Identification and Subgrouping of *Cucumber mosaic virus* with Mouse Monoclonal Antibodies

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ABSTRACT

Hsu, H. T., Barzuna, L., Hsu, Y. H., Bliss, W., and Perry, K. L. 2000. Identification and subgrouping of *Cucumber mosaic virus* with mouse monoclonal antibodies. *Phytopathology* 90:615-620.

Using a mixture of isolates of *Cucumber mosaic virus* (CMV) from subgroups I and II as immunogens, 20 mouse hybridoma cell lines secreting monoclonal antibodies were produced. A reliable method for efficient detection and accurate subgrouping of CMV isolates has been developed. Tests with 12 well-characterized strains of CMV and other

cucumoviruses demonstrated the presence of epitopes that were virus and subgroup specific. Analyses of 109 accessions of CMV isolates collected from various parts of the world revealed 70% were subgroup I, with 20% identified as subgroup II. Seven isolates (6%) did not react with group-specific antibodies but did react with antibodies that recognized all CMV isolates. Differential reactions among isolates suggested a total of 10 epitopes were recognized. The antigenic diversity among subgroup II CMVs was greater than for the subgroup I isolates, even though fewer subgroup II isolates were tested.

Cucumber mosaic virus (CMV) is the type species of the genus *Cucumovirus* and is one of the most widespread plant viruses in the world (22). CMV has a very broad host range of wild and cultivated plants, with more than 800 known hosts (7,22). The virus causes diseases and economic losses in cereals, fruits, vegetables, and ornamentals (19). CMV produces a variety of symptoms, the most typical being a mottle or mosaic. Depending on the host and isolate of the virus, the symptoms also may include growth distortion, blight, fan leaf, ringspot, fruit woodiness, and necrosis followed by death of the host plant. Symptoms incited by CMV can be affected by the presence of satellite RNA (22), e.g., by the CMV-associated RNA5 (CARNA5) (18,30). Diseases caused by CMV have been confused with those caused by other viruses, and in some cases, CMV does not induce obvious symptoms (3,26). The natural spread of CMV is primarily by aphid vectors (9). Seed transmission of CMV is also a significant means of transmission in some weeds and cultivated crop plants (19). Weed hosts function as a reservoir for the virus and serve as sources of inoculum in the regional development of disease epidemics (26).

Serological studies of CMV have shown this species to be antigenically heterogeneous (4-6). CMV isolates were originally characterized as belonging to one of two serological groups (4), although a third serogroup has been reported (6). The serogroup concept subsequently was broadened based on hybridization analyses using complementary DNAs (11,21,24). Although the terms subgroup and serogroup have routinely been used interchangeably, a serogroup designation is based on antigenicity and the subgroup

is inferred. Complications result in the case of some unique viruses that are naturally occurring reassortants of two cucumoviruses or strains (31).

The serological differentiation of CMV isolates is of importance in breeding for disease resistance and in studying disease epidemiology, so specific monoclonal antibodies have been developed (1,12,25,26). One of the goals in their development was to provide tools for serotyping isolates of the virus. Monoclonal antibodies with serotype specificity were described (12,25), but they were tested only with a limited number of strains and, in some cases, did not recognize all members within a subgroup. The objectives of this study were to obtain monoclonal antibodies that are subgroup specific and to test a representative collection of worldwide isolates to validate the utility of the antibodies for serological classification. In this article, we report the establishment of somatic cell hybrids that secrete monoclonal antibodies to CMV and present an analysis of their potential for use in the serotyping of CMV.

MATERIALS AND METHODS

Viruses and antigens. The CMV isolates used were from Agdia, Inc. (Elkhart, IN), the American Type Culture Collection (ATCC; Manassas, VA), L. Barnes (Texas A&M University, College Station), T. Day (Sakata Seed America, Inc., Salinas, CA), J. Fletcher (Crop and Food Research, Christchurch, New Zealand), R. W. Goth (U.S. Department of Agriculture-Agricultural Research Service [USDA-ARS], Beltsville, MD), S. K. Green (Asian Vegetable Research and Development Center, Tainan, Taiwan), J. M. Kaper (USDA-ARS, Beltsville, MD), X. P. Liu (Heilongjiang Academy of Agricultural Sciences, Harbin, China), A. Stein (Agricultural Research Organization, The Volcani Center, Bet Dagan, Israel), and the authors (Table 1). The E isolate of *Peanut stunt virus* (PSV) and the V strain of *Tomato aspermy virus* (TAV) were from the Purdue University collection, and PSV isolate IZ9101 was obtained from J. Staniulis (Botanikos Institutas, Vilnius, Lithuania). Virions of the following were purified and used as immunogens: CMV isolates S, D, Price's 6, NT9, BCM, GC1, GCM, CR M-1, and CR P-1.

