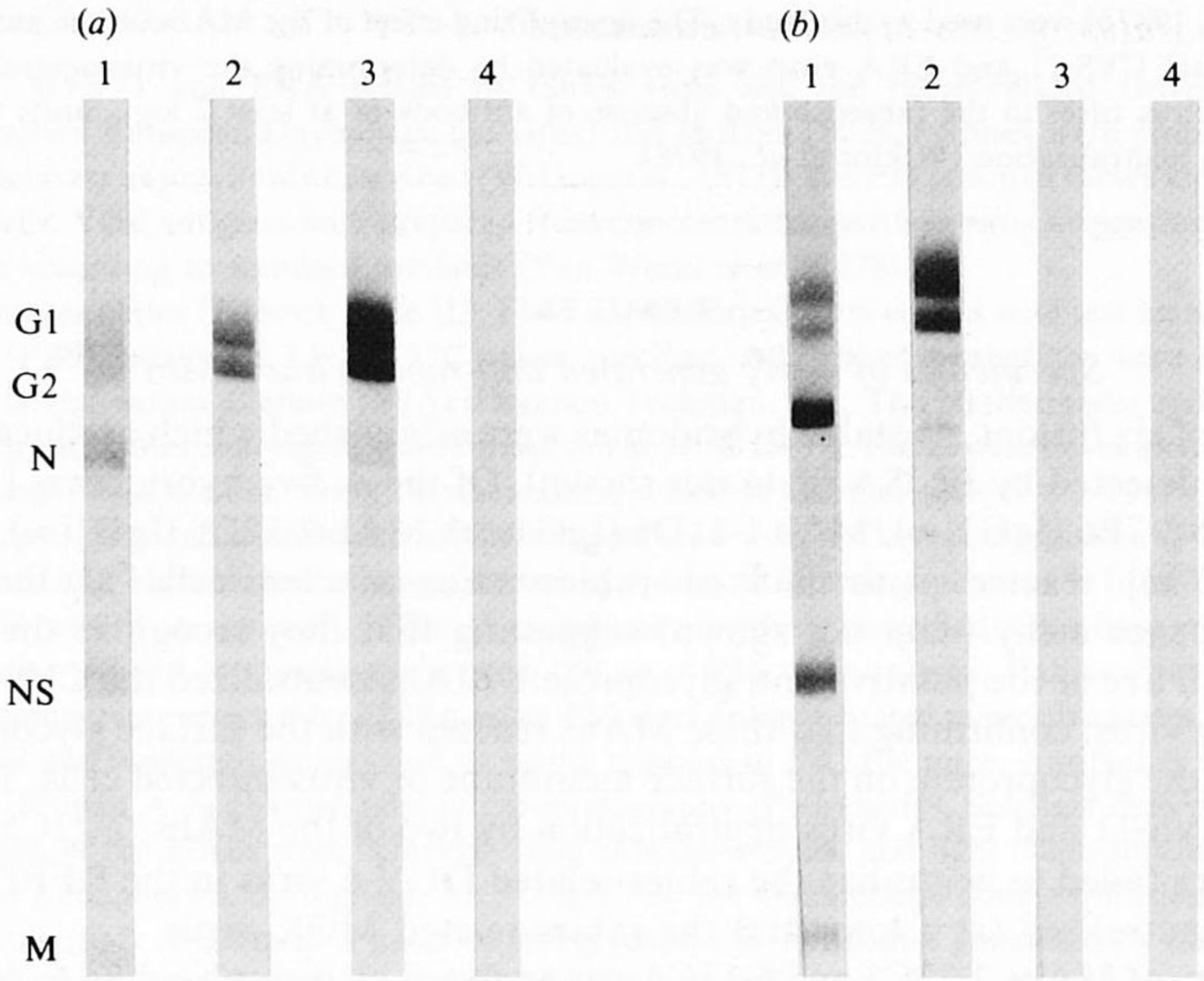

Fig. 1. Western blot analysis of newly generated anti-glycoprotein MAbs in ascites fluid binding to glycoprotein antigen of CVS-11 RV 2-22C5 (*a*) and CVS-11 RV 6-15C4 (*b*). Rabies virus glycoprotein (G1 and G2), nucleoprotein (N), phosphoprotein (NS) and matrix protein (M) detected by polyclonal rabbit anti-rabies virus antibodies (lanes 1), MAb 2-22C5 (lanes 2), MAb 6-15C4 (lanes 3) and control ascites fluid (lanes 4).

