Comparison of Mouse Monoclonal Antibodies and Polyclonal Antibodies of Chicken Egg Yolk and Rabbit for Assay of Carnation Etched Ring Virus

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ABSTRACT

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Serological detection of carnation etched ring virus (CERV) with mouse monoclonal antibodies, polyclonal rabbit antiserum, and chicken egg yolk antibodies was compared by ELISA (enzyme-linked immunosorbent assay) and IEM (immunoelectron microscopy). Hybridoma cells produced more antibodies in ascitic fluid in mice than in a culture medium. Sensitivity of CERV detection was similar in double antibody sandwich ELISA or triple antibody sandwich ELISA on PVC (polyvinyl chloride) plates coated with mouse ascitic fluid at a 1:1,000 dilution. Sensitivity of CERV detection was three- to four-fold lower on PVC plates coated with a 1:250 dilution of rabbit antiserum than with mouse monoclonal antibodies. Plates coated with antibodies from crude egg yolk or partially purified egg immunoglobulins failed to trap the virus. Rabbit antiserum-coated grids gave a linear increase in the number of virions attached to the grid over a concentration range of 3–12 μg/ml. Treatment of grids with protein A before coating with antiserum increased the number of particles attached by five- to 10-fold. Only a few particles were attached at low virus concentrations without protein A treatment. In IEM on monoclonal antibody-coated grids, more particles were attached at higher virus concentrations when grids were treated with protein A, but there was not a linear increase in the number of particles with increasing virus concentration. Carnation etched ring virus particles treated with virus-specific monoclonal antibodies showed specific antibody attachment that was absent on virions treated with Prunus necrotic ringspot virus monoclonal antibody.

Carnation etched ring virus (CERV) is disseminated by vegetative propagation of infected planting materials. Selection of virus-free propagation stock is essential in controlling spread of the disease. Because of the relatively low concentration of CERV in infected carnation tissue, it is sometimes difficult to detect and to identify. The disease often cannot be diagnosed reliably by visual inspection of infected carnations. Although symptoms may appear in one season of the year, plants may be symptomless at another season. Carnation etched ring virus can be detected by sap inoculation of *Saponaria vaccaria* 'Pink Beauty,' but precise control of light and temperature conditions are needed for uniform symptom expression (3). In addition, the presence of other viruses in *S. vaccaria* transmitted from *Dianthus caryophyllus* with mixed infections makes diagnosis of carnation etched ring disease difficult (1).

Although serology provides a precise method for rapid detection of infection by CERV, it is difficult to produce high quality antisera, because only limited quantities of purified virus are available for immunization (7,8). We have produced hybridomas that secrete antibodies to CERV by somatic cell hybridization of mouse myelomas and splenocytes of immunized mice by using the technique described by Köhler and Milstein (6).

The purpose of the research reported here was to compare mouse monoclonal antibodies with rabbit and chicken immunoglobulins by enzyme-linked immunosorbent assay (ELISA) and mouse monoclonal antibodies and rabbit immunoglobulins by immunoelectron microscopy (IEM) in the assay of CERV. A preliminary report was presented (5).

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MATERIALS AND METHODS

Virus and immunization. CERV isolated from *Dianthus caryophyllus* 'Peace River' was propagated in cultivar Pink Beauty *S. vaccaria* by mechanical inoculation (7). Virus was purified from extracts of infected leaf tissue by two cycles of differential centrifugation followed by density gradient centrifugation in 10–40% sucrose (8). The concentration of virus in 0.02 M phosphate, pH 7.2, was determined by using $E^{0.1\%}_{1\,cm,\,260\,nm} = 7$. Unless otherwise indicated, virus suspensions prepared in PBS (0.02 M phosphate, 0.15 M sodium chloride, pH 7.2) were used for immunization.

New Zealand white rabbits were immunized with a combination of intravenous and intramuscular injections. In the first injection, 100 μg of virus was administered intravenously. An intramuscular injection of 100 μg of virus mixed in Freund's complete adjuvant followed 14 days later. The rabbits were bled 2 wk later. The antibody titer was 1:25,000 in indirect ELISA with a concentration of 5 μg of purified CERV per milliliter.

Two chickens were immunized by injecting 75 μg of purified CERV into the wing vein and 75 μg mixed with Freund's complete adjuvant into the breast of each animal. The CERV-specific antibody was detected in the egg yolk on the 8th day after injection and the titer (indirect ELISA) rose from 1:25,000 to 1:162,000 after 30 days, then plateaued for more than 2 wk in both animals. Antibody titers were determined by using 5 μg of purified CERV per milliliter in indirect ELISA.