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Publication no. P-2000-0330-03R

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TABLE 1. Sources of *Cucumber mosaic virus* isolates and their contributors

Isolate	Origin	Location	Contributor ^a	Isolate	Origin	Location	Contributor ^a
A7410			Agdia	P1018	Pepper	Taiwan	Green
C310 (N890)	Gladiolus	Georgia	Agdia	P1347	Pepper	Taiwan	Green
C105	Pepper	Michigan	Agdia	P1360	Pepper	Taiwan	Green
C109	Tomato	California	Agdia	P1369	Pepper	Taiwan	Green
C107	Cucurbit	Indiana	Agdia	P1370	Pepper	Taiwan	Green
C296	Cucurbit	Arizona	Agdia	P1441	Pepper	Taiwan	Green
C297	Cucurbit	Arizona	Agdia	P1471	Pepper	Taiwan	Green
M426	Pepper	Indiana	Agdia	P1624	Pepper	Taiwan	Green
O69	Orchid	Florida	Agdia	P1628	Pepper	Taiwan	Green
P922		USDA	Agdia	P1886	Pepper	Taiwan	Green
Watercress	Watercress		Agdia	P1927	Pepper	Taiwan	Green
PV 29 Type 1	Cucumber	Wisconsin	ATCC	P1929	Pepper	Taiwan	Green
PV 30 CD	<i>Commelina nudiflora</i>	Maryland	ATCC	P2469	Pepper	Taiwan	Green
PV 59	Sugar beet	Arizona	ATCC	P2853	Pepper	Taiwan	Green
PV 242		Maryland	ATCC	P2874	Pepper	Taiwan	Green
PV 243	Mung bean	Missouri	ATCC	P2875	Pepper	Taiwan	Green
PV 289 Q		Australia	ATCC	P2878	Pepper	Taiwan	Green
PV 453 C	Pepper	California	ATCC	Peet	Tomato	Taiwan	Green
PV 539 Py		California	ATCC	RS12	Tomato	Taiwan	Green
PV 540 Y		Ibaraki, Japan	ATCC	T2373	Tomato	Taiwan	Green
PV 542 LS		New York	ATCC	T2374	Tomato	Taiwan	Green
PV 543 O		Japan	ATCC	BCM	Banana	Taiwan	Hsu
PV 544 PY		New Jersey	ATCC	GC1	Gladiolus	Taiwan	Hsu
PV 545 P6		New Jersey	ATCC	GCM	Gladiolus	Taiwan	Hsu
PV 546 M		Cambridge, UK	ATCC	1		United States	Kaper
PV 547 Sny		New York	ATCC	16		Japan	Kaper
PV 548 Fny		New York	ATCC	ICS		Indonesia	Kaper
PV 549 CMV-48			ATCC	Ixora		Philippines	Kaper
PV634 K8	<i>Cucumis metuliferus</i>	Piedmont, Italy	ATCC	D		France	Kaper
S390	<i>Ajuga</i> sp.	Texas	Barnes	R		France	Kaper
CR M-1	Melon	Costa Rica	Barzuna	R76B		England	Kaper
CR P-1	Plantain	Costa Rica	Barzuna	Rm		France	Kaper
T118	Impatiens	Japan	Day	S		South Africa	Kaper
F354 Seed transmissible	Spinach	California	Day	Peet		Taiwan	Kaper
#4 Not seed transmissible	Spinach	California	Day	RS15		Taiwan	Kaper
88	Pepper	New Zealand	Fletcher	Y	Tobacco	Japan	Kaper
90	Lentil	New Zealand	Fletcher	#2		China	Liu
94	Scotch thistle	New Zealand	Fletcher	#3		China	Liu
97	Tomato	New Zealand	Fletcher	#5		China	Liu
98	Spinach	New Zealand	Fletcher	#10		China	Liu
100	Pepper	New Zealand	Fletcher	#11		China	Liu
101	Tomato	New Zealand	Fletcher	#12		China	Liu
107	Pepper	New Zealand	Fletcher	#14		China	Liu
110	Lettuce	New Zealand	Fletcher	Fny	Muskmelon	New York	Perry
116	<i>Gentiana</i> sp.	New Zealand	Fletcher	Sny	Muskmelon	New York	Perry
119	Pea	New Zealand	Fletcher	T	Pepper	Western Australia	Perry
223	Myoga ginger	New Zealand	Fletcher	O	Lupin	Queensland, Australia	Perry
WT		Wisconsin	Goth	Q	Pepper	Queensland, Australia	Perry
#1, 10/26/63	Tobacco	United States	Goth	LS	Lettuce	New York	Perry
F10	Tomato	Taiwan	Green	U	Banana	Queensland, Australia	Perry
M48	Mung bean	Taiwan	Green	T ₁ T ₂ Q ₃	Reassortant with isolate CMV-Q RNA 3		Perry
NT4	Tomato	Taiwan	Green	M	Mutant of Price's 6		Perry
NT9	Tomato	Taiwan	Green	V135			Perry
P3	Pepper	Taiwan	Green	F ₁ F ₂ M ₃	Reassortant with isolate CMV-M RNA 3		Perry
P117	Pepper	Taiwan	Green	V389		Italy	Perry
P184	Pepper	Taiwan	Green	N-1A	Squash	New York	Perry
P212	Pepper	Taiwan	Green	N-1B	Squash	New York	Perry
P427	Pepper	Taiwan	Green	Ala1	Tomato	Alabama	Perry
P522	Pepper	Taiwan	Green	Ala3	Tomato	Alabama	Perry
P600	Pepper	Taiwan	Green	Mal1		USA	Perry
P652	Pepper	Taiwan	Green	1A1	Pepper	Illinois	Perry
P661	Pepper	Taiwan	Green	2A1	Pepper	Illinois	Perry
P888	Pepper	Taiwan	Green	S2A	Squash	New York	Perry
P959	Pepper	Taiwan	Green	Price's 6		New Jersey	Stein

