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Evidence for more than one important, neutralizing site on foot-and-mouth disease virus

Brief Report

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Summary. Using polyclonal sera raised against foot-and-mouth disease virus in susceptible animals, evidence was obtained for the existence of at least one further important antigenic site in addition to the neutralizing site on VP1 140–160.

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Protection against FMDV challenge may be particularly related to antibodies in polyclonal sera that compete with neutralizing monoclonal antibodies (MAs). Improved knowledge about the antigenic sites and the peptides to which these MAs bind may help in the development of a synthetic peptide vaccine against FMDV.

Monoclonal antibody technology provides an excellent means for locating antigenic sites on the virion surface. Neutralization panels of MAs revealed four antigenic sites on rhinovirus 14 [13] and three on polioviruses [11]. Baxt et al. classified a panel of neutralizing MAs against foot-and-mouth disease virus (FMDV) A_{12} into three groups by studying virus adsorption to host cells, virus aggregation, and the efficiency of virus neutralization [1]. Two groups of MAs bound to isolated VP1; the binding of the third group was conformation-specific and the site to which these MAs bound was probably similar to the site described for type 0 that was placed on the VP2/VP3 borderline [1, 9].

For FMDV, emphasis has been put on the amino acids 140–160 of VP1; this antigenic site has been considered important for the induction of protective antibodies [3, 4, 12, 15]. However, by measuring the reactivity of MAs against synthetic peptides, epitopes were shown to be scattered over the entire viral surface [9]. The relevance of these sites in susceptible animals, however, has yet to be established.

Question arises as to the relationship between and significance of members of a panel of neutralizing MAs as compared with the neutralizing antibodies

Table 1. Competition of polyclonal sera for the binding of monoclonal antibodies to FMDV

Antibody	MNT[a] titre	Reactivity in ELISA with trypsin-virus[b]	Competition[c] in ELISA with polyclonal serum of:				
			Convalesc. bovine	Rabbit[d] 1	Rabbit[d] 2	Convalesc. swine	Guinea pig[e]
1 MA4[f]	3.4	—	+ + +	+ + +	+ + +	+ + +	+ + +
MA5	3.7	—	+ + +	+ + +	+ + +	+ + +	+ + +
MA6	4.0	—	+ + +	+ + +	+ + +	+ + +	+ + +
MA11	4.5	—	+ +	+ +	+ +	+ +	+ + +
MA14	4.0	—	+ + +	+ + +	+ +	+ + +	+ + +
2 MA13	3.5	—	—	—	—	+	—
MA18	3.5	—	+	+	+	+ +	+ +
3 MA7	3.7	+	+ + +	+ + +	+ + +	+ + +	+ + +
MA9	6.0	+	+ + +	+ + +	+ + +	+ + +	+ + +
MA10	3.7	+	+	—	+	+	+
MA17	4.0	+	+ + +	+ +	+ +	+ +	+ +
Bovine	4.3	+					
Rabbit 1	2.9	+					
Rabbit 2	2.3	+					
Swine	3.0	+					
Guinea pig	3.2	+					

[a] The virus neutralization titre was determined in a micro-neutralization test [6]. The titres were given as the negative log10 of the ascites fluid dilution giving 50% neutralization

[b] ELISA plates were coated with protein A-purified polyclonal IgG, raised against FMDV. The titre was determined in ELISA with native and trypsin-treated virus. + Less than 0.5 log10 difference

[c] The competition was calculated by summing the deflection from 0% (no competition) for each serum dilution and normalizing the resulting values to the value for the best competing MA, which is defined as 100% (+ + +). — Less than 25%; + 25–50%; + + 50–75%; + + + 75–100% competition

[d] Rabbit sera against FMDV A_{10} were prepared as described [6]

[e] FMDV A_{10} was adapted to guinea pigs by repeated passage. Then guinea pigs were injected six times with virus, subcutaneously and intraveneously at 3-day intervals

[f] Monoclonal antibodies against FMDV A_{10} were prepared essentially as described for FMDV O1 [7]

in a polyclonal serum [2]. An estimation of the quantity of serum antibodies binding to each antigenic site would help to evaluate the importance of each site and the relevance of the MAs. In this study, competition assays were performed with several polyclonal antisera from susceptible animals and members of a panel of eleven neutralizing MAs against FMDV subtype A_{10}. The purpose was to investigate whether the MAs were able to compete with polyclonal antibodies, and thereby to provide a better understanding of the relevance of each antigenic site recognized.

