CHARACTERIZATION OF MONOCLONAL ANTIBODIES AGAINST BROAD BEAN STAIN AND RED CLOVER MOTTLE VIRUSES

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Summary. – Seven monoclonal antibodies (MoAbs) against red clover mottle comovirus (RCMV) and/or broad bean stain comovirus (BBSV) were characterized and used for epitope comparison of both viruses. All tested MoAbs, exclusively of IgG class, were directed to detergent-stable epitopes (metatopes and cryptotopes). Two of them were species-specific, while five others cross-reacted with both viruses to different extent. On the basis of the results of competitive ELISA, we assume different mutual position of common linear epitopes in RCMV and BBSV. They are more closely "packed up" on BBSV, while the BBSV-specific metatope is markedly dominant. The investigation of other RCMV and BBSV isolates by use of our MoAbs confirmed close relationship between both viruses and even showed that it is difficult to determine unambiguously the species of these isolates using immunochemical methods only.

Key words: broad bean stain comovirus; red clover mottle comovirus; monoclonal antibodies; ELISA; competition binding test

Introduction

Owing to high yields either in host plants or in protoplasts the comoviruses represent an appropriate model for investigation of plant virus replication mechanisms, virus – host relations and isometric viral particle structure as well.

Comoviruses are very good immunogens. Close serological relatedness between the RCMV and BBSV (Bruening, 1978) was confirmed using polyclonal antibodies (Musil *et al.*, 1983, Gallo and Musil, 1988, Musil and Gallo, 1993). Also some of MoAbs prepared by us have cross-reacted with both viruses. These MoAbs were directed against detergent-stable linear epitopes localized on analogical V8 protease-digests of the larger coat proteins of RCMV and BBSV, respectively (Šubr *et al.*, 1993).

In this study we were interested in comparison of MoAbs binding abilities to their homologous and heterologous antigens in different ELISA arrangements. Moreover, the reactivity of different RCMV and BBSV isolates with the MoAbs could show how much the respective epitopes are conserved in both viruses.

Materials and Methods

Antigens. RCMV (TpM36 isolate, Musil and Gallo, 1984) and BBSV (VsM isolate, Musil *et al.*, 1978) were propagated, purified and stored as described (Šubr *et al.*, 1993). Some other isolates of RCMV (TpM25, No. 1, 2, 3, 8, 9, 10, 11, 14, 16, 19, 20 – Musil and Gallo, 1984) and BBSV (VsM-Š, F1 – Musil and Gallo, 1986), obtained from Dr. Musil, were used for screening of the MoAbs specifity. For ELISA of viral proteins the viruses were disintegrated by boiling (5 mins) in the presence of 1% SDS and 1% 2-mercaptoethanol.

MoAbs 1 – 7 (Šubr *et al.*, 1993) were prepared according to Gallo and Matisová (1993) by immunization of Balb/c mice with RCMV (MoAbs 2 – 5) and BBSV (MoAbs 1, 6, 7), respectively. The MoAbs isotypization was performed using the ISO-2 (Sigma) ELISA kit.

Immunochemical analyses. Plate-trapped antigen (PTA) ELISA, antibody-trapped antigen (ATA) ELISA, competition binding tests (CBT) and double diffusion tests in agarose gel (Agarose C, Pharmacia) were done according to Gallo and Matisová (1993). In PTA ELISA virus isolates or disintegrated viruses (1 mg/ml) diluted 1:1000 were used as the coating layer. In ATA ELISA anti-BBSV (VsM) and anti-RCMV (TpM36) antisera diluted 1:500 were used as the coating antibodies (Musil *et al.*, 1983).

Results

Basic properties of MoAbs 1 – 7 as well as their reactivities are shown in Table 1. All these MoAbs were of IgG class, most of them of IgG1 subclass and only 2 of them (MoAbs 2 and 5) of IgG2a subclass. The range of PTA ELISA titres of MoAbs using homologous antigens was 16,000 – 512,000.