^a Agdia, Inc., Elkhart, IN; ATCC, American Type Culture Collection, Manassas, VA; L. Barnes, Texas A&M University, College Station; L. Barzuna, USDA-ARS, Beltsville, MD; T. Day, Sakata Seed America, Inc., Salinas, CA; J. Fletcher, Crop and Food Research, Christchurch, New Zealand; R. W. Goth, USDA-ARS; S. K. Green, Asian Vegetable Research and Development Center, Tainan, Taiwan; H. T. Hsu, USDA-ARS; J. M. Kaper, USDA-ARS; X. P. Liu, Heilongjiang Academy of Agricultural Sciences, Harbin, China; K. L. Perry, Agdia, Inc.; A. Stein, Agricultural Research Organization, The Volcani Center, Bet Dagan, Israel.

Purified preparations of the S and D isolates were gifts from J. M. Kaper. All other isolates were purified from inoculated squash, as described by Hsu et al. (17). Previously characterized subgroup I isolates D, Fny, M, O, Sny, Y, and T and subgroup II isolates LS, Q, S, U, and R (21,25,29) were used for initial characterization of monoclonal antibody recognition specificity.

Immunization. A mixture of equal amounts of the nine purified viruses was used for immunization of mice. Three BALB/c female mice were each injected with a total of 100 µg of purified virus according to an immunization schedule previously described (15). Five days after the fourth injection, mice were given a peritoneal injection of 200 µg of purified virus in 0.2 ml of phosphate-buffered saline solution (PBS; 0.01 M phosphate and 0.015 M sodium chloride, pH 7.5). Mice were sacrificed 3 days later, and their spleens were harvested.

Fusion and selection. Single splenocyte suspensions were prepared from immune spleens and fused with FOX-NY myeloma cells, as described previously (14). After fusion, cells were resuspended and cultured in a FOX-NY-conditioned medium (nine parts of complete RPMI-1640 medium and one part of myeloma cell-conditioned medium) supplemented with 10^{-1} mM hypoxanthine, 4×10^{-4} mM aminopterin, 1.6×10^{-2} mM thymidine, 6 mM Hepes, and 2×10^{-2} mM 2-mercaptoethanol, and seeded in 96-well culture plates (16). Twice a week, ≈ 100 µl of culture medium was replaced with a similar amount of the same fresh medium. Two weeks after fusion, aminopterin was omitted from the medium.