A similar approach was followed for Hepatitis A virus [14]. The study showed that binding of human convalescent antibodies was reduced by adding monoclonal antibodies, thus indicating the immunodominance of the site to which these antibodies were directed [14].

The polyclonal antibodies used in this study were tested by an ELISA with either FMDV A_{10} or trypsin-treated virus bound to the plate as described [6]. All polyclonal sera had only a slightly reduced titre against trypsin-treated virus (Table 1). The binding of the majority of the MAs was strongly reduced if FMDV had been trypsin-treated (MA4, 5, 6, 11, 13, 14, and 18), whereas the binding of others was hardly affected. Our panel was thus divided into two main groups. Furthermore, a subdivision was made in the trypsin-sensitive group. MA13 and MA18 strongly and mutually competed for binding to coated FMDV in ELISA (unpublished) and were shown to bind to the C-terminal part of VP1; in contrast, the others from this group all bind to or near the VP1 140–160 trypsin-sensitive sequence [10 and in preparation].

The dilution of each monoclonal antibody that gave just maximal binding

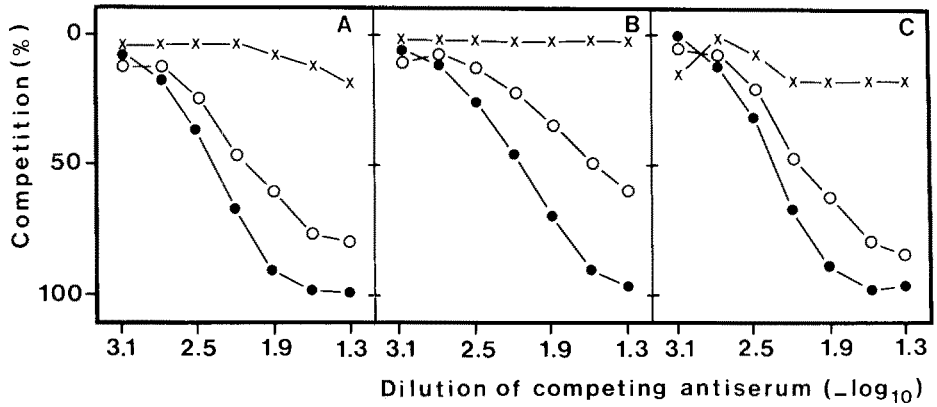

Fig. 1. Competition of polyclonal sera for the binding of monoclonal antibodies to FMDV. MAs were titrated by ELISA. An amount corresponding to a just maximal optical density was mixed with the indicated dilutions of bovine antiserum (**A**), rabbit antiserum (**B**) or guinea pig antiserum (**C**). The mixtures were applied onto virus-coated microtiter plates. After adding conjugate (goat immunoglobulin raised against mouse antibodies and linked to peroxidase) and ortho-phenylene diamine/H_2O_2, the optical densities were read and expressed as a percentage of the optical density obtained with MA alone. × MA13, ○ MA11, and ● MA4

in ELISA with native virus was measured. Monoclonal antibodies at these dilutions were mixed with variable amounts of a polyclonal serum and the mixtures were added to FMDV-coated plates. The residual binding of monoclonal antibody was measured. Figure 1 shows some typical results. The binding of MA4 was readily surpassed by all three polyclonal sera, whereas the binding of MA13 was hardly affected even in the presence of large excess of serum. MA11 behaved in an intermediate fashion. To present the results from all monoclonal antibodies and the five competing sera, the competition was calculated by summing the deflection from 0% (no competition) for each serum dilution and relating the resulting values to the value for the best competing MA (Table 1).

