

Alleged reaction in gel-immunodiffusion of an IgM monoclonal antibody with alfalfa mosaic virus and cucumber mosaic virus is an artefact

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A previously reported spurious serological cross-reaction between alfalfa mosaic virus (AMV) and cucumber mosaic virus (CMV), which had been defined by the reaction in gel-immunodiffusion tests of a single IgM monoclonal antibody (MAb), MAb 8, was no longer detected in the presence of 0.1 M-NaCl. The non-specific reactivity of this IgM was also confirmed in Western

blotting assays. When skimmed milk was used as a blocking agent and as a diluent of antibodies, MAb 8 failed to recognize AMV and CMV coat proteins. Hence, it is concluded that the alleged cross-reaction between AMV and CMV is due to non-specific binding of MAb 8 and that there is no evidence for a serological relationship between these two viruses.

While generating hybridomas to alfalfa mosaic virus (AMV), we isolated an IgM-secreting hybridoma of which the monoclonal antibody (MAb), MAb 8, reacted in gel-immunodiffusion tests with glutaraldehyde-fixed particles of AMV and native and glutaraldehyde-fixed particles of cucumber mosaic virus (CMV) (Hajimorad *et al.*, 1990). AMV and CMV are the type members of two serologically unrelated plant virus groups (Matthews, 1982). However, based on similarity in other properties, it has been proposed that these two groups together with bromoviruses and ilarviruses be considered as four distinct genera within a family called the Tricornaviridae (van Vloten-Doting *et al.*, 1981). The finding of a MAb raised against AMV but recognizing CMV could be considered as evidence for the antigenic relatedness of these two virus groups.

Here we report the results of a more detailed investigation on the properties of this MAb which led us to confirm the non-specific nature of its alleged cross-reactivity.

Antigens, polyclonal antibodies and MAbs were those

described previously (Hajimorad & Francki, 1989, 1991; Hajimorad *et al.*, 1990; Wahyuni *et al.*, 1992). An IgM MAb (MAb 9) raised against a secreted protein of *Rhizoctonia solani* and produced *in vivo* (Matthews & Brooker, 1991) was used as a control IgM. Western blotting (Hajimorad *et al.*, 1990) and immunodiffusion tests were done as described (Hajimorad & Francki, 1989), and except where indicated, 0.1 M-NaCl was incorporated in the gel.

We previously reported that in immunodiffusion tests an IgM MAb against AMV (MAb 8) reacted more efficiently with particles of CMV than with those of AMV (Hajimorad *et al.*, 1990). In a quantitative gel-immunodiffusion experiment, 0.4 µg of a purified preparation of this antibody immunoprecipitated 10 µg of glutaraldehyde-fixed particles of CMV, whereas 6.4 µg of MAb 8 was required to precipitate the same amount of AMV. In gel-immunodiffusion, MAb 8 also precipitated other cucumoviruses, but not host components. Under similar assay conditions, eight other ascitic fluids containing different subclasses of MAbs against AMV (Hajimorad *et al.*, 1990) as well as MAb 9 failed to precipitate particles of any CMV strains. Surprisingly MAb 8 produced spurs between glutaraldehyde-fixed virus preparations of CMV and particles of other cucumoviruses as well as between native and glutaraldehyde-fixed particles of CMV (Hajimorad *et al.*, 1990). Despite the ability of MAb 8 to produce spurs, it is unlikely to be a mixture of antibodies (Hajimorad *et al.*, 1990).

Non-specific immunoprecipitation in a gel can be prevented by NaCl and after non-specific precipitin lines have formed, they dissolve when washed in saline