Monoclonal antibodies. Two types of enzyme-linked immunosorbent assays (ELISA) were used for identification of hybridomas secreting antibodies specific to CMV (15). In one procedure, coating antibody solutions (2 µg/ml) were prepared by mixing equal amounts of polyclonal antisera prepared against CMV-D (subgroup I) and CMV-S (subgroup II). Mixtures of each antigen used for immunization (1 µg/ml) were prepared in PBS and incubated in antibody-coated wells before the culture medium was added to each well. The second procedure involved sensitizing ELISA plates with the same mixture of antigens prepared in 0.1 M carbonate coating buffer at pH 9.6. Alkaline phosphatase-labeled goat antimouse immunoglobulins were used to detect mouse antibodies in both procedures. A double-antibody sandwich (DAS)-ELISA employing purified rabbit antibodies (mixture of equal amounts of 1 µg each of anti-CMV-D and anti-CMV-S per ml) and homologous enzyme conjugates was used as a control. Immunoglobulin class was determined with commercial isotyping kits (Sigma-Aldrich Chemical Co., St Louis). Single-cell clonings were similar to those previously described, except that FOX-NY-conditioned medium was used (14). Large-scale production of monoclonal antibodies in ascitic fluids was done by Cocalico Biological Co. (Streamtown, PA).

Antibody specificities and CMV serotyping. Antibodies were titrated by DAS-ELISA, using horseradish peroxidase (HRP)-immunoglobulin conjugates. Individual antibody concentrations that gave between 1.5 and 2.0 absorbance (492 nm) values 15 min after addition of substrate were determined and used in triple-antibody sandwich (TAS)-ELISA (Table 2) for determination of reactivities of mouse monoclonal antibodies to CMV isolates. The polyclonal antisera for CMV, PSV, and TAV were from Agdia. ELISA plates were coated with a mixture of equal amounts of rabbit immunoglobulins prepared against CMV-D and CMV-S. Individual viral antigens were prepared from fresh leaf tissues (1:10, wt/vol) or dried leaf tissue (1:100, wt/vol) by grinding in a mortar and pestle with Agdia extraction buffers. Purified virus was diluted to 200 ng/ml in the same extraction buffer. After the addition of individually optimized monoclonal antibodies (Table 2) to the plates, HRP-labeled rabbit antimouse immunoglobulin antibodies (0.3 µg/ml) in DeMan-Rogosa-Sharpe (MRS) buffer (Agdia) were immediately added and incubated together with the monoclonal antibodies. Phenylenediamine dihydrochloride at 0.1 mg/ml in a hydrogen peroxide solution, pH 5.0, was used as substrate. Heal-

thy tobacco plants, plants infected with *Potato A virus* (PVA), and buffer alone were used as controls. All tests were duplicated. Reagents were used (100 µl per test well) in Nunc Maxisorp microtiter plates (A/S Nunc, Roskilde, Denmark).

RESULTS

Twenty mouse hybridoma cell lines secreting monoclonal antibodies to CMV were established, and the immunoglobulin classes and subclasses for each were determined (Table 2). The antibodies were produced in ascitic fluids in mice, and the optimum dilution of the antibody solutions for use in ELISA ranged from 1:1,000 to 1:32,000 (Table 2).

In testing the recognition specificity of the monoclonal antibodies, a DAS-ELISA was used as a reference in all assays. This used a mixture of polyclonal rabbit antisera prepared to CMV-D (subgroup I) and CMV-S (subgroup II), and these polyclonal antibodies detected all strains of CMV in both subgroups (Tables 3 and 4). In TAS-ELISA, assays with extracts from control plants, either healthy or PVA infected, yielded optical density (492 nm; OD_{492nm}) readings in the range of 0.029 to 0.072. Scores of 2 indicate absorbance values >0.4 , and scores of 1 indicate absorbance values ranging from 0.1 to 0.4. Absorbance values <0.1 were scored as 0.

