Use of Monoclonal Antibodies to Characterize Grapevine Leafroll Associated Closteroviruses

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ABSTRACT

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Stable hybridoma cell lines secreting monoclonal antibodies to the NY-1 isolate of grapevine leafroll associated closteroviruses (GLRaV) were produced by fusing spleen cells of immunized BALB/c mice and mouse myeloma cell line SP2/0-AG14. The monoclonal antibodies reacted with the NY-1 isolate and other type III isolates, but not with type I, II, and IV isolates. The reactions were the same in five different kinds of enzyme-linked immunosorbent assays (ELISA), immunosorbent electron microscopy, dot-immunoblotting, and Western blotting assays. Sensitivity of the monoclonal antibodies were very good for the detection of the virus in grape leaf tissue in double antibody sandwich ELISA. With the double gold labeling electron microscopy technique, we were able to detect serologically distinct particles of GLRaV in single leafroll affected grapevines. A sensitive Western blotting assay was developed to estimate the molecular weight of virus coat protein of the GLRaV from partially concentrated samples using the monoclonal antibodies.

Grapevine leafroll (GLR) disease is one of the most important diseases of grapes, and it occurs wherever grapes are grown (7). Although the causal agent of this graft-transmitted, viruslike disease has not been determined, various types of virus particles have been associated with the GLR disease, including isometric- (3), potyvirus- (26,27), and closteroviruslike particles (20). In recent years, however, closteroviruslike particles have been consistently associated with GLR disease (8,12,19,30,31). In this paper, we will refer to the grapevine leafroll associated closteroviruslike particles as GLRaV.

A few of these closteroviruses have been purified from GLR-infected grapes and specific polyclonal antibodies produced (8,12,30,31). Molecular weights of the viral coat protein and dsRNA isolated from GLR-infected grape tissue have been determined (12,19). GLRaV has been transmitted by mealy bugs from GLR-diseased grapevines to healthy grapevines, which subsequently developed typical GLR symptoms (24,25). Previous studies indicate that serologically distinct types of GLRaV exist. The types are referred to as I, II, III, and IV (12,22). However, two factors have hindered more detailed studies on the biochemical and biophysical properties of the GLRaV and the production of large quantities of high quality antibodies. First, there are no herbaceous host plants available for propagation of the virus, and, second, the virus yield from grapevines is extremely low.

Polyclonal antisera against the GLRaV have been used successfully for detection of the virus in enzyme-linked immunosorbent assay (ELISA) (8,12,28,30). However, the antisera have two limitations. They react with grape leaf proteins, so it is necessary to preabsorb the antisera with healthy grape leaf extracts, and the supply of antisera is limited.

Hybridoma technology has been used widely in plant virology (9,10,17,21), enabling plant virologists to conduct studies which could not be done with polyclonal antibodies (1,5,13–15,18,22). Thus, hybridoma technology appeared to be a good approach with which to further characterize GLRaV. In this study, we produced monoclonal antibodies to the NY-1 isolate of GLRaV, which were then used to detect GLRaV from crude grape extracts in ELISA, differentiate mixed infection of serologically distinct GLRaV types, and further characterize the virus coat protein. A preliminary report of this work has been published (11).

MATERIAL AND METHODS

Virus. The primary isolates used in this study were GLR-diseased grapevines from New York (designated NY-1, from cultivar Pinot noir; NY-2, cultivar Himrod), Arkansas (AK-1, cultivar Chardonnay), China (China-1, cultivar Bei-Mei), and California (CA-1, cultivar Emperor; CA-2, cultivar Melon; CA-3, cultivar Blackrose; CA-4, cultivar Thompson seedless; CA-5 cultivar Italia; and CA-6 cultivar Mission) (12). The above isolates have been classified into type I (AK-1), II (China-1, CA-

1, CA-2), III (NY-1, NY-2, CA-5, AK-1), and IV (CA-3, CA-4) (12). The NY-1 isolate is the type member of GLRaV type III (12). Purified virus was obtained by differential centrifugation, CsS_4-sucrose gradient centrifugation (12), and dialyzed against potassium phosphate buffered saline (PBS, 0.05 M, pH 7.2; 0.15 M NaCl) prior to immunization.

