Differences Among Monoclonal Antibodies to Barley Yellow Dwarf Viruses

H. T. Hsu, J. Aebig, and W. F. Rochow

First two authors, American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD 20852; third author, ARS, U.S. Department of Agriculture, Plant Pathology Department, Cornell University, Ithaca, NY 14853.

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ABSTRACT

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Monoclonal antibodies of hybridoma clones derived from mice injected with either the RPV or MAV isolate of barley yellow dwarf virus (BYDV) were evaluated in a variety of tests with five previously characterized BYDV luteoviruses. Five cell lines were from mice injected with RPV, and eight lines were from MAV-injected mice. Three of the 13 cloned hybridomas ceased producing antibodies; three others produced antibodies that reacted with healthy oat antigen. Of the seven virus-specific antibodies, which represented four antibody isotypes, three reacted only with RPV and one only with MAV. Two additional MAV-derived antibodies reacted with both MAV and PAV. One reacted with MAV and SGV. Specificity of the reactions was consistent in two kinds of enzyme-linked immunosorbent assays and in preliminary neutralization studies carried out in test tubes or within aphid vectors. The potential value of these monoclonal antibodies for use in luteovirus research is discussed.

Additional key words: cell hybridization, murine monoclonal antibodies, neutralization, somatic techniques.

Serological procedures have been especially useful for studying the luteoviruses which are not mechanically transmissible to plants, develop to only low concentration in infected plants, and are subject to all of the difficulties of using aphid vectors in virus transmission work. Useful procedures have ranged from a kind of neutralization of infectivity (10,17) to enzyme-linked immunosorbent assay (EIA) (15,18). Despite the many advantages of these procedures, their use is limited by the difficulties of preparing sufficient quantities of purified virus to make antiserum in animals, the limited supplies of antisera that have been made, the need for a variety of antisera because of serological specificity among luteoviruses, and the variations among antisera that usually occur from animal to animal.

The somatic cell hybridization technique introduced by Köhler and Milstein (12) has been used to generate continuous cell lines producing monoclonal antibodies against many antigens. The availability of monoclonal antibodies (2,3,6,21) would facilitate and accelerate studies of plant luteoviruses such as barley yellow dwarf virus (BYDV).

The purpose of this study was to establish somatic cell hybrids secreting monoclonal antibodies to the RPV and MAV isolates of BYDV, which are serologically distinct luteoviruses (1,16). We evaluated 13 cell lines, detected differences among the antibodies, and considered their potential for use in studies of luteoviruses. A preliminary report (11) was published, together with a summary of a similar study (5).

MATERIALS AND METHODS

Virus and immunization. The five characterized BYDV isolates have been classified into two distinct groups: RPV and RMV in group 2; and MAV, PAV, and SGV in group 1 (9,18,19). The RPV and MAV isolates were selected for use because they represent the two groups of BYDV (19). Purified virus preparations made in Ithaca and sent to Rockville were used to immunize six 20-g BALB/c mice, three each for RPV and MAV. Two intraperitoneal injections ~2–4 wk apart were made, first with 10 μg of virus in 0.15 ml of PBS (0.02 M phosphate and 0.15 M sodium chloride, pH 7.4) and 0.15 ml of complete Freund's adjuvant, and then with the same dose but with incomplete Freund's adjuvant. One week after the second injection the same amount of purified virus was injected into the tail vein. The mice were sacrificed 3 days later and their spleens were harvested.

In addition to RPV and MAV used for immunization, three other BYDV isolates were used to evaluate the monoclonal antibodies. These were RMV, PAV, and SGV, which were previously described (18,19). Clarified preparations were made by first extracting virus from 3 g of fresh oat tissue in 9 ml of PBS containing 0.05% Tween-20 and 0.02% sodium azide with a PT-20 probe of a Brinkmann Polytron homogenizer (Brinkmann Instrument, Inc., Cantiague, NY 11590) for 6 sec. Homogenates were then clarified by shaking with 9 ml of chloroform and centrifuging at low speed. Purified virus preparations were made as described previously (16).