Purified virus preparations were used to immunize 20-gram BALB/c mice. Injection schedules were similar to those used for barley yellow dwarf virus (4). Two intraperitoneal injections were made 4 wk apart using 20 μg of virus in 0.15 ml of PBS and 0.15 ml of adjuvant. Freund's complete adjuvant was used for the first injection and Freund's incomplete adjuvant for the second. One week after the second injection, 30 μg of purified virus was injected into the tail vein. The mice were sacrificed 3 days later and their spleens were harvested.

Cells and media. The mouse myeloma cell line P3/NS1/1-Ag4-1 (originally obtained from the Cell Distribution Center, Salk Institute, La Jolla, CA) was maintained in RPMI-1640 medium (GIBCO Laboratories, Grand Island, NY) supplemented with 13% fetal bovine serum and 1 mM Na-pyruvate (RPMI medium) as previously described (4).

Hybridoma production. Methods used for cell fusions were similar to those described previously (2). Spleen cell suspensions were prepared by injecting culture medium into spleen tissue to dislodge most cells into the medium. Spleen cells and myeloma cells were washed twice by resuspension in serum-free RPMI medium and centrifugation at 400 *g* for 5 min unless otherwise indicated. Five parts of spleen cells were mixed with one part P3/NS1/1-Ag4-1 myeloma cells and were fused with polyethylene glycol (PEG, 4,000 molecular weight). Fusion was carried out in a 50-ml conical centrifuge tube in a water bath at 37 C by dropwise addition of 1.5 ml of prewarmed 45% PEG in serum-free medium over a period of 45 seconds, followed by a 75-second incubation with gentle agitation. Two 1-ml aliquots of serum-free medium were added over 1-min periods at 37 C with a 1-min waiting interval between each addition. Final dilution of PEG was made by adding 20 ml of serum-free medium over a period of 2 min. Fusion was completed in 8 min.

After centrifugation at 400 *g* for 5 min, the supernatant was removed by aspiration. Cells suspended in HAT medium (9) at a density of 5×10^5 cells per milliliter were distributed (0.2 ml per well) in 96-well plates and incubated in a 5–6% CO_2 atmosphere at 37 C in humidified incubators.

Hybrid cells were selected for CERV antibody activities by using indirect ELISA with CERV-sensitized PVC (polyvinyl chloride) plates and alkaline phosphatase-labeled goat anti-mouse immunoglobulins. The positive hybridomas were cloned by using the limiting dilution methods described previously (10) and the cell lines were selected for expansion, liquid nitrogen storage, and ascitic fluid production.

Production of antibodies. After being cloned by limiting dilution methods, the hybridoma cell lines were established and the specific subclasses of monoclonal antibodies produced were identified by testing concentrated culture supernatants with subgroup-specific antibodies in Ouchterlony double diffusion tests in 0.65% agarose in PBS (4). Antibodies were produced in mice by injecting hybridoma cells (10^7 cells in 0.3–0.5 ml of serum-free RPMI medium) intraperitoneally into pristane-primed BALB/c mice. Ascitic fluids were collected 2–4 wk after transplantation of tumor cells. Monoclonal antibodies produced by three cell lines (22A2C3, 28B4F6, and 28E4C6) were selected for this study. Relative activities of monoclonal antibodies produced in vitro and in vivo were compared by indirect ELISA.

Enzyme-linked immunosorbent assay. Mouse antibodies were titrated by using indirect ELISA. Polyvinyl chloride plates were coated with 50 μl per well of 5 μg of purified CERV per milliliter in 0.1 M carbonate coating buffer at pH 9.6 at room temperature for 1 hr or at 4 C overnight. Serial dilutions of 150 μl mouse antibodies were incubated for 1 hr at room temperature followed by 50 μl of alkaline phosphatase-labeled goat anti-mouse immunoglobulins, all in PBS-Tween (PBS containing 0.05% Tween-20). The enzyme conjugate was also incubated for 1 hr and this was followed by 150 μl of substrate. Plates were washed three times with PBS-Tween between each incubation step. Absorbance was measured at 405 nm in a Titertek Multiskan ELISA reader (Flow Laboratories Inc., McLean, VA).

For detection of viruses, double antibody sandwich and triple antibody sandwich tests were used. In double antibody sandwich ELISA tests, PVC plates were coated with either CERV-specific rabbit or mouse antibodies followed by serial dilutions of either purified virus or infected leaf extracts. Alkaline phosphatase-labeled mouse 22A2C3 monoclonal antibodies were used as the detecting antibodies with both antisera. In triple antibody sandwich ELISA tests, CERV-specific 22A2C3 mouse antibodies or rabbit antiserum were used to sensitize PVC plates followed by incubation with viral antigens. Chicken egg yolk anti-CERV immunoglobulins were incubated as the second antibody. Alkaline phosphatase-conjugated goat anti-chicken globulins were used as the third and final detecting antibody. All incubations were for 1 hr at room temperature. Plates were washed three times with PBS-Tween between each step.