Production of monoclonal antibodies to NY-1 isolate of GLRaV. Six-week-old BALB/c mice (Jackson Laboratories, Bar Harbor, ME) were immunized (300 μl intraperitoneal and 200 subcutaneous) with purified virus preparations mixed 1:1 with Freund's complete adjuvant. Booster injections (500 μl intraperitoneal) 2 wk after immunization were prepared with Freund's incomplete adjuvant. Three days prior to fusion, 250 μl intraperitoneal booster injections were administered. Mouse spleens were removed 5 wk after initial immunization, and 10^8 splenocytes were fused with 10^7 Sp 2/0-AG14 myeloma cells (American Type Culture Collection, Rockville, MD) in 1 ml of 50% polyethylene glycol (MW 1450), as described (2). Hybridoma cells secreting an antibody which reacted to NY-1 infected but not to healthy grapevines were cloned and subcloned by limiting dilution method. Antibodies were produced in ascitic fluid by intraperitoneal injection of 11-wk-old pristane-primed BALB/c mice with 2×10^6 hybridoma cells in 500 μl PBS. Immunoglobulin was purified from cell culture medium and ascitic fluid using a protein-A column kit with high salt high pH running buffer (Bio-Rad Laboratories, Richmond, CA). Immunoglobulin class and subclass of monoclonal antibody were identified by Ouchterlony double-diffusion tests. Rabbit-anti-mouse IgG1, IgG2a, IgG2b, IgG3, IgM, and the 2 light chains were purchased from Pharmacia, Inc. (Piscataway, NJ).

ELISA. Hybridoma cells were screened by double antibody sandwich (DAS) indirect ELISA. Polystyrene ELISA plates were precoated with 1 μg/ml of anti-NY-1 polyclonal immunoglobulins from a rabbit (30). The plates were stored at 4 C and used within a month. For screening tests, plates were incubated with crude leaf extracts from NY-1-infected or healthy grapevines at 4 C overnight; then, undiluted culture media containing monoclonal antibody were incubated at 37 C for 3 hr; finally, phosphatase conjugated with goat-anti-mouse IgG (1:1500 dilution, Sigma Chemical Co., St, Louis, MO) was incubated at 37 C for 1 hr. Antisera collected from immunized mice were used as positive controls, and PBS buffer and culture media containing monoclonal antibody to other viruses were used as negative controls. Simple indirect ELISA was also used to test the monoclonal antibody. With this test, every step was the same as the DAS indirect ELISA, except that plates were coated with purified virus preparations.

Three types of DAS direct ELISA were used to evaluate monoclonal antibody cell lines: both coating and conjugated antibodies were monoclonal antibodies, the coating antibody was a monoclonal antibody and the conjugated antibody was anti-NY-1 polyclonal immunoglobulins, and the coating antibody was anti-NY-1 polyclonal immunoglobulins and the conjugated antibody was the monoclonal antibody linked to alkaline phosphatase enzyme. Conjugation of both antibodies were done as described previously (4). Isolates of different GLRaV types were used in all five kinds of ELISA tests. Virus extraction buffer and healthy grape leaf preparations were included as negative controls. A reaction was considered positive only if the absorbance was at least 0.100. This threshold was much higher than the twice-background range of healthy controls (0.005–0.04).

Immunogold labeling. Reaction of the monoclonal antibodies with different isolates was evaluated in an immunogold labeling electron microscopy assay. In this assay, 1 μl purified virus preparation was placed on Formvar-filmed 400 mesh copper grids. The grids were sequentially incubated on a drop of undiluted monoclonal antibody culture medium for 5 min, on a drop of goat-anti-mouse IgG gold conjugate (Sigma Chemical Co.) for 5 min, and on a drop of 1% uranyl acetate for 10 sec. Gold particles in the goat-anti-mouse IgG gold conjugate were 10 nm in diameter; the conjugate was diluted 1:20 in PBS-T (PBS containing 0.05% Tween 20). Grids were examined with a JEM-100SX electron microscope (JEOL LTD, Tokyo, Japan). All steps were performed at 21 C, and a 20-drop rinse of the grids with PBS was done after each step, except after staining. Before staining, the grids were rinsed with 20 drops of distilled water. In "double gold labeling" tests, after the incubation with the first antibody (from mouse) and the first conjugate (goat-anti-mouse IgG gold), the grids were incubated on rabbit antibody (1:20 in PBS-T) and then on goat-anti-rabbit IgG gold conjugate (Sigma Chemical Co., 1:20 in PBS-T), in which gold particles were 5 nm in diameter. Papaya ringspot virus, GLRaV isolates of different types, and polyclonal antibodies and monoclonal antibodies to these viruses were used as controls in gold labeling tests.