Cells and media. The mouse myeloma cell lines P3x63AG8.653 (obtained from Washington Veterans Administration Medical Center, Washington, DC 20422) and P3/NS1/1-Ag4-1 (obtained from Cell Distribution Center, Salk Institute, La Jolla, CA 92037) were maintained in RPMI-1640 medium (GIBCO Laboratories, Grand Island, NY 14072) supplemented with 15% fetal bovine serum (FBS) and 1 mM Na-pyruvate (RPMI medium). Hybrid cell lines derived by fusing myeloma cells with mouse spleen cells were initially maintained in HAT medium (RPMI medium containing 10^{-4} M hypoxanthine, 4×10^{-7} M aminopterin, and 1.6×10^{-5} M thymidine (13), and then HT medium (HAT medium containing no aminopterin). Once established, hybridomas were grown in RPMI medium. For liquid nitrogen storage, cells were resuspended in RPMI medium containing 50% FBS and 7.5% dimethylsulfoxide.

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Cell fusions. Spleen cell suspensions were prepared by injecting RPMI medium into intact spleen tissue to dislodge most cells into the medium. Remnant spleen tissues were further triturated with forceps and scissors. Cells were centrifuged (400 *g*, 5 min, unless otherwise stated) after removal from cell suspension with a pipette leaving the large tissue pieces behind. Spleen cells were mixed with myeloma cells (taken from log growth phase) at a 5:1 ratio in serum-free RPMI medium and then co-pelleted. This process was repeated and the medium was aspirated completely. Fusion was carried out in a 50-ml conical centrifuge tube in a water bath at 37 C by adding dropwise 1.5 ml of prewarmed 45% PEG (4000 molecular weight) in serum-free medium over a period of 45 sec, followed by 75 sec of incubation with gentle agitation. The volume of the cell suspension was increased twice by adding 1 ml of serum-free medium over a 1 min period at 37 C with a 1 min waiting interval between each addition. The final volume was further increased by adding 20 ml of serum-free medium over the course of 2 min. Fusion was completed in 8 min. After centrifugation, the supernatant was removed by aspiration. Cells were resuspended in HAT medium to a density of $\sim 5 \times 10^5$ cells per milliliter, distributed in 96-well culture plates (0.2 ml per well), and incubated in a 5% CO_2 atmosphere at 37 C in humidified incubators.

Myeloma cells P3x63AG8.653 were used in earlier fusions in which hybridomas had plate numbers of 1 to 8. For later fusions (plates numbered 9 or higher) we used cells P3/NS1/1-AG4-1.

Nomenclature of hybridomas and antibodies. The following system was used to identify hybridomas. Cell lines prefixed with RPV were derived from splenocytes taken from RPV-immunized mice. Similarly, MAV designated those cell lines derived from MAV-sensitized mice. Each cell line and monoclonal antibody produced by that cell line will be referred to by the appropriate three-letter prefix and a number.

Immunoglobulin class determination. Immunoglobulin class and subclass of monoclonal antibodies were identified by Ouchterlony double diffusion tests of concentrated culture supernatants in agar gel (0.65% agarose in PBS). Antibodies were twice precipitated and concentrated from culture fluid with 0.9 volume saturated $(NH_4)_2SO_4$ and dialyzed extensively against PBS. Rabbit antisera to mouse IgG1, IgG2a, IgG2b, IgG3, and IgM, and their homologous antigens, were purchased from Litton Bionetics (Kensington, MD 20795).

Enzyme-immunosorbent assay (EIA). In Rockville, indirect EIA was used to screen antibody activity. Polyvinyl chloride or polystyrene plates previously coated with 10 μg/ml of RPV- or MAV-specific rabbit globulins at pH 9.6 were sent from Ithaca. Clarified antigen preparations that had been evaluated for serological specificity were also sent from Ithaca. Plates were stored at 4 C in Rockville and were usually used within 2 mo. For use, plates were incubated with the clarified extracts from RPV- or MAV-infected plants, followed by 150 μl of hybridoma culture fluid per well, then 50 μl of alkaline phosphatase-labelled goat immunoglobulins to mouse IgG and IgM (Kirkegaard & Perry Laboratories, Inc., Gaithersburg, MD 20879), and finally 150 μl of substrate. Each reagent was incubated at room temperature for 1 hr. Plates were read at 405 nm in a Multiskan Microplate Reader (Flow Laboratories, Inc., McLean, VA 22102).