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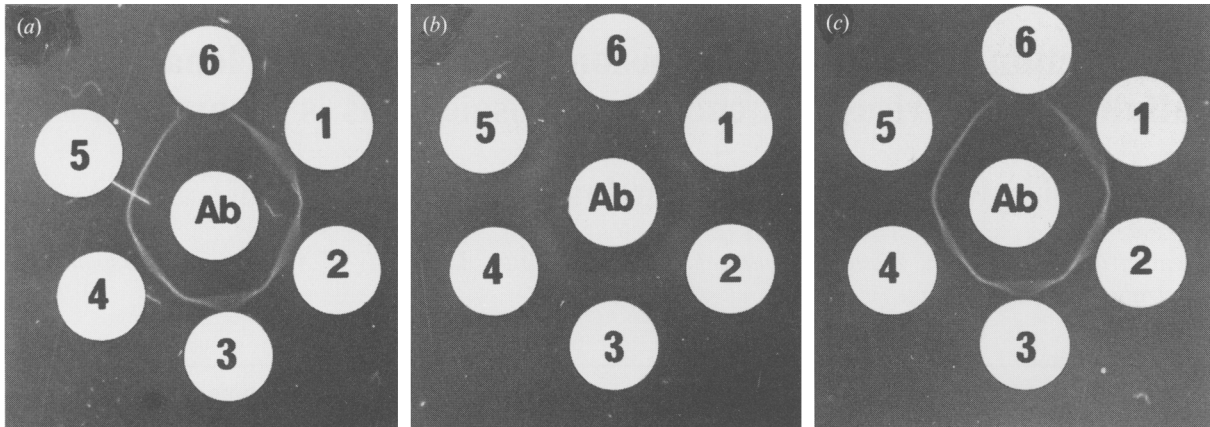


Fig. 1. Agarose gel-immunodiffusion tests in the absence (a) or presence (b) of 0.1 M-NaCl and using MAb 8. Central wells (Ab) were filled with a preparation of 1 mg/ml of purified MAb 8. (c) Gel from (a), but after soaking in PBS for 30 days before photography. Wells 1 to 5 were loaded with 1 mg/ml of preparations of glutaraldehyde-fixed virus of five CMV strains and well 6 with a preparation of total plant protein extract from uninfected *Nicotiana clevelandii* leaves.