Western blotting. Concentrated virus preparations were used. For each sample, virus was purified from 2 g of grape tissue (usually stem phloem tissue) by differential centrifugation (30). The high-speed pellet was resuspended in 200 μl 1 × degradation buffer (0.4 M Tris-HCl, pH 6.8, with 2.5% sodium dodecyl sulfate, 5% 2-mercaptoethanol, and 5% sucrose). Sodium dodecyl sulfate polyacrylamide gel electrophoresis and silver staining were done as previously described (12). Western blot was carried out as described (12), with major modifications. Immobilon transfer membrane (Millipore Corporation, Bedford, MA) was used instead of nitrocellulose membrane in Western blot. The membrane was first wet with 100% methanol, then soaked in transfer buffer at 21 C for 30 min. After overnight transfer (22 V, constant voltage), the Immobilon membrane was soaked in 2% Carnation nonfat milk powder in PBS (MP-PBS) at 37 C for 1 h for "blocking"; incubated with monoclonal antibody in 2% MP-PBS (3 μg/ml) at 37 C for 2 h; washed with PBS-T three times, 10 min/each; incubated in protein-A gold conjugate solution (Bio-Rad Laboratories) at 21 C in dark for 1 h; washed with PBS-T three times, 10 min/each; TBS-T (0.02 M Tris, pH 7.5, 0.5 M NaCl, 0.05% Tween 20) two times, 10 min/each; distilled water five times, 1 min/each; citrate buffer (0.2 M, pH 3.7) once for 5 min; incubated in enhancement solution (0.2 M citrate acid, pH 3.7, with 80 mM hydroxyquinone, and 5 mM silver lactate) at 21 C in the dark for 5–15 min; and, finally, incubated in fixing solution for 5 min at 21 C, rinsed with distilled water, air-dried, and photographed. Papaya ringspot virus, cucumber mosaic virus, and polyclonal antibodies against these viruses were used in Western blot as controls. There were no nonspecific reactions.

Dot-immunoblotting. Nitrocellulose membrane (Schleicher and Schuell, BA84, Keene, NH) was first soaked in PBS for 30 min; then, concentrated virus (5 μl) was applied to the membrane by using a Bio-Rad Minifold apparatus. The membrane was blocked, incubated with specific antibodies, and washed as in Western blot. Then the membrane was incubated with alkaline phosphatase

TABLE 1. Properties of the monoclonal antibodies elicited by the NY-1 isolate of grapevine leafroll associated closterovirus[a]

Monoclonal antibody	Hybridoma cell lines	Isotype	Dilution end points in ELISA[b]		Decoration in ISEM[c]
			Indirect	Direct	
MAbNY1.1	F244D2C10F6	IgG1	1:1,562,500	>1:8,000	+
MAbNY1.2	F241D5F12A2	IgG1	1:500	<1:500	−
MAbNY1.3	F254C5H8B9	IgG1	1:1,526,500	>1:8,000	+
MAbNY1.4	F256G6B4F3	IgG1	1:312,500	>1:8,000	+

[a] Ascitic fluids were used in all characterization tests of the monoclonal antibodies (MAbs). Culture media were used in isotyping the monoclonal antibodies.

[b] In indirect enzyme-linked immunosorbent assay (ELISA), rabbit polyclonal antibody to the NY-1 isolate was used to trap virus, and the monoclonal antibodies were used as detecting antibodies. In direct ELISA, the monoclonal antibodies were coated on plates and rabbit antibody-enzyme conjugate was used to detect trapped virions.

[c] Concentrated virus was coated on grids; the grids were incubated on a drop of 1:25 diluted ascitic fluid or antiserum (in phosphate-buffered saline solution containing 0.05% Tween 20 for 5 min and rinsed with 20 drops of distilled H_2O. Then, the grids were stained with 1% uranyl acetate for 10 sec and rinsed as before. ISEM = immunosorbent electron microscopy.

conjugated with goat-anti-mouse IgG (1:1000 dilution in MP-PBS) at 21 C for 2 h, washed, and, finally, incubated in substrate (nitro blue tetrazolium chloride/5-bromo-4-chloro-3-indolyl-phosphate *p*-toluidine salt) at 21 C for about 15 min. The reaction was stopped by rinsing the membrane in distilled water.

RESULTS

Production of monoclonal antibodies. The virus immunogen was purified from about 3 kg of NY-1 infected grape leaf tissues of Pinot noir. Examination with electron microscopy showed that the purified preparation had about 100 virus particles per grid square (400 mesh grid) but virus concentration was too low to measure with a spectrophotometer. Two cell fusions were made simultaneously. More than 90% of the 1152 wells, into which the fusion products were distributed (6 plates per fusion), contained growing colonies. The media from those wells were tested by the DAS indirect ELISA. For one fusion (F-25), 235 out of 240 tested wells had supernatant fluids containing virus-specific antibodies, while only 5 wells contained antibodies to healthy plant components. The cell lines were preserved by freezing the plates (29). However, for another fusion (F-24), only 8 out of 180 tested wells had virus-specific antibodies; and 2 tested wells contained nonspecific antibodies to both virus and healthy preparations. Ten cell lines from two fusions were cloned by the limiting dilution method. Four stable antibody-secreting hybridoma cell lines (designated MAbNY1.1 to MAbNY1.4) were selected for further characterization (Table 1). All belonged to the IgG1 subclass. The ascitic fluids of MAbNY1.1, 3, and 4 had high titers in ELISA; whereas MAbNY1.2 had low titer. The MAbNY1.1, 3, and 4 decorated the NY-1 virions in electron microscopy tests, but MAbNY1.2 did not. About 5 mg of IgG1 was purified from each ml of ascitic fluid with the high salt high pH running buffer system, whereas the IgG1 yield averaged only about 0.8 mg/ml when the regular low salt low pH running buffer (6) was used. About 5 mg of IgG was obtained from 1,000 ml of the culture medium supernatant.