In Ithaca, the monoclonal antibodies were evaluated both by double sandwich and indirect EIA procedures. The double sandwich tests were done with four different virus-specific polyclonal rabbit globulins as described previously (18) except that 100 μl of reagent was used in each well, precoating was at 10 μg of globulin per milliliter, conjugated globulin was usually used at a dilution of 1:800 of stock, and a Dynatech model 2-580 Micro-ELISA reader at 405 nm was used. This procedure was also used to evaluate each virus preparation made for use in these tests. Monoclonal antibodies at various dilutions were substituted for the polyclonal ones to test use of the antibodies in the first coating step of EIA. In indirect tests, wells were coated with one of the four polyclonal antibodies (about 6 hr at 37 C), virus was incubated overnight (4 C), and monoclonal antibody was incubated for 3 hr (37 C). The reaction was measured by incubating anti-mouse labelled globulin for 3 hr (37 C) before substrate was added (45 min at room temperature). Controls included preparations made from healthy oats, and preparations containing 100–250 ng of homologous virus per well.

Ascitic fluid productions. For production of antibodies in ascitic fluid, BALB/c mice were primed intraperitoneally with 2,6,10,14-tetramethylpentadecane (Pristane; Aldrich Chemical Co., Milwaukee, WI 53201) 1 wk before intraperitoneal injection of hybridomas. About 0.3 ml of 10^7 cells per milliliter in serum-free medium was injected into each mouse. Mice were observed for 2 wk for ascites development. Usually ascitic fluid could be collected between 10 and 15 days after intraperitoneal transplantation of tumor cells.

For convenience, when BALB/c mice were not readily available, hybridomas were frozen in a liquid nitrogen freezer. Before injection, thawed cells were diluted 10-fold and pelleted by centrifugation. The cells were resuspended in serum-free RPMI medium and injected directly into Pristane-primed mice for the production of ascitic fluid.

RESULTS

Hybridomas. A total of four independent fusions were made, two for each virus using the spleen from one RPV- or MAV-immunized mouse each time. Eight to eleven 96-well plates were used for each spleen with 0.2 ml of cell suspension in HAT medium in each well. Initially, the large amount of cell debris and

TABLE 1. Summary of tests of 13 monoclonal antibodies: Isotype and titration of murine ascitic fluid against the RPV or MAV isolates of barley yellow dwarf luteovirus and summary of the reactivity of each antibody

Monoclonal antibody	Cell line	Antibody isotype	Reciprocal of dilution endpoint of ascitic fluid in indirect EIA with homologous virus trapped by polyclonal globulin	Antigen with which antibody was found to react
RPV1	RPV10C5D8	IgG2a	7,812,500	RPV
RPV2	RPV15F7E4	IgM	62,000	RPV
RPV3	RPV14A3G9	IgG2a	1,638,400	RPV
RPV4	RPV14G10F9	IgM	102,400	Oat component
RPV5	RPV14D5D2	IgG3	0	None
MAV1	MAV14B1F4	IgG3	25,600	MAV
MAV2	MAV9A3D3	IgG2a	62,500	MAV,PAV
MAV3	MAV14B3H7	IgG2a	409,600	MAV,PAV
MAV4	MAV17A8A10	IgG2b	1,562,500	MAV,SGV
MAV5	MAV14C11A11	IgG1	12,500	Oat component
MAV6	MAV9E9H1	IgM	12,500	Oat component
MAV7	MAV17A8D7	None	0	None
MAV8	MAV6B8E7	IgM	0	None

asynchrony in the killing of the nonfused myeloma cells by HAT obscured appearance of hybrid cells. In about 1 wk, small colonies of cells (10–100 cells) with the typical morphology of hybridomas began to appear in ~5–10% of wells. At the end of the 3rd wk, colonies which varied from 1–3 mm in diameter could be easily observed by lifting plates and examining from the bottom. The percentage of wells with growth of hybridomas increased to about 5–25%. Wells with hybridomas were marked and 0.1 ml of culture fluid was replaced with HT medium when colonies were about 2–3 mm in diameter or when the color of the medium changed to yellow. Culture supernatants were replaced completely at least once during feeding procedures prior to testing hybridoma antibody activity. This process removed non-hybridoma-derived antibody produced by unfused plasma cells that were plated into wells at the beginning of culture and continued to secrete antibody for several days.