Enzyme-labeled antibodies. Alkaline phosphatase was used to label the mouse antibodies. Monoclonal antibodies 22A2C3 which are IgG2b immunoglobulins were purified from mouse ascitic fluid by elution from a protein A-Sepharose CL-4B column (Pharmacia Fine Chemicals, Piscataway, NJ) with 0.1 M glycine buffer at pH 3.2. Immunoglobulins were precipitated with 0.9 part of saturated $(NH_4)_2SO_4$. Antibodies (1 mg of protein per milliliter) were conjugated to alkaline phosphatase (2 mg) in the presence of 0.2% glutaraldehyde at room temperature for 3 hr.

Alkaline phosphatase-labeled goat anti-mouse IgG and IgM (H + L), alkaline phosphatase-labeled goat anti-rabbit IgG (H + L), and alkaline phosphatase-labeled goat anti-chicken IgG (H + L) immunoglobulins were purchased from Kirkegaard & Perry Laboratories, Inc., Gaithersburg, MD.

Leaf tissue preparation. Inoculated leaves of *S. vaccaria* showing red ring local lesions were harvested 18 days after inoculation and one part of tissue was extracted by grinding the leaves in 10 parts of 0.02 M tris buffer containing 0.15 M NaCl, pH 7.6, and squeezing the slurry through cheesecloth. The extract was centrifuged at 5,000 *g* for 10 min and the supernatant was serially diluted in PBS-Tween before being tested. Leaves of *D. caryophyllus* were similarly prepared for ELISA testing. Some leaves from CERV-infected plants showed mild necrotic etch symptoms, but most were symptomless. Leaves from uninoculated *S. vaccaria* and *D. caryophyllus* were similarly prepared as control samples. Control samples gave $A_{405\ nm}$ readings ranging from 0 to 0.01. Data are presented as corrected values by subtracting absorbance of healthy controls from test samples for each experiment.

Immunoelectron microscopy (IEM). Purified virus from infected *S. vaccaria* was used in comparative studies of rabbit antiserum and mouse ascitic fluid in IEM. Initial experiments were performed with known concentrations of purified virus. Formvar-carbon coated 400-mesh grids were used for trapping the virus particles. Dilutions of rabbit antiserum at 1:100 were prepared in 0.05 M sodium phosphate buffer, pH 7.0. Mouse ascitic fluid was diluted 1:1,000 in the same buffer. Grids were incubated with antiserum for 1 hr, washed with 30 drops of phosphate buffer, and charged with CERV antigen for 1 hr. A final rinse with 30 drops of phosphate buffer was followed by staining with 2% phosphotungstic acid neutralized with NaOH. Comparisons were made between grids treated with antiserum only and those treated with protein A (0.1 mg/ml) (Pharmacia Fine Chemicals, Piscataway, NJ) for 10 min before exposing the grid to rabbit antiserum or ascitic fluid. Control grids were treated with purified Prunus necrotic ringspot virus (PNRV)-specific monoclonal antibodies (2) with and without protein A treatment.

Particle counts were made by photography on 8.26 × 10.16-cm (3.25 × 4.0-inch) lantern slide plates at a magnification that included 175 μm^2 of a single grid opening. A micrograph was taken from each of three grid openings and the particle count was an average of counts made from six fields on two grids.

Virus particles were also attached to grids and a second antibody treatment was applied to determine if the virions could be decorated with the monoclonal antibody. The decorating antibody was diluted 1:500 and 1:1,000 and incubated for 45 min on the virion-coated grids.

RESULTS

Immunoglobulin subclass identification. Three subclasses of monoclonal antibody specific to CERV were identified by using individual isotype-specific antiserum (Table 1). The subclass with the lowest antibody titer was IgM. Antibodies 22A2C3, 28B4F6, and 28E4C6 in subclasses IgG1 and IgG2b had higher titers and were used in subsequent studies.

Antibody evaluation. The titer of culture fluid from monoclonal antibody-secreting cell lines was 10^3 to 10^4 times lower than that of ascitic fluid produced from mice (Fig. 1). The titers of ascitic fluids

produced from three of these cell lines was $1:1.6 \times 10^6$ for two cell lines and $1:6.0 \times 10^6$ for the third cell line.

In double antibody sandwich ELISA, ascitic fluid from all three cell lines (28E4C6, 28B4F6, and 22A2C3) showed similar reaction curves over the range from 0.62 to 5.0 μg of purified CERV per milliliter (Fig. 2). All three ascitic fluids used in the coating step at a 1:1,000 dilution showed similar patterns when enzyme-labeled monoclonal antibodies 22A2C3 were used as the detecting antibody at 1 μg/ml.