Comparison of isolates. Serological relationships of six representative GLRaV isolates belonging to different types (12) were examined using monoclonal antibodies in five different ELISA procedures, dot-immunoblot, immunosorbent electron microscopy, and Western blot analyses. All assays gave clear, similar results (e.g., Fig. 1); that is, the monoclonal antibodies to the NY-1 isolate reacted only with the NY-1 and other type III isolates.

Detection of mixed infection. A double immunogold labeling electron microscopy procedure was used to detect serologically distinct virions of the AK-1 isolate. In this test, virions were sequentially labeled with monoclonal antibodies from mouse hybridoma cells (to the NY-1 isolate, the type member of type III of GLRaV), then goat-anti-mouse gold conjugate (10 nm), then polyclonal antiserum from a rabbit (to type I of GLRaV, 8), and finaly goat-anti-rabbit gold conjugate (5 nm). All of the virions observed were labeled; most of them were labeled with the 10-nm gold particles, and only a few were labeled with the 5-nm gold particles. As expected, in single gold labeling experiments, most virus particles were labeled with 10-nm gold particles when antibodies to the NY-1 isolate and goat-anti-mouse gold conjugate were used, but a few virus particles were labeled with 5-nm gold particles when antibodies to type I isolate and goat-anti-rabbit gold conjugate were used. The technique was also used to investigate five Cyprus GLRaV isolates (obtained from Dr. N. Ioannou, Cyprus). Results showed that four isolates were doubly labeled with both antibodies (i.e., two groups of virus particles each labeled differently, Fig. 2), and one isolate was singly labeled with mouse anti-NY-1 monoclonal antibody. Identical results were obtained when these Cyprus samples were tested by DAS direct ELISA with specific antibodies to type III (NY-1) or to type II (Table 2). Polyclonal antibodies and monoclonal antibodies to the NY-1 isolate did not react with papaya ringspot virus and GLRaV isolates of other types in gold labeling tests, and vice versa.

Estimation of coat protein molecular weight. The Immobilon membrane with protein-A gold and silver enhancement system was the most sensitive procedure when two kinds of membrane (nitrocellulose, Immobilon) and two kinds of conjugate (goat-anti-mouse phosphatase, protein-A gold and silver enhancement) were compared in Western blot analysis (data not shown). With this Western blot assay, we were able to estimate the coat protein molecular weight with concentrated virus preparations from 2 g of tissue. Ten isolates from different countries were analyzed and found to have the same molecular weight coat protein (ca. 43 kDa). Some examples are presented in Fig. 3.

Comparison of monoclonal and polyclonal antibodies. The effectiveness of monoclonal and polyclonal antibodies in virus detection from crude extracts in DAS direct ELISA were compared using conjugate and virus dilution end points. Both antibody systems were equally good in conjugate dilution tests. The monoclonal antibody conjugate could be diluted up to 1:32,000. In virus dilution end point tests, the polyclonal antibody system was more sensitive, although both systems were useful for detecting virus from crude tissue extracts (Fig. 4). Very similar results were obtained when the monoclonal and polyclonal antibodies were compared for virus detection from crude extracts in DAS indirect ELISA using virus dilution end points.

DISCUSSION

Our results demonstrate the advantages of monoclonal antibodies in characterizing closteroviruses associated with grapevine leafroll disease, an economically important and widespread disease