From RPV fusions, 272 wells were tested for antibody activity and 75 were found to be positive to RPV. From MAV, 93 of 635 wells tested were positive. During expansion of the positive cultures many were lost for undetermined reasons. After a second or third test for antibody activity, 31 cell lines were selected for RPV and 15 for MAV.

Three cell lines from each virus were cloned immediately while they were being expanded for liquid nitrogen storage. The remaining cell lines were also expanded and stored in a liquid nitrogen freezer. Further clonings of the remaining hybridomas were carried out three to four cell lines at a time after revival from liquid nitrogen.

Cloning. Cloning was done by limiting dilution of about 0.3 cell per 100 μl per well using conditioned medium in which mouse fibroblast cells, L929 (obtained from T. A. Chen, Rutgers University, New Brunswick, NJ 08903), had grown for 24 hr using medium supplemented with 50 ng of fibroblast growth factor per milliliter (Collaborative Research, Inc., Lexington, MA 02173). In most cases, 5–30% of the plated wells showed growth. Thirty percent growth still achieves 95% probability that a given well contains only a single clone (4). Antibody activity for RPV or MAV was confirmed by using indirect EIA. Cell populations were expanded for storage at liquid nitrogen temperature and/or production of ascitic fluid.

In most cases, several cloned cell lines derived from each original cell line were selected for study. In all experiments, we obtained consistent results for each of the sister clones. This is another indication of monoclonality. For this report we have chosen to present data for only one clone from each of the original cell lines, as summarized in Table 1.

Immunoglobulin subclass identification. Subclasses of monoclonal antibody specific to RPV and MAV were identified using individual isotype specific antiserum (Table 1). No precipitin line was observed for antibody MAV7 in agar gel when tested against rabbit anti-mouse IgM, IgG1, IgG2a, IgG2b, and IgG3 immunoglobulins. Ascitic fluid produced from this cell line also was negative in tests with MAV; it was then employed as a negative control in further tests. Two other cell lines that were positive in screening tests later proved to be negative in tests for monoclonal antibodies in mouse ascitic fluid (Table 1, RPV5, MAV8).

Monoclonal antibody specificity. Titers of antibody in ascitic fluid were first determined against homologous virus by indirect EIA (Table 1). Then ascitic fluid preparations shipped from Rockville were tested in parallel in Ithaca against all five previously characterized luteoviruses. Each of five sets of clarified antigen preparations used in these tests was first evaluated with four polyclonal antibodies to assess its general serological specificity (Table 2). These tests showed that none of the preparations were contaminated and that each reacted as expected from results of many previous comparisons (15,18). Similar results in tests of purified preparation were obtained except that a weak heterologous reaction between RPV and RMV was evident (18). This cross reaction was not usually observed in tests with clarified antigen preparations.

Each monoclonal antibody was tested by indirect EIA at dilutions of 1:100 to 1:62,500 in parallel against four luteoviruses in one series of comparisons. Many of the monoclonal antibodies gave strong homologous reactions at the 1:62,500 dilution. In these preliminary tests we observed large differences in specificity among the different antibodies. Some reacted only with homologous virus, some reacted strongly with the homologous virus and weakly with one or more of the other isolates, and three of the antibodies reacted with all four isolate preparations. Further comparisons

TABLE 2. Serological specificity of clarified preparations used to test monoclonal antibodies

Luteovirus preparation used as antigen	Absorbance at 405 nm in double sandwich EIA reactions with indicated polyclonal globulins from rabbit antiserum[a]			
	RPV	RMV	MAV	PAV
RPV	1.088	0.007	0.002	0.002
RMV	0.008	0.463	0.002	0.001
MAV	0.017	0.007	1.428	0.104
PAV	0.001	0.004	0.204	1.173
SGV	0.001	0.012	0.073	0.034
Control	0.001	0.011	0.000	0.001

[a] Mean of separate reactions of five different preparations of each antigen made during a period of several months from infected (or uninfected as control) oats.