Monoclonal antibodies, when tested with 12 well-characterized CMV isolates, could be assigned to three major categories based on their specificities (Table 3). Three monoclonal antibodies (4H10B6, 4H10B12, and 6D11B9) reacted with all members of CMV in subgroup I but did not react with CMV strains in subgroup II. Four monoclonal antibodies (6D11D12, 7B3D3, 7B3D9, and 60B3E7) reacted with CMV strains in subgroup II but did not react with CMV in subgroup I, although 60B3E7 failed to recognize one of the subgroup II strains. A number of the monoclonal antibodies reacted with all 12 isolates from both subgroups; the 3 strongest reactions strongly (10F10F9, 44E9A7, and 62B7A3) are indicated in Table 3. Antigenic variations were apparent among members of CMV in subgroup II; fewer variations were observed in subgroup I.

To establish the general applicability of the monoclonal antibodies, a variety of CMV isolates from worldwide collections were tested. Most of the CMV isolates from culture collections were previously uncharacterized, but others have been well studied. Some isolates may be duplicates and were maintained independently in the different collections. Approximately 70% of the isolates (76/109) were assigned to subgroup I, whereas 20% (22/109) of the isolates were identified as subgroup II (Table 4). All isolates of CMV in

TABLE 2. *Cucumber mosaic virus* antibodies, immunoglobulin (Ig) classes and subclasses of monoclonal antibodies, and dilutions of ascitic fluids used in the study

Antibody	Class and subclass	Optimum dilution
Polyclonal		1/1,300
10F10F9	IgG1	1/16,000
44E9A7	IgM	1/32,000
62B7A3	IgG2a	1/4,000
23C10E4	IgG1	1/4,000
34H11C10	IgG1	1/16,000
6G6B4	IgG1	1/4,000
9D11F11	IgG1	1/4,000
15E6F2	IgG1	1/4,000
42G5D3	IgM	1/16,000
14C5A4	IgG2a	1/32,000
44E4B7	IgG2a	1/16,000
14D12H9	IgG2a	1/16,000
46D12B12	IgM	1/1,000
4H10B6	IgG1	1/8,000
4H10B12	IgG1	1/32,000
6D11B9	IgG1	1/32,000
6D11D12	IgG1	1/16,000
7B3D3	IgG1	1/8,000
7B3D9	IgG1	1/1,000
60B3E7	IgM	1/16,000

the our study tested positively in TAS-ELISA with monoclonal antibodies 10F10F9, 44E9A7, and 62B7A3 (Table 4). All of the monoclonal antibodies also were tested for their recognition of TAV and PSV, and, with one exception, results were negative, and ELISA values were comparable to those of the controls. The exception was antibody 62B7A3, which reacted weakly with TAV and recognized one of the two PSV isolates (Table 4). Seven CMV isolates (C296, M48, PV243, PV634, M426, #4 not seed transmiss-

sible, and 116) were unique in that they were not recognized by either subgroup I- or subgroup II-specific monoclonal antibodies (Table 4). These unique isolates tested negative for TAV and PSV in DAS-ELISA with polyclonal rabbit antisera, with the exception of #4 not seed transmissible, which was not available in sufficient quantity for additional testing. Four CMV isolates (S2A, C105, Mal1, and C109) reacted with both subgroup I- and subgroup II-specific antibodies, although in each case reactions with one of the two

TABLE 3. Reactions of monoclonal antibodies to previously characterized strains of *Cucumber mosaic virus* (CMV) of known subgroup^a

Cell line or antibody	CMV subgroup I			CMV subgroup II				
	D ^b	Fny ^c	M	LS	Q	S	U	R
Polyclonal	2	2	2	2	2	2	2	2
10F10F9	2	2	2	2	2	2	2	2
44E9A7	2	2	2	2	2	2	2	2
62B7A3	2	2	2	2	2	2	2	2
23C10E4	2	1	2	2	2	2	2	2
34H11C10	2	2	2	2	2	2	2	0
6G6B4	2	2	1	2	1	2	1	2
9D11F11	2	2	1	2	1	2	1	2
15E6F2	2	2	1	2	1	2	1	2
42G5D3	2	2	2	1	2	2	1	1
14C5A4	2	2	2	2	2	1	0	0
44E4B7	2	2	2	2	2	1	0	0
14D12H9	2	2	2	2	2	1	0	0
46D12B12	2	2	1	2	2	0	0	0
4H10B6	2	2	2	0	0	0	0	0
4H10B12	2	2	2	0	0	0	0	0
6D11B9	2	2	2	0	0	0	0	0
6D11D12	0	0	0	2	2	2	2	2
7B3D3	0	0	0	2	2	2	2	2
7B3D9	0	0	0	2	2	2	2	2
60B3E7	0	0	0	0	1	2	1	1

^a Enzyme-linked immunosorbent assay reactions were scored as 2 for measurements greater than 0.4; 1 for measurements of 0.1 to 0.4; and 0 for measurements less than 0.1. The optical density for buffer wells ranged from 0.009 to 0.070. The optical density for healthy or *Potato A virus*-infected tobacco ranged from 0.029 to 0.072. Reactions that are virus or subgroup specific are boxed.