In a heterologous double antibody sandwich ELISA test, rabbit polyclonal antibodies were used to replace mouse monoclonal antibodies for coating plates, and enzyme-labeled monoclonal antibodies were used to detect the virus. At virus concentrations from 0.62 to 5 μg/ml the optimal coating condition was a 1:250 dilution with rabbit antiserum. Higher dilution of rabbit antiserum used for coating gave lower $A_{405\ nm}$ readings. Higher concentrations of coating rabbit antiserum, however, showed little improvement in sensitivity. With monoclonal antibodies at a dilution of 1:1,000 of coating antibody, and using the same range of virus concentrations, $A_{405\ nm}$ values were three to four times higher. Carnation etched ring virus was not detected by double antibody sandwich ELISA when plates were coated with either crude extracts of egg yolk from immunized chickens or with partially purified and concentrated chicken antibodies.

In triple antibody sandwich ELISA tests, serial dilutions of CERV were incubated on plates coated with a 1:1,000 dilution of mouse monoclonal antibodies. The plates were then incubated with CERV-specific chicken antibodies at a 1:100 dilution followed by enzyme-labeled goat anti-chicken globulins before the addition of substrate. The curves were similar to those obtained for mouse double antibody sandwich ELISA shown in Fig. 2. When coating monoclonal antibodies were substituted with rabbit antiserum at a dilution of 1:250 in triple antibody sandwich ELISA, the minimum amount of CERV detected was about 1.25 μg/ml.

Detection of CERV in infected plants. CERV could be detected in extracts from infected leaf tissues by either double antibody sandwich ELISA using monoclonal antibodies (Fig. 3) or by triple antibody sandwich ELISA (Fig. 4) by using chicken anti-CERV antibodies followed by alkaline phosphatase-labeled goat anti-chicken globulins. In double antibody sandwich ELISA, coating individually with three different monoclonal antibodies all gave similar results when enzyme-labeled mouse monoclonal antibody 22A2C3 was used as the detecting antibody (Fig. 3). When the same extracts were tested in triple antibody sandwich ELISA on 22A2C3 antibody-coated plates followed by 1:100, 1:200, 1:500, and 1:1,000 dilutions of chicken antibodies and 1 μg of enzyme-labeled goat anti-chicken conjugate per milliliter, chicken antibodies gave lines of similar slope over the entire range of dilutions tested (Fig. 4). The highest concentration of CERV detected in infected leaf tissue was with a chicken antibody dilution of 1:100. At chicken antibody dilutions of 1:200 and 1:500, there was a corresponding decrease in the sensitivity of detection. When the chicken antibody was diluted to 1:1,000, the level of CERV detected in infected *D. caryophyllus* greatly decreased.

TABLE 1. Subclasses of mouse monoclonal antibodies from hybridoma cell lines prepared for carnation etched ring virus (CERV) and reciprocal titers of ascitic fluids produced after tumor transplantation

CERV cell lines	Antibody secreted	Ascitic fluid titers by ELISA (reciprocal)
1E6F8	IgM	25,600
14F9B12	IgM	25,600
14F9G8	IgM	25,600
22A2A7	IgG2b	409,600
22A2C3	IgG2b	1,638,400
22A2H8	IgG2b	1,638,400
28B4E7	IgG1	6,553,600
28B4E12	IgG1	6,553,600
28B4F6	IgG1	1,638,400
28E4B4	IgG1	1,638,400
28E4C6	IgG1	1,638,400

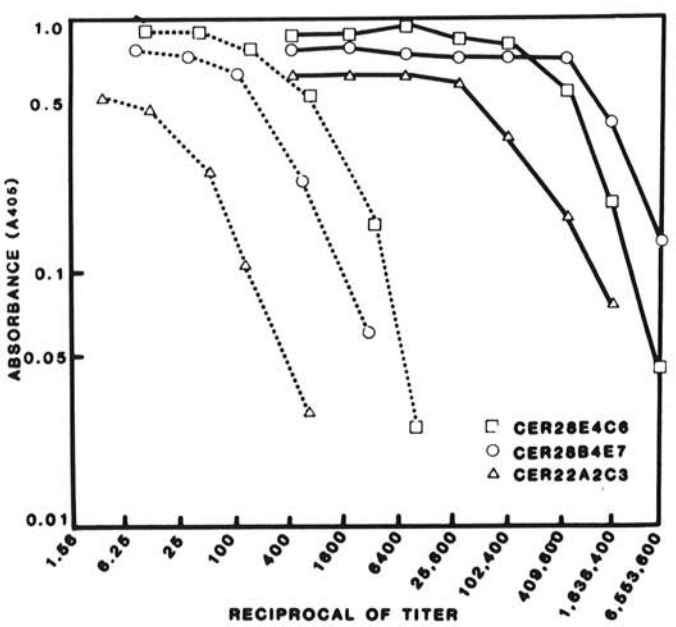

Fig. 1. Titration curves of carnation etched ring virus-specific monoclonal antibodies obtained by using indirect ELISA. Serial dilutions of culture fluid (dotted lines) or mouse ascitic fluid (solid lines) were incubated in plates coated with viral antigen at 5 μg/ml. Antibody was detected by alkaline phosphatase-labeled goat anti-mouse IgG + IgM.