TABLE 3. Tests of 13 monoclonal antibodies in reactions with clarified preparations of five luteoviruses in indirect enzyme immunosorbent assays

Monoclonal antibody	Absorbance at 405 nm in reaction with ascitic fluid preparation diluted 1:2,500 and virus trapped by homologous polyclonal globulin[a]						
	Buffer control	Healthy control	RPV	MAV	PAV	RMV	SGV
RPV1	0.000	0.001	1.288	0.002	0.001	0.001	0.003
RPV2	0.001	0.010	1.192	0.004	0.000	0.005	0.007
RPV3	0.002	0.008	0.633	0.020	0.011	0.011	0.018
RPV4	0.001	0.319	0.237	0.288	0.138	0.237	0.365
RPV5	0.005	0.019	0.029	0.027	0.013	0.014	0.028
MAV1	0.003	0.024	0.026	0.630	0.017	0.015	0.025
MAV2	0.005	0.020	0.038	0.540	0.101	0.015	0.015
MAV3	0.003	0.023	0.015	1.227	0.122	0.018	0.018
MAV4	0.000	0.002	0.016	0.595	0.008	0.002	0.031
MAV5	0.000	0.954	0.695	0.727	0.757	0.845	0.761
MAV6	0.009	0.106	0.135	0.067	0.070	0.063	0.046
MAV7	0.000	0.001	0.009	0.001	0.001	0.003	0.004
MAV8	0.004	0.010	0.015	0.016	0.008	0.002	0.013

[a] Mean of two wells following a 45-min reaction at room temperature with antimouse-labelled globulin. SGV was trapped by heterologous polyclonal MAV globulin.

were expanded to include parallel controls of extracts of healthy plants and of buffer in each plate. When these controls were included, it became apparent that the broad-spectrum antibodies reacted with healthy extracts as well as with clarified preparations of all the luteoviruses (Table 3, RPV4, MAV5, and MAV6). When direct comparisons of the different antibodies were made in indirect EIA tests, all at dilutions of 1:2,500, differences among the 13 cell lines were observed (Table 3). Although three of the preparations reacted in a nonspecific fashion with both healthy and clarified virus preparations, three of the cell lines were inactive (Table 3, RPV5, MAV7, and MAV8), and the other seven had virus-specific reactions. Three of the preparations from RPV cell lines reacted only with RPV (Table 3, RPV1, RPV2, and RPV3). Similarly, one of the MAV cell lines produced antibodies that reacted only with MAV (Table 3, MAV1). Two of the antibodies from MAV cell lines reacted strongly with MAV and weakly with PAV, the virus known to be serologically related to MAV (Table 3, MAV2 and MAV3). One of the antibodies (Table 3, MAV4) reacted strongly with MAV and weakly with SGV.

In another experiment, the virus-specific monoclonal antibodies were compared in similar indirect EIA assays at a dilution of 1:5,000. Known amounts of RPV or MAV from purified virus preparations were used as antigen to evaluate sensitivity and to compare reactivity of antibodies for homologous antigen. All seven antibodies gave strong reactions with 100 ng of homologous virus; clear reactions often occurred with as little as 10 ng of virus (Table 4). For the RPV cell lines, the relative activity of the antibodies was generally related to the dilution endpoint that was observed in initial screening of the preparations (Tables 1 and 4). Differences among activities of the MAV cell lines were, however, less pronounced and varied from the initial titration endpoint (Tables 1 and 4).