^b Strains O, Sny, and Y produced results similar to strain D.

^c Strain T produced results similar to strain Fny.

TABLE 4. Reactions of monoclonal antibodies to *Cucumber mosaic virus* isolates from various collections and designation of proposed recognized epitopes (PE)^a

	Cucumber mosaic virus isolate																											
Cell line or antibody	1A1 ^b	T118	#14	BCM ^c	S2A	C105	Mal1	C109	F354 ST ^d	R76B	S390	100	PV242 ^e	88	119 ^f	97	98	110 ^g	PV289	PV549	Rm	C296	M48 ^h	PV634	M426	#4 NST ⁱ	PE	
Polyclonal	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	1	1	2	2	2	2	2		
10F10F9	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	1	
44E9A7	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	1	
62B7A3 ^j	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	1	2	1	2	2	2	2	
23C10E4	1	0	1	2	2	2	2	2	2	2	0	2	2	2	2	2	1	1	1	2	2	2	0	1	1	0	3	
34H11C10	2	2	2	1	2	2	2	2	2	2	2	2	1	2	2	0	1	1	1	1	0	2	1	2	2	1	4	
6G6B4	2	1	1	0	2	2	2	1	2	1	1	2	1	1	1	1	1	0	1	0	0	2	1	0	0	0	5	
9D11F11	2	1	1	0	2	2	2	2	2	1	1	1	1	1	1	1	0	0	1	0	0	2	1	0	0	0	5	
15E6F2	2	1	0	0	2	2	2	1	2	1	1	2	1	2	1	1	1	0	1	0	0	2	1	0	0	0	5	
42G5D3	2	2	2	2	2	2	1	1	2	1	1	2	0	2	2	2	1	1	0	0	0	0	0	0	0	1	6	
14C5A4	2	2	2	1	2	2	2	2	2	1	2	1	1	1	0	0	0	0	0	0	0	2	1	2	0	0	7	
44E4B7	2	2	2	1	2	2	2	2	2	1	2	1	1	1	0	0	0	0	0	0	0	2	1	2	0	0	7	
14D12H9	2	2	2	1	2	2	2	2	2	1	2	1	1	0	0	0	0	0	0	0	0	2	1	2	0	0	7	
46D12B12	2	2	1	1	2	2	1	1	2	1	2	0	0	0	0	0	0	0	0	0	0	2	1	1	1	0	7	
4H10B6	2	2	2	2	2	2	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8	
4H10B12	2	2	2	1	2	2	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8	
6D11B9	2	2	2	1	2	2	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8	
6D11D12	0	0	0	0	1	1	2	2	2	2	2	2	2	2	2	2	1	1	1	1	1	0	0	0	0	0	9	
7B3D3	0	0	0	0	1	1	2	2	2	2	2	2	2	2	2	2	1	1	1	1	1	0	0	0	0	0	9	
7B3D9	0	0	0	0	1	1	2	2	2	2	2	2	2	2	2	2	1	1	1	1	1	0	0	0	0	0	9	
60B3E7	0	0	0	0	1	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	10	

^a Enzyme-linked immunosorbent assay reactions were scored as 2 for measurements greater than 0.4, 1 for measurements from 0.1 to 0.4, and 0 for measurements less than 0.1. The optical density for the buffer wells ranged from 0.009 to 0.070. The optical density for healthy and *Potato A virus*-infected tobacco ranged from 0.029 to 0.072. Reactions that are virus or subgroup specific are boxed.

^b The following isolates produced results similar to 1A1: 16, 2A1, Ala1, Ala3, C107, C297, C310, F10, ICS, Ixora, N-1A, N-1B, NT4, NT9, O69, P3, P117, P184, P212, P427, P522, P600, P652, P661, P888, P959, P1018, P1347, P1360, P1369, P1370, P1441, P1471, P1624, P1628, P1886, P1927, P1929, P2469, P2853, P2874, P2875, P2878, Peet, RS12, T2373, T2374, Tobacco #1, V135, F.F.M., V389, #2, #3, #5, #10, #11, #12, PV29, PV30, PV59, PV453, PV539, PV540, PV543, PV544, PV545, PV546, PV547, PV548, WT, and A7410.