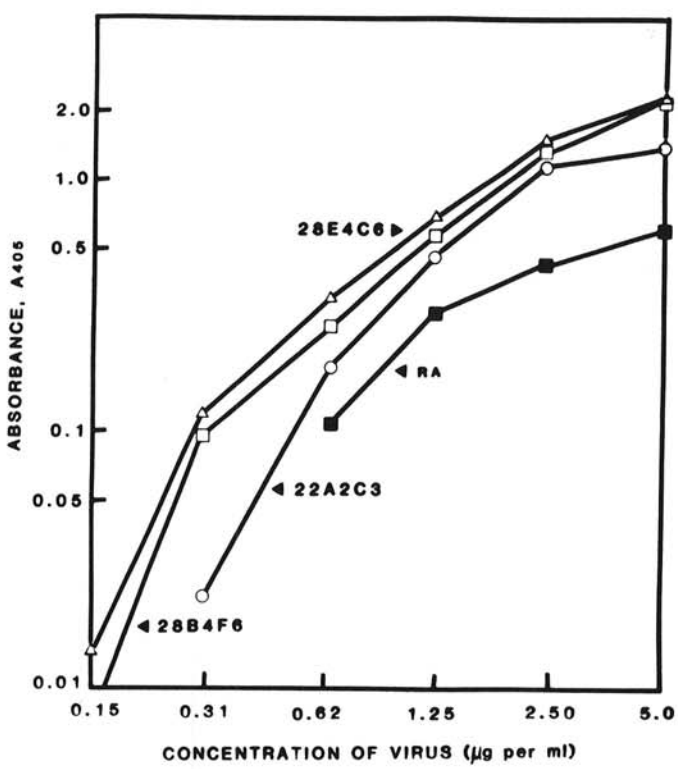

Fig. 2. Absorbance in double antibody sandwich ELISA (DAS-ELISA) showing a linear response in A_{405} values over a range of carnation etched ring virus concentration from 0.62 to 5 μg/ml. Open symbols represent homologous mouse-mouse monoclonal antibodies in DAS-ELISA. Solid squares show heterologous rabbit polyclonal-mouse monoclonal antibodies in DAS-ELISA.

Immunoelectron microscopy. The CERV rabbit antiserum diluted 1:100 resulted in high concentration of attached particles, whereas, only a low background particle count was observed with normal serum. At antiserum dilutions of 1:800 and greater only a few particles were observed. Purified CERV tested in the concentration range from 3 to 12 μg/ml showed a linear increase in particle attachment (Fig. 5). Treatment of grids with protein A increased the number of particles attached to the grids (Fig. 5). A linear increase was also observed on grids treated with protein A and a four- to five-fold increase in particle attachment was observed compared with grids that had received no protein A treatment.

Mouse ascitic fluid (22A2C3) gave optimum particle attachment at a dilution of 1:1,000. Virion attachment to purified monoclonal antibody (1.8 μg/ml) was linear over a concentration range from 3.0 to 12 μg/ml of purified antigen when grids were treated with protein A (Fig. 6). A similar linear response was obtained with the crude ascitic fluid on grids treated with protein A. Grids treated with purified monoclonal antibodies without protein A treatment showed a nonlinear response of virion attachment (Fig. 6). Fewer virus particles (about a three-fold reduction) attached to the grids at the 12 μg/ml concentration of antigen with both purified monoclonal antibody and crude ascitic fluid without protein A treatments compared with grids treated with protein A. A similar reduction in particle attachment occurred with purified monoclonal antibody at a virus concentration of 9 μg/ml, but there was more than a five-fold difference with crude ascitic fluid with and without protein A at this antigen concentration. At virus concentrations of 3 and 6 μg/ml there was a six- to eight-fold difference in the number of particles attached with both purified and crude monoclonal antibody without protein A treatment compared to the presence of protein A. Only low numbers of virions were attached to grids treated with or without protein A and