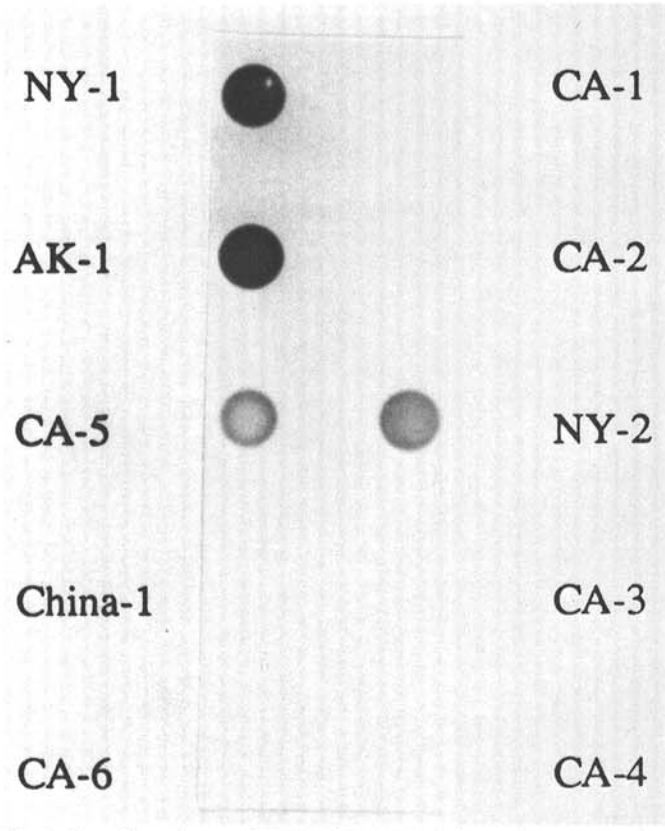

Fig. 1. Reaction of an anti-NY-1 (MAbNY1.3) in dot-immunoblotting assay with grapevine leafroll associated closterovirus isolates from New York (designated NY-1, from cultivar Pinot noir; NY-2, cultivar Himrod), Arkansas (AK-1, cultivar Chardonnay), China (China-1, cultivar Bei-Mei), and California (CA-1, cultivar Emperor; CA-2, cultivar Melon; CA-3, cultivar Blackrose; CA-4, cultivar Thompson seedless; CA-5 cultivar Italia; and CA-6 cultivar Mission). Partially purified virus preparations were spotted onto nitrocellulose membrane. The membrane was incubated with the monoclonal antibody (3 μg/ml) for 2 hrs, with phosphatase conjugated with goat-anti-mouse IgG (diluted at 1:1,000) for 2 hr, and, finally, with substrate at 37 C for 15 min.

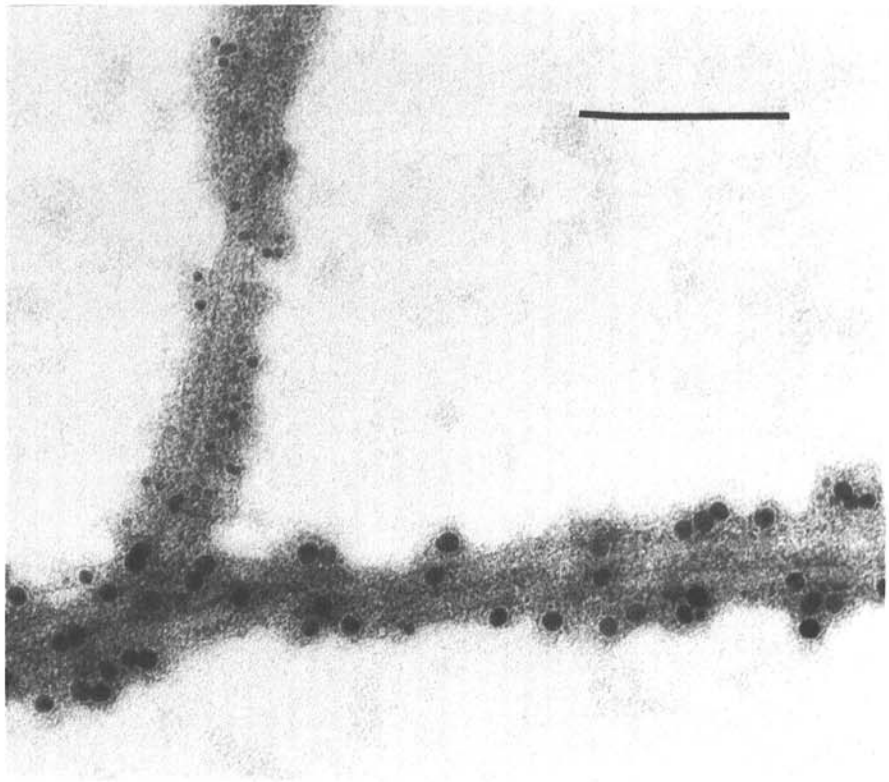

Fig. 2. Double immunogold labeling of closteroviruslike particles from a Cyprus isolate of grapevine leafroll associated closteroviruses (GLRaV) in electron microscopy. Virions were sequentially labeled with mouse monoclonal antibodies (to the NY-1 isolate), goat-anti-mouse gold conjugate (gold particles each 10 nm in diameter), rabbit polyclonal antiserum prepared to a GLRaV type II isolate, (Boscia, Hu, Golino, and Gonsalves, *unpublished*), goat-anti-rabbit gold conjugate (gold particles each 5 nm in diameter). Note that two virions are labeled by the larger gold particles. Bar = 100 nm.

TABLE 2. Reaction of GLRaV Cyprus isolates to two different antibodies in enzyme-linked immunosorbent assay (ELISA) and in double gold labeling electron microscopy (EM)

	ELISA[b]		Gold labeling EM[c]	
Isolates[a]	III	II	10 nm	5 nm
NY-1	+	−	+	−
SS9	−	+	−	+
Cyprus 217	+	+	+	+
Cyprus 220	+	+	+	+
Cyprus 221	+	+	+	+
Cyprus 222	+	+	+	+
Cyprus 231	+	−	+	−

[a]The Cyprus grapevine leafroll associated closterovirus (GLRaV) isolates had been assayed by grape woody indicator to be grapevine leafroll (GLR) positive. The isolates and the positive controls are grown in a greenhouse in Geneva, NY.