Similar results were obtained with all polyclonal sera tested. The binding of MA13 was hardly influenced by the addition of polyclonal serum. The binding of MA10 and MA18 was lowered to 30–40%, whereas MA11 and MA17 competed about half as much as the best competing MAs (MA 4, 5, 6, 7, 9, and 14). The results shown in Table 1 suggest that polyclonal sera contain antibodies that compete with the monoclonal antibodies for binding to the virus. Table 1 further shows that no correlation exists between the binding of MAs in the presence of polyclonal sera and the neutralization titre of each MA.

It may be argued that competition with polyclonal sera is not only dependent on the concentration of antibodies in sera and ascitic fluids but on the affinity of both types of antibodies as well. Absence of competition could, therefore, be due to a high affinity of the noncompeting MAs.

The relative affinities of MAs towards FMDV were measured as described [16]. The results indicated the highest affinities for MA10, 11, 13, and 18, followed by MA4 and 9, which were higher than MA5 and 14. The lowest affinities were measured for MA6, 7, and 17. This means that although the affinities of MA10 and MA11 are very similar, their competing ability is quite different (Table 1). Furthermore, the competition of MA11 and MA17 was comparable, whereas their affinities were at the extremes.

Thus, the difference in competing ability between MAs cannot be explained by their affinities alone, but reflects the concentration of competing antibodies in polyclonal sera. That MA10, 11, 13, and 18—two of which are bad competitors—have the highest affinities may be explained by the fact that only MA13 and 18 (and MA11) strongly bind to synthetic peptides [10 and in preparation]. This implies that MAs binding to sequential epitopes are poorly represented in polyclonal sera.

The results strongly suggest two important antigenic sites on FMDV: the first one, represented by group 1 MAs, involves VP1 140–160, the second one, represented by group 3 MAs probably lies outside VP1 as these MAs bind to trypsin-treated virus (Table 1). Work is in progress to map mutations of non-neutralizable variants raised agaisnt these MAs and preliminary results indicate that group 3 MAs bind to an antigenic site not related to the site of group 1 MAs.

Species specificity was not observed in this study. Icenogle et al. showed the immunodominance of a site around amino acid 100 of VP1 of poliovirus type 3 [5]. This dominance, however, could only be established in mouse sera and not in sera from other species [5]. Although no competition could be performed with mouse antiserum under the experimental conditions, the latter serum behaved not differently from other sera in binding to native and trypsinized virus (not shown).

Uniformity of reactions of the different sera in the competition experiments leads to the following conclusions:

First, MAs of two competition groups had polyclonal counterparts. The exceptions were MA13 and MA18, either because they were not represented in the sera, or the polyclonal antibodies had a low affinity for the MA13/MA18 site.

Although MA13 and MA18 are able to neutralize FMDV, the site to which MA13 and MA18 bind apparently is unimportant in the induction of neutralizing activity in polyclonal antisera raised against whole virus. This finding is consistent with the earlier observation that this minor neutralizing site, located at the C-terminus of VP1, is responsible for the induction of neutralizing activity of isolated VP1 [8].

An antigenic site that is recognized by MA13 and 18 is presumably less relevant for peptide vaccine development, since very little antibody with an affinity comparable to that of MA13 and 18 is present in polyclonal sera from susceptible animals.

Second, important neutralizing sites of FMDV, as defined by polyclonal sera raised in susceptible species, are not restricted to trypsin-sensitive sites, such as site 140–160 in VP1.

The additional site, defined by group 3 MAs and probably located on VP2 or VP3, may be an important and thus far unrecognized candidate for synthetic peptide vaccines.

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