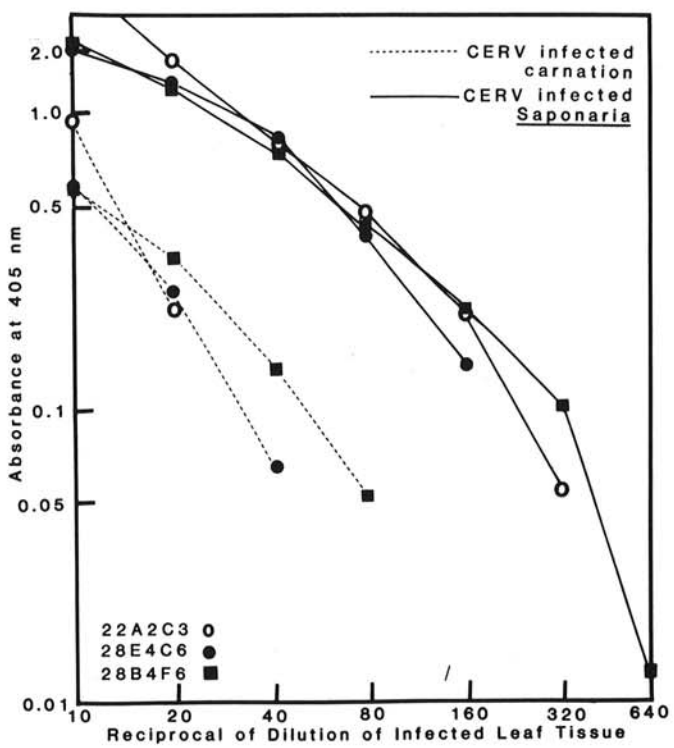

Fig. 3. Detection of carnation etched ring virus from infected *Dianthus caryophyllus* and *Saponaria vaccaria* in DAS-ELISA. Serial dilutions of infected leaf tissue extracts were incubated on PVC plates coated with mouse 22A2C3 monoclonal antibody at 1:1,000 dilution of unfractionated ascitic fluid. Alkaline phosphatase-labeled mouse 22A2C3 monoclonal antibodies at 1 μg/ml were used to detect CERV. The background control obtained with healthy tissue at each dilution was subtracted from comparable dilutions of extracts from diseased plants.

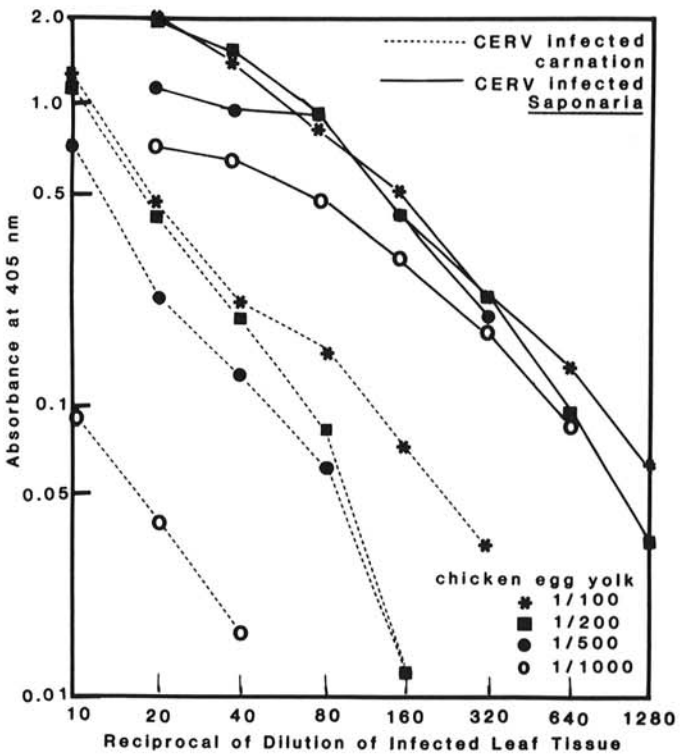

Fig. 4. Detection of carnation etched ring virus from infected *Dianthus caryophyllus* and *Saponaria vaccaria* in triple antibody sandwich ELISA. Serial dilutions of infected leaf tissue extracts were incubated on PVC plates coated with mouse 22A2C3 monoclonal antibody at 1:1,000 dilution of unfractionated ascitic fluid. Enzyme-labeled goat anti-chicken immunoglobulin conjugates (1 μg/ml) were used to detect CERV-specific chicken antibodies after the addition of chicken egg yolk at dilutions of 1:100, 1:200, 1:500, or 1:1,000. $A_{405\ nm}$ values were obtained as described in Fig. 3.