No MoAb gave precipitate lines in double immunodiffusion test. MoAbs 1, 2, 6 and 7 were able to react with their homologous antigen in ATA ELISA in which rabbit polyclonal antisera were used for coating. The paratopes of these MoAbs were directed against surface virion structures – the metatopes. The other MoAbs (3, 4 and 5) were anti-cryptotopes – they bound only to denatured viral proteins or viruses deformed due to their binding onto the diagnostic plate surface in PTA ELISA (Van Regenmortel, 1990).

Table 1. Basic characteristics and reactivities of MoAbs 1 – 7

MoAb	X	ELISA titer (×1000)								Isotype	Epitope type	Homologous antigen
		RCMV		BBSV		RCMVp		BBSVp				
		PTA	ATA	PTA	ATA	PTA	ATA	PTA	ATA			
5	∞	64	–	–	–	8	–	–	–	IgG2a	C	RCMV
3	32	256	–	8	–	512	64	256	4	IgG1	C	RCMV
4	8	512	–	64	–	512	2	128	2	IgG1	C	RCMV
1	8	256	4	32	1	512	32	256	8	IgG1	M	BBSV
2	1	512	2	512	2	1024	512	1024	256	IgG2a	M	RCMV
7	0.13	2	–	16	128	4	–	256	1	IgG1	M	BBSV
6	0	–	–	32	4	–	–	256	2	IgG1	M	BBSV

X – the ratio of MoAbs' titers in PTA ELISA using RCMV and BBSV as antigen (corresponding to fluent change of epitope type from RCMV to BBSV). According to the falling X value MoAbs were sorted and lined in the table. RCMVp, BBSVp – viral proteins prepared by disintegration of RCMV and BBSV, respectively. C – cryptotope, M – metatope.

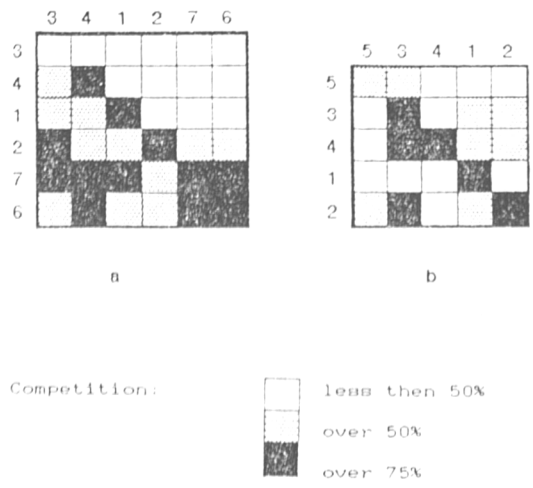

Fig. 1
Results of CBT ELISA for BBSV (a) and RCMV (b)
MoAbs are sorted as in Table 1. Rows – competing MoAbs, columns – competed MoAbs.

The results of CBT ELISA are shown in Fig. 1. Only MoAbs reacting with BBSV and RCMV, respectively are included in Fig. 1a and 1b. Using BBSV MoAb 3 did not block the binding site of any other MoAb, its own one included and epitope III was blocked by every other MoAb. We explain this phenomenon by very low affinity of MoAb 3 to its epitope on BBSV.

On the whole, reciprocal binding site blocking by MoAbs was considerably asymmetrical. With RCMV, only MoAbs 5 and 1, and 5 and 4 (no competition) showed equivalent relations and with BBSV MoAbs 2 and 6, and 2 and 7 (partial competition), and 6 and 7 (full competition) did so. Markedly asymmetrical influences were observed between MoAbs 3 and 4 with RCMV, and between MoAbs 1 and 7, 2 and 3, 3 and 7, 4 and 6, 4 and 7 with BBSV (Fig. 1).

The reactivity of MoAbs with other 16 RCMV and BBSV isolates in PTA ELISA is demonstrated in Fig. 2. A weak MoAb 7 binding ability to 5 of 14 RCMV isolates investigated was detected. Five RCMV isolates gave no reaction with MoAb 5. All four BBSV isolates reacted with MoAbs 6 and 7, and none with MoAb 5.