Table 1. *Neutralizing activity* of newly generated MAbs against rabies and rabies-related viruses*

	Rabies virus strain		Rabies-related virus	
MAb	CVS-11	ERA	DUV-6	MOK
2-22C5	>59049	19683	<3	243
6-15C4	6561	6561	<3	<3

* Reciprocal dilution of MAbs capable of reducing virus titre by 2 $\log_{10}$.

Table 2. *Neutralization of various neutralization escape variant rabies viruses with MAbs 2-22C5 and 6-15C4*

		Neutralization index*		
Antigenic site	Rabies virus variant	Homologous MAb†	MAb 2-22C5	MAb 6-15C4
I	CVS-11 RV 509-6	0·0	4·4	2·6
II	ERA RV 231-22	0·0	2·1	3·5
	ERA RV 101-1	0·0	2·1	2·8
	ERA RV 162-3	0·0	2·3	3·0
III	ERA RV 194-2	0·0	2·0	2·6
	ERA RV 718-4	0·0	2·1	3·9
	ERA RV 507-1	0·0	2·1	4·0
	ERA RV 176-2	0·0	2·8	2·5
	ERA RV 193-2	0·0	2·7	3·4
IV	ERA RV 110-3	0·0	2·6	3·3
	CVS-11 RV V7‡	0·0	2·7	2·9

* Neutralization index was calculated as described by Wiktor & Koprowski (1978). A titre reduction by a given MAb of 2·0 $\log_{10}$ or more is regarded as neutralization.

† Homologous MAb is the MAb used for the selection of each of the rabies variant viruses.

‡ CVS-11 RV V7 was not neutralized by any MAb representing antigenic sites I to III of CVS-11 virus, and sites I to V of ERA virus.

Table 3. *Neutralization of CVS-11 and variant viruses CVS-11 RV 2-22C5 and CVS-11 RV 6-15C4 with the panel of anti-glycoprotein antibodies*

		Neutralization index*		
Antigenic site	MAb	CVS-11	CVS-11 RV 2-22C5	CVS-11 RV 6-16C4
I	509-6	>3·0	1·5	>3·0
II	231-22	>3·0	>3·0	>3·0
	220-8	>3·0	1·8	>3·0
	1119-14	1·6	1·3	0·7
	1107-1	>3·0	>3·0	2·7
	101-1	>3·0	>3·0	>3·0
	162-3	>3·0	2·8	>3·0
	719-3	>3·0	2·8	>3·0
	1116-1	>3·0	2·8	>3·0
	1121-2	>3·0	>3·0	>3·0
	1111-1	>3·0	>3·0	>3·0
III	1112-1	>3·0	2·8	>3·0
	613-1	>3·0	2·1	>3·0
	1117-8	1·8	1·4	0·8
	240-3	>3·0	1·7	>3·0
	226-1	0·4	0·4	0·7
	194-2	>3·0	2·8	>3·0
	248-8	>3·0	>3·0	>3·0
	523-11	>3·0	>3·0	>3·0
	1105-3	>3·0	>3·0	>3·0
	1113-1	>3·0	>3·0	>3·0
	1122-3	1·0	0·7	1·0
	718-4	>3·0	0·8	>3·0
	1109-3	>3·0	>3·0	>3·0
	1114-2	>3·0	>3·0	>3·0
	507-1	>3·0	2·8	>3·0
	120-6	0·1	0·4	0·2
	1103-4	0·0	0·1	0·0
	127-5	0·0	0·4	0·0
	904-4	2·1	0·7	2·5
	176-2	1·4	0·5	0·2
	193-2	1·0	0·4	1·3
	1108-1	2·0	0·4	1·0
IV	110-3	0·9	0·7	1·0
V	1120-1	2·6	1·8	2·4
Uncl.†	1118-6	1·7	1·4	0·3
	504-1	0·3	0·2	0·3
	508-9	0·3	0·1	0·9
	419-2	0·3	0·4	0·4
	422-2	0·3	0·4	0·3
Unknown	2-22C5	>3·0	0·2	>3·0
	6-15C4	>3·0	1·1	0·0