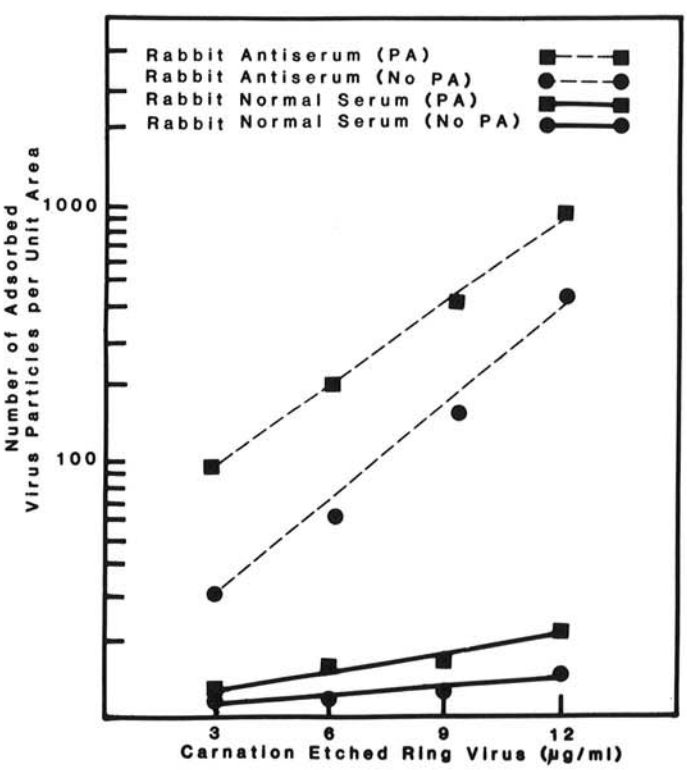

Fig. 5. Serological trapping of purified CERV by rabbit polyclonal antiserum or control normal serum, both at 1:100 dilution, on grids treated with and without protein A (PA).

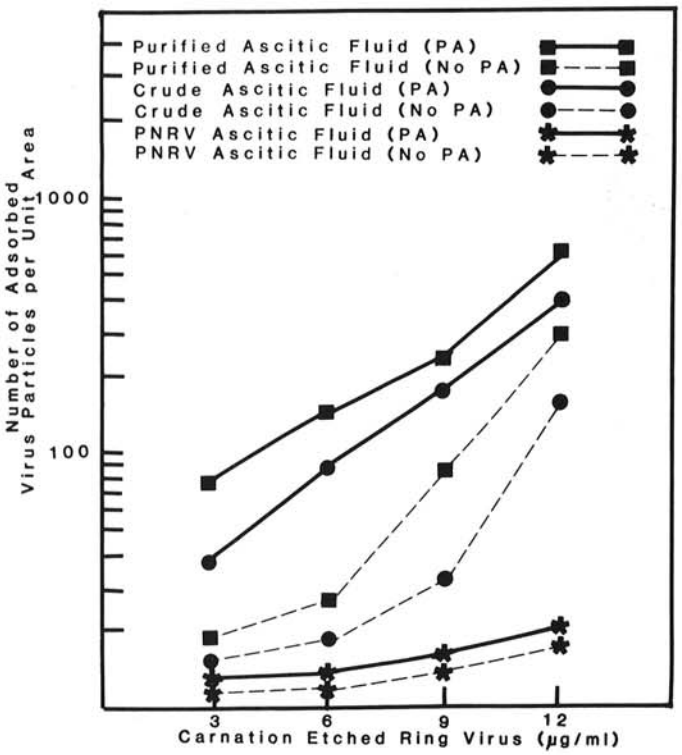

Fig. 6. Serological trapping of purified CERV at several concentrations by purified mouse monoclonal antibodies CER A22C3 or crude ascitic fluid at 1:1,000 dilution on grids treated with and without protein A (PA). Grids treated with purified Prunus necrotic ringspot virus monoclonal antibodies from ascitic fluid served as controls.

ascitic fluid containing monoclonal antibodies to Prunus necrotic ringspot virus.

Virions treated with the second monoclonal antibody specific to CERV showed decoration (Fig. 7A) typical of the antibody coating observed with rabbit polyclonal antiserum. The coating was present with both the 1:500 and 1:1,000 dilution of the second antibody. Monoclonal antibodies specific to Prunus necrotic ringspot virus did not show the decoration effect (Fig. 7B).

DISCUSSION

By using the mouse hybridoma technique (6) high-titer antibodies to CERV can be produced by cell lines generated from fusion of myelomas with splenocytes of immunized mice. These cell lines can be maintained in cultures or stored frozen indefinitely at liquid nitrogen temperature. When needed, cells can be revived and antibodies can be produced in mice by injecting the cells intraperitoneally. Ascitic fluid can be collected 2–3 wk after tumor transplantation.

The method also offers other advantages over the conventional technique of producing antisera in rabbits or chickens. Only small quantities of antigens are required to stimulate the immune response in mice. A total of about 70 µg of purified CERV antigen in three injections was used for each mouse. This is a particular advantage for CERV and other viruses which are difficult to obtain in sufficiently high concentration for antiserum production in rabbits. Once the antibody-secreting mouse cell lines were produced, no further immunization was required. Each hybridoma cell line produces antibodies of defined specificity so that the quality of serological reagent is assured.