In testing SGV in indirect EIA comparisons, the antigen was trapped in plates by heterologous MAV globulin because we do not yet have a homologous antiserum for SGV. In all tests with MAV cell lines, there appeared to be a consistent reaction of antibody MAV4 to SGV. To study this reaction further, two separate experiments were done with a series of preparations of SGV and all of the active MAV monoclonal antibodies. These tests confirmed the consistent reaction of only one of the MAV antibodies with SGV (Table 5). Other studies had previously suggested a serological relationship between SGV and MAV (18).

Monoclonal antibodies in the coating step of EIA. The antibodies were next compared to determine whether they could be used in the initial coating step of the EIA double sandwich procedure. A series of dilutions of each antibody was made in coating buffer (0.05 M sodium carbonate, pH 9.6) and incubated at 37 C for 6 hr. Clarified preparations of each of the five antigens were incubated overnight at 4 C, together with preparations of healthy oats as controls. Labelled homologous polyclonal globulins were reacted next for about 5 hr, except for SGV for which the heterologous MAV-globulin was used. Results showed that all seven monoclonal antibodies absorbed in the initial coating step of the procedure (Table 6). Moreover, the same specificities found in the previous indirect assays occurred in these tests (Table 6). None of the antibodies that reacted with oat component trapped viral antigens when used in the initial EIA coating step.

Additional evaluations of the seven virus-specific monoclonal antibodies were made with known amounts of purified, homologous antigens to compare activity of the antibodies in the initial coating step. All antibodies reacted strongly with 100 ng of virus and most also gave strong reactions with 10 ng of virus (Table 7).

Neutralization studies. Preliminary comparisons of the antibodies were also made to evaluate their usefulness in the kind of neutralization of infectivity assays based on reducing virus transmission when aphids feed through membranes on treated inocula (10,17). Antibodies diluted 1:50 or 1:100 were mixed in test tubes with preparations of MAV or RPV, incubated at 37 C for 30 min, kept overnight at 4 C, and then assayed by two methods. In one method, unreacted virus was assayed in double sandwich EIA procedures. In the other method, the virus-antiserum mixture was combined with an equal volume of 40% sucrose, and aphids were allowed to feed on the preparations through membranes before

TABLE 4. Test of seven monoclonal antibodies in reactions with known amounts of homologous luteovirus in indirect enzyme immunosorbent assays

Monoclonal antibody	Absorbance at 405 nm in reaction with ascitic fluid preparation diluted 1:5,000 and partially purified virus trapped by homologous polyclonal globulin[a]			
	Buffer control	Healthy control	100 ng virus	10 ng virus
Group A				
RPV1	0.000	0.000	1.181	0.565
RPV2	0.000	0.000	0.805	0.423
RPV3	0.001	0.000	0.892	0.105
MAV7	0.002	0.008	0.033	0.025
Group B				
MAV1	0.001	0.000	0.340	0.039
MAV2	0.000	0.001	0.500	0.059
MAV3	0.000	0.000	0.638	0.077
MAV4	0.000	0.002	0.445	0.069
MAV8	0.000	0.002	0.025	0.002
RPV5	0.000	0.001	0.013	0.001

[a] Mean of two wells following a 45-min reaction at room temperature with antimouse-labelled globulin. Group A was tested with RPV; Group B with MAV. MAV7, MAV8, and RPV 5 were used as controls with the group indicated.

TABLE 5. Reaction of anti-MAV monoclonal antibodies with SGV in indirect enzyme immunosorbent assays

Ascitic fluid tested at dilution of 1:2,500[a]	Absorbance at 405 nm in reaction with four preparations of SGV[b]					
	Buffer control	Healthy control	SGV 1	SGV 2	SGV 3	SGV 4
MAV2	0.004	0.016	0.024	0.022	0.020	0.020
MAV3	0.002	0.012	0.016	0.026	0.024	0.015
MAV4	0.001	0.004	0.103	0.110	0.122	0.130
MAV7	0.002	0.001	0.006	0.004	0.005	0.004
MAV Polyclonal	0.000	0.000	0.026	0.036	0.050	0.063
MAV1	0.001	0.006	0.009	0.006	0.007	0.011
MAV4	0.002	0.005	0.114	0.127	0.164	0.166
MAV8	0.001	0.003	0.005	0.006	0.006	0.005
MAV Polyclonal	0.003	0.000	0.003	0.025	0.028	0.032

[a] The MAV polyclonal globulin was used as double sandwich control in each plate as shown. MAV7 and MAV8 were included as controls.