[b]Antibodies specific to type III (NY-1) and to type II (SS9 isolate) were used in the double antibody sandwich direct ELISA. Crude virus preparations were made by grinding 0.1 g petiole tissue in 2 ml of ELISA extraction buffer. "+" means absorbance readings were above 0.8 and "−" means absorbance readings were below 0.1 60 min after the substrate was added into the wells.

[c]Partially concentrated virus preparations were used. Virions were sequentially labeled with monoclonal antibodies (to type III), goat-anti-mouse gold conjugate (10 nm), polyclonal antiserum (to type II), and goat-anti-rabbit gold conjugate (5 nm). "+" means virions were labeled clearly (as shown in Fig. 2); "−" means virions were not labeled.

of grapevines, in the following aspects. Previously, in antibody decoration electron microscopy tests, we found that virions in the AK-1 isolate could be decorated with different antibodies (12). It was possible that the AK-1 isolate contained a mixture of different types, or the same virions might have different epitopes (15,16). Results from double gold labeling tests provide direct, definitive evidence for the mixed infection nature of the AK-1 isolate. Additionally, results of double labeling tests were confirmed by ELISA tests with Cyprus isolates of GLRaV. Our investigation with a number of other GLRaV isolates has shown that mixed infections are common.

Previously, the coat protein molecular weight of the NY-1 isolate was estimated with purified virus preparations prepared from large quantities of tissue (12). However, the Western blot method developed in this study reduced the amount of tissue needed from 40 g to only 1–2 g. Also, we previously had found that the coat protein molecular weight of the GLRaV NY-1 isolate was about 43K, which is much higher than that of other closteroviruses. Results obtained from 10 other GLRaV isolates verified our previous coat protein molecular weight estimation. Since the procedure was also successful for other types of GLRaV and citrus tristeza virus, it should have broad applications for other hard-to-purify closteroviruses.

Very low virus yields have limited the routine production of high titer antisera to GLRaV. Furthermore, these virus preparations contain significant host contaminants, which necessitates the absorption of the polyclonal antisera (30). In this study, a large quantity of specific, high titered monoclonal antibodies to the virus were produced from mouse ascitic fluids. Our results indicate that the monoclonal antibodies are useful in DAS ELISA for virus detection from crude extracts. These monoclonal antibodies should facilitate the detection, identification, and study of this economically important grape disease.

Previously, we and others found that serologically distinct closteroviruslike particles are associated with grapevine leafroll disease (8,12,23,30). This posed a potential problem for the routine use of serology for diagnosis. The monoclonal antibodies we characterized only reacted with the NY-1-like isolates (type III). However, we screened only a small number of the hybridoma cell lines. We will now screen the other cell lines in an effort to identify broader reactivity lines.