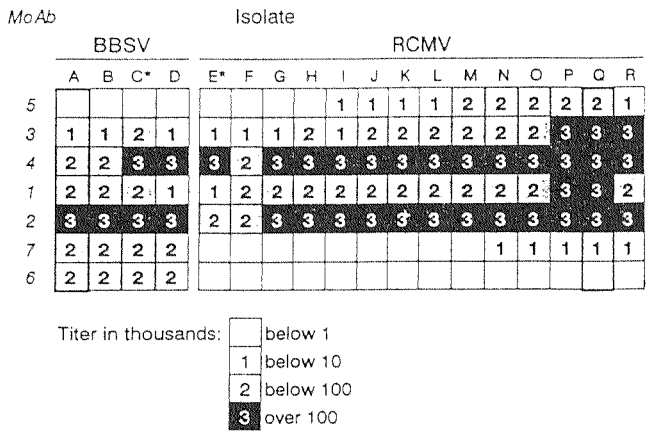

Fig. 2

MoAbs binding ability to different RCMV and BBSV isolates

MoAbs are sorted as in Table 1. Isolates VsM (A, C), VsM-Š (B), FI (D), TpM36 (E, Q), No. 12 (F), No. 10 (G), No. 8 (H), No. 3 (I), No. 2 (J), No. 20 (K), TpM25 (L), No. 16 (M), No. 14 (N), No. 9 (O), No. 19 (P) and No. 11 (R) were used. C*, E* – original VsM and TpM36 isolates, respectively. A, Q – VsM and TpM isolates, respectively, after 10 – 15 passages in pea plants by mechanical inoculation.

Discussion

A close serological relatedness between BBSV and RCMV was described earlier (Bruening, 1978) and confirmed by us using MoAbs prepared independently against RCMV (MoAbs 2, 3, 4 and 5) and BBSV (MoAbs 1, 6 and 7). Respective epitopes were designated I – VII according to MoAbs 1 – 7.

All prepared MoAbs were directed against detergent-stable linear epitopes – cryptotopes (MoAbs 3, 4 and 5) and metatopes (MoAbs 1, 2, 6 and 7). The respective epitope types recognized by MoAbs (Van Regenmortel, 1982) were identified by their reactivity in PTA and ATA ELISA (Dekker *et al.*, 1989). All MoAbs were able to bind to denatured viral proteins in immunoblot analysis (Šubr *et al.*, 1993) and in ELISA so that all the epitopes I – VII had a linear character. No MoAb was directed against a neotope, which was probably as a result of low number of prepared MoAbs. For comparison, Joisson and Van Regenmortel (1991) isolated 10 MoAbs against bean pod mottle virus and all of them were anti-cryptotopes, Wang *et al.* (1992) prepared 9 MoAbs against cowpea mosaic virus – all were anti-neotopes, and Kalmar and Eastwell (1989) obtained 15 MoAbs against cowpea mosaic and cowpea severe mosaic viruses distinguishing all epitope types.

As reported earlier 4 MoAbs (MoAb 1, 2, 3 and 4) reacted not only with their homologous antigens, but cross-reacted with the other virus in immunoblot analysis (Šubr *et al.*, 1993). Further investigation showed also a weak cross-reactivity of MoAb 7 with RCMV in PTA ELISA, which was not detected in immunoblots. It might be of nonspecific nature, but further experiments showed different level of MoAb 7 reaction with different RCMV isolates. According to this result only two of analyzed MoAbs were species-specific: MoAb 5 (RCMV) and MoAb 6 (BBSV). Exclusive BBSV-specifity of alkaline phosphatase-labelled MoAb 7 was indicated in CBT ELISA which might be caused by a drop of the MoAb affinity after labelling.

The reactivity of MoAbs with RCMV and BBSV did not correlate with their relation to their homologous antigen in every case. When a complete virus particle suspension was used, MoAb 2 reacted equally well with both viruses, and MoAb 1 reacted with RCMV even at 8-fold higher titer than with its homologous antigen (BBSV) in PTA ELISA. MoAbs 3, 4 and 7 reacted better with their homologous antigens.

MoAb 7 was the only one which reacted better in ATA than in PTA ELISA. It is probably directed against a metatope which became partially blocked by virus binding onto the plastic plate. All other metatopes (I, II, VI) showed the reverse tendency.