* Neutralization index for CVS-11 parent virus and the variant viruses RV 2-22C5 and RV 6-15C4 was calculated as described (Wiktor & Koprowski, 1978). A titre reduction by a given MAb of 2·0 $\log_{10}$ or more is regarded as neutralization.

† Uncl., Unclassified.

Mapping of new antigenic sites by competitive binding assays

To determine further whether the epitopes recognized by MAbs 6-15C4 and 2-22C5 could be mapped within any of the known antigenic sites or to a unique antigenic site on the rabies virus glycoprotein, a competitive binding assay which examines mutual immunoreactivities among MAbs was used. For this assay, the antigen used to measure antibody binding was the same as that used to immunize mice for production of the newly generated MAbs. Out of the original panel of 40 anti-glycoprotein MAbs previously described (Wiktor & Koprowski, 1978; Flamand *et al.*, 1980; Lafon *et al.*, 1983, 1984), 17 did not react with the PM virus antigen in the ELISA test, nor could they neutralize PM virus (data not shown). The 23 remaining MAbs from the

Table 4. *Antigenic site mapping of newly generated anti-glycoprotein MAbs in competitive binding assay*

Antigenic site	Inhibitor MAb*	HRP-conjugated MAb†	
		2-22C5	6-15C4
Unknown	2-22C5	+ + +	–
	6-15C4	–	+ + +
I	509-6	+ + +	–
II	1119-14	+	–
	1107-1	–	–
	1121-2	–	–
III	1112-1	–	–
	613-2	–	–
	226-11	–	–
	194-2	–	–
	1105-3	–	+
	1113-1	–	–
	1122-3	+	+
	718-4	–	–
	1109-3	–	–
	1114-2	–	–
	507-1	–	–
	120-6	+	–
	1103-4	–	–
	176-2	+	+
	193-2	+	–
	1108-1	–	–
IV	110-3	+	–
V	1120-10	+	–
Uncl.‡	1118-6	+	–

* Dilution 1:10.

† + + +, 85 to 100% homologous inhibition; +, 40 to 60% homologous inhibition; –, ⩽40% homologous inhibition.

‡ Uncl., Unclassified.

panel were tested as competitors for the binding of HRP-conjugated MAbs 2-22C5 and 6-15C4 to plate-bound PM virus antigen (Table 4). Of the panel of 17 MAbs, only MAb 509-6, specific for antigenic site I, competed effectively (85 to 100% inhibition) with the binding of HRP-conjugated MAb 2-22C5 to rabies virus (PM strain); the same level of inhibition was obtained with the homologous antibody as control. The binding of HRP-conjugated MAb 2-22C5 was either uninhibited or inhibited to a much lower extent (<60%) by MAbs defining other antigenic sites. The data suggest that the epitope recognized by MAb 2-22C5 resides in antigenic site I. None of the MAbs, apart from the homologous MAb 6-15C4, inhibited the binding of HRP-conjugated MAb 6-15C4 to rabies virus, indicating that the epitope delineated by this antibody is distinct from all previously recognized epitopes.