Less CERV antigen was attached to PVC plates treated with rabbit antiserum than with mouse monoclonal antibodies. This could be due to a lower concentration of virus specific antibodies in rabbit antiserum, although it was used at a coating dilution of 1:250. Failure of chicken egg yolk antibodies to trap CERV antigens could be due to the inaccessibility of chicken antibodies to antigenic determinants on the virions when antibodies were

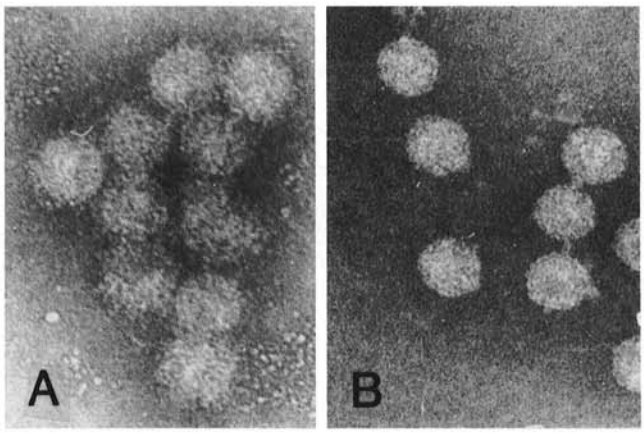

Fig. 7. Treatment of CERV with **A,** 22A2C3 monoclonal antibody as a second antibody and **B,** Prunus necrotic ringspot virus monoclonal antibody as a second antibody. Note the decoration of virions in A and not in B.

immobilized. Factors such as the presence of inhibitors or interfering substances in extracts of chicken egg yolk might also contribute to failure during coating. When chicken egg yolk antibodies were used as detecting antibodies in monoclonal antibody-coated plates followed by enzyme-labeled goat anti-chicken immunoglobulins, a dose-response curve similar to that of mouse double antibody sandwich ELISA was obtained. When rabbit antiserum was substituted for mouse monoclonal antibodies in triple antibody sandwich ELISA, the level of sensitivity for CERV detection was reduced. The reduced sensitivity of rabbit antiserum in ELISA could be attributed to less CERV-specific antibodies or antibodies of lower affinity.

The number of CERV particles attached to grids treated with rabbit antiserum was linearly related to virus concentration with and without protein A treatment. Only a low number of virions attached to grids coated with normal serum. The grids were rinsed with buffer after being sensitized with antiserum and again after incubation of the antigen.

Ascitic fluid-treated grids with or without protein A treatment showed a high concentration of attached virions at the highest virus concentration tested, although the grids were washed with buffer after antibody treatment. This may result from antigen saturation of the grid surface, as further increases in concentration of virus did not result in a significant increase in particle attachment. At lower concentrations of virus, the buffer wash following antibody incubation decreased particle attachment. The lack of linearity in virion attachment in the absence of protein A with both purified and crude monoclonal antibodies over the range of virus concentrations tested may be related to the poor spreading of monoclonal antibody and reduced affinity for the grid surface.

LITERATURE CITED

1. Hakkaart, F. A. 1974. Detection of carnation viruses with the test plant *Saponaria vaccaria* 'Pink Beauty.' Pages 35-46 in: Proc. Third Int. Symp. on Virus Disease of Ornamental Plants. Acta Hortic. 36. R. H. Lawson and M. K. Corbett, eds. Int. Soc. Hortic. Sci., The Hague, The Netherlands.
2. Halk, E. L., Hsu, H. T., Aebig, J., and Franke, F. 1984. Production of monoclonal antibodies against three ilarviruses and alfalfa mosaic virus and their use in serotyping. Phytopathology 74:367-372.
3. Hearon, S. S., and Lawson, R. H. 1981. Effects of light intensity, photoperiod, and temperature on symptom expression and host and virus ultrastructure in *Saponaria vaccaria* infected with carnation etched ring virus. Phytopathology 71:645-651.
4. Hsu, H. T., Aebig, J., and Rochow, W. F. 1984. Differences among monoclonal antibodies to barley yellow dwarf viruses. Phytopathology 74:600-605.
5. Hsu, H. T., and Lawson, R. H. 1984. Comparative detection of carnation etched ring virus using mouse monoclonal antibodies and chicken and rabbit antiserum. (Abstr.) Phytopathology 74:861.

6. Köhler, G., and Milstein, C. 1975. Continuous culture of fused cells secreting antibodies of predefined specificity. Nature 256:495-497.
7. Lawson, R. H., and Civerolo, E. L. 1976. Purification of carnation etched ring virus and comparative properties of CERV and cauliflower mosaic virus nucleic acids. Acta Hortic. 59:49-59.
8. Lawson, R. H., and Civerolo, E. L. 1978. Carnation etched ring virus: purification, stability of inclusions, and properties of the nucleic acid. Phytopathology 68:181-188.
9. Littlefield, J. W. 1964. Selection of hybrids from matings of fibroblasts in vitro and their presumed recombinants. Science 145:709-710.
10. McKearn, T. J. 1980. Cloning of hybridoma cells by limiting dilution in fluid base. Page 374 in: Monoclonal Antibodies; Hybridomas: A New Dimension in Biological Analysis. R. H. Kennett, T. J. McKearn, and K. B. Bechtol, eds. Plenum Press, New York.