MoAb 2 reactet equaly well with RCMV and BBSV, thus the sequence homology of epitope II is apparently conserved in them. However, according to CBT ELISA results its location and orientation with regard to other epitopes is not the same in both viruses. Inverse dominance of epitopes II and IV was ascertained – MoAb 4 blocked partially the MoAb 2 binding site in RCMV but not BBSV.

The reactivity of MoAb 2 with viral proteins in ATA ELISA was markedly the best of all MoAbs (titers 512,000 – 256,000). Consecutive antigen dilution led also to dilution of detergent and thus the coated antibodies probably did not bind isolated coat proteins but oligomeres of them. The protein – protein interaction is the crucial force stabilizing mature comovirions (Kaper, 1972) and comoviral coat proteins strongly tend to aggregate (Wu and Bruening, 1971). Good reaction of MoAb 2 with disintegrated viruses in ATA ELISA demonstrated the epitope II to be well accessible in protein oligomers. On the contrary, epitopes IV, VI and VII are more or less hidden in those structures. Since metatope VII was very well distinguished in native viral particles, the aggregation ability of the coat proteins was not limited to protein parts participating in mutual interactions inside mature virions but it was also linked to protein parts located on the surface of virions. The aggregation of virions *in vitro* and/or *in vivo* may be caused by such interactions.

Only MoAb 5 reacted with viral protein more weakly than with the virus, probably in consequence of direct blocking of epitope V with SDS.

The CBT ELISA differences in MoAbs using RCMV and BBSV as antigen resulted from different antibody-epitope affinities in both viruses, caused either by another

arrangement of coat protein structures (differences in accessibility and mutual localization of epitopes) or simply by homology level of epitopes (sequence homology because of exclusively linear character of investigated epitopes). The first possibility is apparently true, because the differences in MoAbs (1, 3, 4) affinities were substantially less expressed when disintegrated viruses were used. We assume that the respective antigenic structures are better "packed up" in BBSV – the cryptotopes III and IV become less exposed after virus binding onto the plate and the RCMV-metatope I has rather the character of a cryptotope in BBSV.

MoAbs 6 and 7 bound considerably overlapping epitopes creating an expressively dominant antigenic area not present in RCMV. However, a part of epitope VII, different of the site recognized by MoAb 6, was conserved in some RCMV isolates.

Gallo and Musil (1988) estimated the group- and species-specific antibodies ratio 1:1 – 1:2 in rabbit antisera against RCMV, and 1:4 – 1:8 in those against BBSV ("group-specific" did mean RCMV and BBSV cross-reacting). The idea of BBSV with better "packed up" and worse accessible "group"-specific (common with RCMV) epitopes and with significant species-specific metatope fits well these results.

Screening of some other RCMV and BBSV isolates yielded about one third of RCMV isolates able to react with MoAb 7. MoAb 5 recognized five RCMV isolates well, five weakly and four not at all. On the contrary, the species-specifity of MoAbs 5 and 6 was fully demonstrated by all BBSV isolates. However, 3.5-fold less BBSV isolates were tested in comparison with RCMV. In conclusion, MoAb 6 was the only one reacting strictly species-specifically (a marked reaction with BBSV, no reaction with any RCMV isolate).

Our results show that the "passage" between both viruses is more or less continuous. Moreover, an expressed MoAbs binding ability appeared during propagation (15 passages) of TpM36 isolate in *Pisum saticum*. Further investigation of epitope change rate in comoviruses should be done to prove this observation.

RNA viruses are known to display a marked genome variability as a result of mutations without possibility of reparation (Holland *et al.*, 1982). Whereas the genes essential for virus replication (mostly the RNA-dependent RNA polymerase genes) are considerably conserved, the selection pressure is substantialy weaker in the case of coat protein genes and their non-lethal mutability is higher. This fact results in broad spectrum of immunologically different virus strains (serotypes). Epitope mapping of different comovirus serotypes and pathotypes may be used for clearing up their evolutionary linkage and origin.

However, RCMV and BBSV are very closely related and immunochemical methods – even the use of MoAbs – do not lead to their clear-cut categorization into different species.

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