[b] Mean of two wells following a 45-min reaction at room temperature with antimouse-labelled globulin. The SGV preparations were separate clarified preparations of 3-g samples of different sources from the greenhouse for numbers 1–3; number 4 was a partially purified SGV concentrate. Virus was trapped in wells with heterologous MAV globulin.

TABLE 6. Tests of seven monoclonal antibodies in initial coating step of enzyme immunosorbent assays in double sandwich method with five barley yellow dwarf luteoviruses

Monoclonal antibody	Reciprocal of ascitic fluid dilution tested	Absorbance at 405 nm in reaction with clarified antigen preparation shown[a]					
		Healthy	RPV	MAV	PAV	RMV	SGV
Tests:							
RPV1	12,500	0.001	0.836	−0.031	−0.033	−0.025	−0.031
RPV2	500	0.005	0.314	0.001	0.005	0.008	0.006
RPV3	5,000	0.001	1.124	0.003	0.005	0.000	0.001
MAV1	5,000	0.010	0.012	1.439	0.000	0.011	0.001
MAV2	2,500	0.001	0.001	1.196	0.032	0.003	0.008
MAV3	5,000	0.014	0.003	1.448	0.219	0.017	0.003
MAV4	2,500	−0.020	−0.025	0.436	−0.028	−0.016	0.069
Controls:							
RPV5	5,000	0.002	0.001	0.002	0.001	0.002	0.001
MAV Polyclonal	10 μg/ml	0.002	0.000	1.439	0.793	0.006	0.134
RPV Polyclonal	10 μg/ml	0.003	1.406	0.003	0.008	0.191	0.002

[a] Mean of two wells following a 45-min reaction with labelled homologous polyclonal globulin from rabbit. For SGV, reaction is that of heterologous MAV globulin. For healthy controls, globulins used were for RPV or RMV.

TABLE 7. Tests of seven monoclonal antibodies (diluted 1:5,000) in initial coating step of enzyme immunosorbent assays in double sandwich method with partially purified, homologous luteovirus

Monoclonal antibody	Absorbance at 405 nm in reaction with homologous antigen preparation shown[a]		
	Healthy control	100 ng virus	10 ng virus
RPV1	0.022	1.186	0.175
PRV2	0.009	0.125	0.029
RPV3	0.017	0.857	0.067
Polyclonal RPV globulin	0.024	1.287	0.098
MAV1	0.001	1.361	0.212
MAV2	0.008	1.307	0.297
MAV3	0.005	1.459	0.164
MAV4	0.007	0.769	0.201
Polyclonal MAV globulin	0.001	1.331	0.146
Polyclonal PAV globulin	0.000	0.352	0.103

[a] Mean of four wells following a 45-min reaction with labelled homologous polyclonal globulin from rabbit. For cell lines RPV1, RPV2, and RPV3, RPV was used; for all others, virus was MAV. The polyclonal globulin controls were used at 10 μg/ml for coating.

transfer to test plants to evaluate biological activity of the treated virus. In several experiments with RPV, EIA assays showed that only RPV-specific antibodies reacted; MAV antibodies gave results no different from those of the inactive ascitic fluid controls. Although no RPV was detected in these preparations in EIA assays, infectivity tests with *Rhopalosiphum padi* showed no reduction in transmission following virus acquisition by aphids fed through membranes. Only when virus preparations were diluted before use was there any evidence of a reduction in virus transmission by *R. padi*. Results of tests with MAV were similar. Incubation with MAV antibodies, but not with RPV antibodies, greatly reduced the amount of MAV detected in EIA assays, but did not prevent transmission of virus from the same preparations when *Macrosiphum avenae* were allowed to feed on the treated inocula through membranes. A reduction in transmission when diluted virus was used, however, suggested that the antibodies can be used to neutralize virus in such tests if the ratio between amounts of antibody and virus is properly controlled.