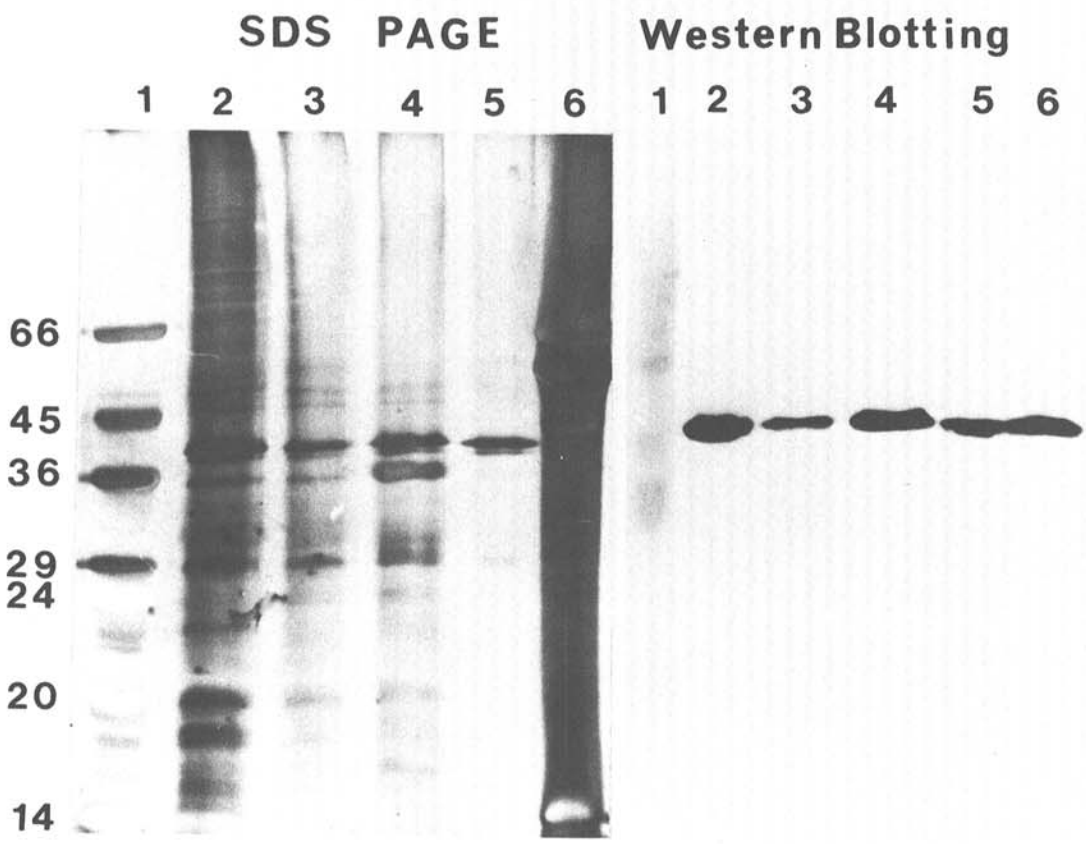

Fig. 3. Estimation of virus coat protein molecular weight from partially concentrated virus preparations in Western blotting assay using monoclonal antibodies to the NY-1 isolate of grapevine leafroll associated closteroviruses. Virus preparations were purified from 2 g of grape stem phloem tissue after one high-speed centrifugation. The pellets were resuspended with 200 μl of Tris buffer 0.1 M, pH 8.2, containing 10% sucrose. The virus preparations were analyzed on sodium dodecyl sulfate polyacrylamide gel electrophoresis (5% stacking and 12% separating acrylamide gels, Left). In the Western blot (Right), Immobilon membrane and the protein-A gold-silver enhancement system were used. Lane 1: molecular weight markers. Lane 2: the NY-1 isolate. Lane 3: the NY-2 isolate. Lane 4: M 2, an isolate from Japan. Lane 5: the CA-5 isolate. Lane 6: the AK-1 isolate. The molecular weight of the virus coat protein was about 43 kDa.

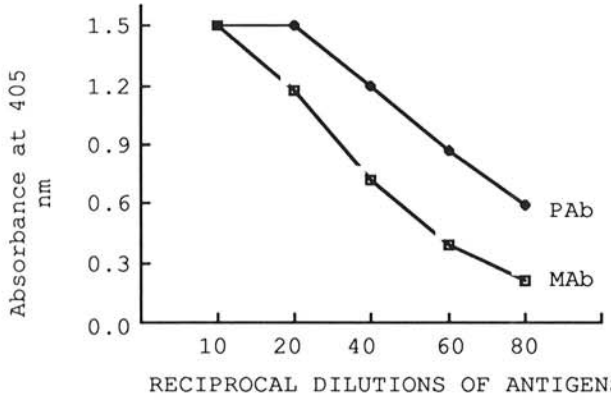

Fig. 4. Comparison of sensitivity of monoclonal and polyclonal antibodies in double antibody sandwich direct enxyme-linked immunosorbent assay at various dilutions of crude extracts of grapevine leafroll associated closterovirus type III (NY-1) infected grape leaf tissue. Same coating antibody concentrations (1 μg/ml) and conjugate dilutions (1:1000) were used for both antibodies. A standard two-day procedure was used. Absorbance readings were obtained 45 min after the substrate was added. The average absorbance readings for buffer and healthy crude extracts were below 0.02. PAb = polyclonal antibody. MAb = monoclonal antibody.

LITERATURE CITED

1. Al Moudallal, Z., Briand, J. P., and Van Regenmortel, M. H. V. 1982. Monoclonal antibodies as probes of the antigenic structure of tobacco mosaic virus. EMBO J. 1:1005-1010.
2. Bishop, A. L., Burr, T. J., Mittak, V. L., and Katz, B. H. 1989. A monoclonal antibody specific to *Agrobacterium tumefaciens* biovar 3 and its utilization for indexing grapevine propagation material. Phytopathology 79:995-998.
3. Castellano, M. A., Martelli, G. P., and Savino, V. 1983. Virus-like particles and ultrastructural modifications in the phloem of leafroll affected grapevines. Vitis 22:22-39.
4. Clark, M. F., and Adams, A. N. 1977. Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. J. Gen. Virol. 34:475-483.
5. Diaco, R., Hill, J. H., and Durand, D. P. 1986. Purification of soybean mosaic virus by affinity chromatography using monoclonal antibodies. J. Gen. Virol. 67:345-351.
6. Goding, J. W. 1986. Monoclonal Antibodies: Principles and Practice. Academic Press, New York.
7. Goheen, A. C. 1988. Leafroll. Page 52 in: Compendium of Grape Diseases. R. C. Pearson and A. C. Goheen, eds. APS Press, St. Paul, MN.
8. Gugerli, P., Brugger, J. J., and Bovey, R. 1984. L'enroulement de la vigne: Mise en évidence de particules virales et developpement d'une methode immuno-enzymatique pour le diagnostic rapide. Rev. Suisse Vitic. Arboric. Hortic. 16:299-304.
9. Halk, E. L., Hsu, H. T., Aebig, J., and Fanke, J. 1984. Production of monoclonal antibodies against three ilarviruses and alfalfa mosaic virus and their use in serotyping. Phytopathology 74:367-372.
10. Hsu, H. T., Aebig, J., and Rochow, W. F. 1984. Differences among monoclonal antibodies to barley yellow dwarf viruses. Phytopathology 74:600-605.
11. Hu, J. S., Boscia, D., and Gonsalves, D. 1989. Use of monoclonal antibodies in the study of closteroviruses associated with grape leafroll disease. (Abstr.) Phytopathology 79:1189.