DISCUSSION

In this paper, we have examined the antigenic structure of the rabies virus glycoprotein with an extended panel of anti-glycoprotein MAbs. The additional mouse MAbs, two of which were used in this study, were generated against the PM strain of rabies virus, but they reacted at high titres with the ERA and CVS-11 strains (Table 1). Before this study, the antigenic structure of the rabies virus glycoprotein was analysed using a panel of virus-neutralizing MAbs and a number of laboratory-selected mutant viruses, which allowed the description of at least 12 unique epitopes on the glycoprotein of the CVS-11 strain and 17 epitopes on the glycoprotein of the ERA strain of the rabies virus (Lafon *et al.*, 1983, 1984). These permitted the delineation of three functionally independent antigenic sites on the glycoprotein that are common to the CVS-11 and ERA strains (type-common sites, I to III) and two additional independent sites on the ERA strain (type-specific sites, IV and V). With the additional MAbs described in this paper,

we present evidence for the existence of another antigenic site on the glycoprotein of the CVS-11 and ERA strains, defined by MAb 6-15C4 (antigenic site VI), which is both independent and unique. By comparative neutralization analysis of the pre-existing and newly generated antigenic variants of the CVS-11 and ERA strains of rabies virus, it was shown that the epitope defined by MAb 6-15C4 (i) was present in all other antigenic variants that define the remaining epitopes on the glycoprotein recognized by the panel of virus-neutralizing MAbs (Table 2) and (ii) that all MAbs from the extended panel of antibodies, except the homologous 6-15C4 virus-neutralizing antibody, that neutralized the parent virus also neutralized the variant CVS-11 RV 6-15C4 (Table 3). These findings clearly indicate that the epitope recognized by this MAb resides in a novel, not previously defined, antigenic site. The competitive binding experiments, in which MAb 6-15C4 when conjugated to HRP could not be inhibited significantly by any of the MAbs directed to any of the previously recognized antigenic sites, further strengthened this conclusion. Since not all MAbs used in this study had been raised against the same virus strain, the bias in these competition assays towards a better reactivity of the PM virus-derived MAbs with the homologous PM virus antigen in the coated well of the assay plate cannot fully be excluded, although the reactivity of the HRP-conjugated MAb 6-15C4 was also measured with a heterologous antigen coating the well and no appreciable difference was detected (data not shown).

Analysis of the MAb 2-22C5, which in the competition binding assay recognized an epitope that appeared to be located within antigenic site I (Table 4), showed that MAb 2-22C5 neutralized all the other variant viruses, including the representative variant of antigenic site I, CVS-11 RV 509-6, which was neutralized to the greatest extent (Table 2). However, when MAbs defining other epitopes, including MAb 509-6 which defines antigenic site I, were tested for neutralization of CVS-11 RV 2-22C5, many were unable to neutralize the variant virus as efficiently as the parent virus (Table 3). This indicated that the alteration in the epitope recognized by MAb 2-22C5 not only affected the putative site I location but also affected epitopes within the antigenic sites II and III. Therefore, it is difficult to define operationally the epitope represented by MAb 2-22C5 and map it to any of the known antigenic sites. It is interesting to note that the glycoprotein of CVS-11 RV 2-22C5, which resists neutralization by MAb 2-22C5, still binds this MAb in Western blot analysis (Fig. 1).

The most important result in this study was the observation that MAbs 6-15C4 and 2-22C5 recognized the reduced and alkylated rabies virus glycoprotein in the Western blot analysis. It represents the first identification of an epitope in the rabies virus glycoprotein that appears to be linear and independent of structural conformation in this viral protein; all previously identified epitopes in the rabies virus glycoprotein have been sensitive to mild denaturation and unrecognizable after SDS–PAGE (Dietzschold *et al.*, 1982; Wunner *et al.*, 1985). The apparent conformation-independent nature of the epitope recognized by MAb 6-15C4 has been further confirmed (in a separate study to map the epitope within the primary structure of the glycoprotein) by showing that it reacted with a CNBr cleavage fragment of the rabies virus glycoprotein in a Western blot (data not shown). The identification of a linear epitope within the amino acid sequence of the glycoprotein molecule has prompted us to reconsider the importance of such a determinant in the generation of a peptide vaccine that would be effective against the challenge of rabies virus.

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