Preliminary evaluations of some of the monoclonal antibodies were also made to determine whether they reacted with virus within aphids. Previous studies with polyclonal antibodies have shown that virus-specific antiserum injected into the hemocoel of aphids before they are permitted to acquire virus consistently reduced transmission of the homologous virus (20). In one series of experiments a similar result occurred with antibody MAV4 (Table 1). When 0.02 μl of a 1:50 dilution of this antibody was injected into *M. avenae*, and then these injected aphids were allowed to feed for 24 hr on MAV-infected leaves, fewer aphids transmitted MAV compared with those injected in parallel with a 1:50 dilution of antibody MAV7, one of the control preparations (Table 1). In one experiment, 33 of 40 control aphids transmitted MAV; 16 of 40 aphids injected with antibody MAV4 transmitted in parallel. In a second experiment, the corresponding numbers were 33 and 20, respectively. In a similar test with *R. padi* and antibody RPV1 (Table 1), 20 of 40 control aphids transmitted RPV; only two aphids injected with the RPV-specific antibody transmitted RPV from opposite halves of the same source leaves.

DISCUSSION

Three of the 13 cell lines investigated produced antibody to an oat component and not to a common determinant on BYDV. Although the virus preparations injected into mice were from sucrose density gradient fractions, we know the virus is not pure. Polyclonal antisera prepared to several isolates of BYDV also contained antibodies to host contaminants (1,17,18). A strong immunogenic activity of the oat component may offset its relatively low concentration in the final virus preparations. Since we used only clarified virus to do initial screenings for antibody production from hybridomas, antibodies against the oat component were detected initially along with those against the viruses. These three antibodies against an oat component may eventually prove to be useful in final purification steps for producing "pure" virus.

The seven virus-specific monoclonal antibodies will have many uses in our efforts to understand mechanisms of specificity between plant luteoviruses and their aphid vectors. The MAV-specific antibodies should be useful in studying details of the interaction of MAV and PAV within *Sitobion avenae* (Fabricius) because it should permit differentiation of MAV from PAV (8). Together with the antibodies specific for RPV they should also provide improved tools for studying the role of heterologous encapsidation as a mechanism of dependent virus transmission from mixed infections of RPV and MAV (14). These antibodies should be helpful in attempts to identify specific sites on luteovirus capsid proteins in studies of the relationship of such sites to capsid interaction with aphid membranes (7,8). Although we do not know much about the neutralizing ability of these different antibodies, preliminary tests suggest that they will be useful in aphid-injection and membrane-feeding assays. Another potential application of the monoclonal antibodies is in virus purification work (21). Antibodies can be bound to solid supports and used to prepare an affinity column. Luteovirus bound to the solid supports by the specific antibodies might then be eluted and obtained in relatively pure form (21).

For diagnostic tests it appears that mixtures of these different antibodies would be necessary because of the marked serological specificity among some luteoviruses found in field samples (15,18,19). Use of mixtures of monoclonal antibodies has the advantage that binding of one monoclonal antibody at one virus site may enhance binding of a second antibody at another site (21). Antibodies that cross react with several viruses, such as those described by Diaco et al (5), might be more useful for diagnosis than those described here.

We do not know whether monoclonal antibodies recognize the same antigenic determinant. For example, we plan to investigate the three RPV-specific antibodies to study this question. However, antibodies that apparently recognize three different epitopes on MAV have been produced. Of the four antibodies (Table 1), MAV1 reacts only with MAV, MAV2, and MAV3 react with MAV and PAV, and MAV4 reacts with MAV and SGV.

Previous serological, cytological, and chemical studies of the five BYDV isolates have shown that RPV and RMV should be classified into one group and MAV, PAV, and SGV in another (9,18,19). Results of experiments with the MAV monoclonal antibodies support this division; those antibodies that react with PAV and SGV, as well as with MAV, show that common antigenic determinants do occur among these three viruses.

This work confirms suggestions about the usefulness of monoclonal antibodies in plant virology (2,3,6). The application of monoclonal antibodies in luteovirus research should provide significant new information on the relationships of luteoviruses and the mechanisms of specificity of their aphid vectors.

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