12. Hu, J. S., Gonsalves, D., and Teliz, D. 1989. Characterization of closterovirus-like particles associated with grapevine leafroll disease. Phytopathol. Z. 128:1-14.
13. Hu, J. S., and Rochow, W. F. 1988. Anti-idiotypic antibodies against an anti-barley yellow dwarf virus monoclonal antibody. Phytopathology 78:1302-1307.
14. Hu, J. S., Rochow, W. F., and Dietert, R. R. 1985. Production and use of antibodies from hen eggs for the SGV isolate of barley yellow dwarf virus. Phytopathology 75:914-919.
15. Hu, J. S., Rochow, W. F., Palukaitis, P., and Dietert, R. R. 1988. Phenotypic mixing: Mechanism of dependent transmission for two related isolates of barley yellow dwarf virus. Phytopathology 78:1326-1330.
16. Hyatt, A., McPhee, D. A., and White, J. R. 1988. Antibody competition studies with gold-labelling immunoelectron microscopy. J. Virol. Methods 19:23-32.
17. Jordan, R., and Hammond, J. 1986. Analysis of antigenic specificity of monoclonal antibodies to several potyviruses. (Abstr.) Phytopathology 76:1091.
18. Koenig, R., and Torrance, L. 1986. Antigenic analysis of potato virus X by means of monoclonal antibodies. J. Gen. Virol. 67:2145-2151.
19. Mossop, D. W., Elliot, D. R., and Richard, K. D. 1985. Association of closterovirus-like particles and high molecular weight double-stranded RNA with grapevines affected by leafroll disease. N.Z. J. Agric. Res. 28:419-425.
20. Namba, S., Yamashita, S., Doi, Y., and Yano, R. 1979. Grapevine leafroll virus, a possible member of closteroviruses. Ann. Phytopathol. Soc. Jpn. 45:497-502.
21. Rochow, W. F., Hu, J. S., Forster, R. L., and Hsu, H. T. 1987. Parallel identification of five luteoviruses that cause barley yellow dwarf. Plant Dis. 71:272-275.
22. Ronald, W. P., Tremaine, J. H., and Mackenzie, D. J. 1986. Assessment of southern bean mosaic virus monoclonal antibodies for affinity chromatography. Phytopathology 76:491-494.
23. Rosciglione, B., and Gugerli, P. 1986. Maladies de l'enroulement et du bois strié de la vigne: Analyse microscopique et sérologique. Vitic. Arboric. Hortic. 18(4):207-211.
24. Rosciglione, B., and Gugerli, P. 1989. Transmission of grapevine leafroll disease and an associated closterovirus to healthy grapevine by the mealybug *Planococcus ficus*. (Abstr.) Phytoparasitica 17:63.
25. Tanne, E., Ben-Dov, Y., and Raccah, B. 1989. Transmission of closterovirus-like particles by mealybugs (*Pseudococcidea*) in Israel. (Abstr.) Phytoparasitica 17:63.
26. Tanne, E., Naveh, L., and Sela, I. 1989. Serological and molecular evidence for the complexity of the leafroll disease of grapevine. Plant Pathol. 38:183-189.
27. Tanne, E., Sela, I., Klein, M., and Harpaz, I. 1977. Purification and characterization of a virus associated with the grapevine leafroll disease. Phytopathology 67:442-447.
28. Teliz, D., Tanne, E., Gonsalves, D., and Zee, F. 1987. Field serological detection of viral antigens associated with grapevine leafroll disease. Plant Dis. 71:704-709.
29. Wells, D. E., and Bill, W. F. 1986. A method for freezing hybridoma in 96-well microculture plates. Methods Enzymol. 121:417-422.
30. Zee, F., Gonsalves, D., Goheen, A., Kim, K. S., Pool, R., and Lee, R. F. 1987. Cytopathology of leafroll-diseased grapevines and the purification and serology of associated closteroviruslike particles. Phytopathology 77:1427-1434.
31. Zimmermann, D., Walter, B., and Legall, O. 1988. Purification de particules virales associées à l'enroulement de la vigne et mise au point d'un protocole ELISA permettant leur détection. Agronomie 8(8):731-741.