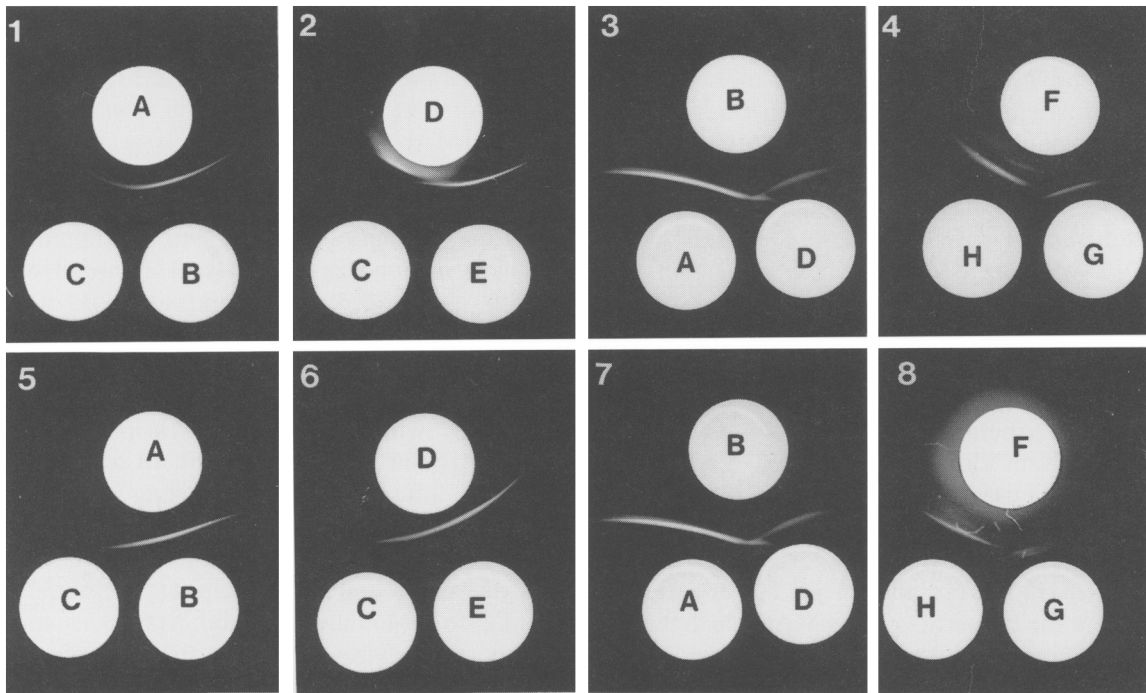


Fig. 2. Agarose gel-immunodiffusion tests in the absence (panels 1 to 4) or presence (panels 5 to 8) of 0.1 M-NaCl. Glutaraldehyde-fixed or native particles of CMV strain Twa or native particles of AMV strain N20 were loaded in wells A, D and F, respectively. Wells E, B and G were loaded with polyclonal antibodies raised against native and glutaraldehyde-fixed particles of CMV, and native particles of AMV, respectively. Purified MAbs 8 and 5 (1 mg/ml) were loaded in wells C and H, respectively. All antigens were used at concentrations of 1 mg/ml.

(Crowle, 1973; Altschuh & van Regenmortel, 1983). When 0.1 M-NaCl was incorporated into the agarose gel, the reaction of MAb 8 with CMV particles disappeared (Fig. 1, compare a with b). The immunoprecipitin lines that formed in the absence of NaCl remained stable when washed with PBS (Clark & Adams, 1977) even after a period of 1 month (Fig. 1c). The reaction of

MAb 8 with glutaraldehyde-fixed particles of CMV resembled the reaction of homologous polyclonal antibodies (Fig. 2, panel 1). Its reaction with native particles of CMV differed from the reaction of homologous polyclonal antibodies (Fig. 2, panel 2). The ability of MAb 8 to react with both types of CMV virions as well as glutaraldehyde-fixed particles of AMV (not shown)

was abolished in the presence of 0.1 M-NaCl (Fig. 2, compare panels 1 and 2 with 5 and 6). However, a genuine immunoprecipitin reaction of AMV with MAb 5 (Hajimorad *et al.*, 1990) was not affected by salt (Fig. 2, compare panel 4 with 8) and produced a spur when compared with polyclonal antibodies using native AMV particles as the test antigen. When comparing antigenic reactivity of native and glutaraldehyde-fixed particles of CMV using anti-CMV serum, certain epitopes on native particles were not detected (Fig. 2, panels 3 and 7). This was irrespective of the presence (Fig. 2, panel 3) or absence (Fig. 2, panel 7) of 0.1 M-NaCl in the gel. The lack of certain epitopes on native CMV and their presence on glutaraldehyde-fixed particles under gel-immunodiffusion conditions may explain why a spur is formed between these two types of virions when they are reacted side by side with MAb 8 (Hajimorad *et al.*, 1990).

To analyse further the spurious cross-reactivity of MAb 8, Western blotting was used. When BSA was used as the blocking agent, MAb 8 reacted with AMV and CMV coat proteins irrespective of whether ascitic fluid or purified antibody was used (not shown). MAb 8 also reacted with coat proteins of a number of other cucumoviruses and unrelated plant viruses except tobamoviruses. Under similar assay conditions control MAb 9 failed to react with any of the proteins tested. Blocking with BSA may be unsatisfactory and can result in non-specific reactions (Johnson *et al.*, 1984; Zimmermann & van Regenmortel, 1989; Dietzgen & Zaitlin, 1991) that can be abolished by the use of skimmed milk. When skimmed milk instead of BSA was used as a blocking agent and as a diluent of antibodies, MAb 8 failed to react with any of the antigens tested including AMV (not shown). Furthermore, a total of 45 (21 anti-AMV; 24 anti-CMV) sera collected from the late stages of immunization did not show any heterologous cross-reactivity between AMV and CMV (not shown), when 0.1 M-NaCl was incorporated in the gel and skimmed milk was used as the blocking agent in Western blotting. Although AMV and CMV have many features in common, we conclude that there is no evidence of a serological relationship between them.

These observations indicate that the previously reported alleged reaction in gel-immunodiffusion of MAb 8 with CMV and AMV is an artefact. This is another example of spurious serological cross-reactivity between unrelated plant viruses as a result of un-

satisfactory assay conditions in gel-immunodiffusion and Western blotting.

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