^c CMV P922 produced results similar to BCM.

^d Samples T₁T₂Q₃, RS15, and 107 produced results similar to F354 ST (seed transmissible).

^e PV542 produced results similar to PV242.

^f Samples Watercross, 94, 101, and 90 produced results similar to 119.

^g Sample 223 produced results similar to 110.

^h PV243 produced results similar to M48.

ⁱ Sample 116 produced results similar to #4 NST (not seed transmissible).

^j The epitope identified by the 62B7A3 antibody also is present on a *Peanut stunt virus* isolate (IZ9101) obtained from Lithuania and a *Tomato aspermy virus* isolate (V-TAV). Absorbance (492 nm) was greater than 3.0 for IZ9101 and averaged 0.126 for V-TAV.

types of subgroup-specific antibodies were relatively weak (Table 4). The absorbance values for S2A and C105 tested with the subgroup I-specific antibodies were >3.0, whereas the values with the subgroup II-specific antibodies were always <0.2 (data not shown). On the other hand, absorbance values of Ma11 and C109 with subgroup I-specific antibodies were generally <0.2, whereas the subgroup II-specific antibodies were between 1.6 and 3.0 (data not shown).

DISCUSSION

Results obtained in tests of all 121 strains and isolates revealed patterns that suggest there are at least 10 epitopes on virions of CMV (Table 4). These epitopes are inferred by the observation that subsets of the monoclonal antibodies recognized the same isolates, usually with similar intensities of signals. For instance, the first two monoclonal antibodies listed in Table 4, 10F10F9 and 44E9A7, reacted with every isolate of CMV tested and are hypothesized as recognizing the same epitope, designated epitope 1. The third antibody, 62B7A3, had a similar pattern, but it also reacted with a TAV and PSV isolate; therefore, its recognized epitope is designated epitope 2. Other antibodies recognized different subsets of the collection of isolates, and their recognized epitopes are designated 3 through 10. The three monoclonal antibodies that are subgroup I-specific are thought to recognize the same epitope (epitope 8). Similarly, the epitope unique to the subgroup II strains is designated epitope 9. The antibody 60B3E7 is subgroup II specific, but it recognized only a small number of subgroup II isolates; therefore, its epitope is given a separate designation, epitope 10. Interestingly, at least a part of epitope 3 recognized by monoclonal antibody 23C10E4 has been identified. It is a linear sequence of at least six coat protein-specific amino acids sufficient to confer binding (13).

Analyses of antibody reactivity of a large number of isolates in this study have identified those whose coat proteins appear to be different from the majority. The four isolates that are recognized by both subgroup I- and subgroup II-specific antibodies appear to be unique, although it is possible that these isolates originated from infections with a mixture of CMV strains belonging to both subgroups; mixed infections are not uncommon in natural populations of CMV (10,23). Other examples would be the seven isolates that are not recognized by either set of the subgroup-specific antibodies. It would be of interest to see if the sequencing of these coat protein genes revealed common changes at the positions of otherwise highly conserved amino acids.

A variety of properties have been used to group CMV isolates into two major subgroups, including antigenic specificity, symptomatology, peptide mapping, and nucleic acid analyses (2,8,20,21,24,29). A recent example of these methods is the development of reverse transcriptase-polymerase chain reaction-based assays (27,28). Although serological methods facilitate the rapid processing of large numbers of samples, they suffer from the drawback of analyzing, via the encoded coat protein, only a small portion of the viral genome. In our study, the identification of virus subgroup using serological methods has yielded results consistent with those obtained by analyses of the entire genome. This indicates that these monoclonal antibodies can be used reliably to type a majority of CMV isolates to a subgroup. Furthermore, two of the antibodies recognize all CMV isolates tested but do not cross react with other cucumoviruses. Given the number of isolates used in this study, it is expected that these virus- and subgroup-specific monoclonal antibodies will be generally applicable in both diagnostic work and epidemiological studies.

ACKNOWLEDGMENTS

We thank L. Barnes, T. Day, J. Fletcher, R. Goth, S. Green, J. M. Kaper, X. P. Liu, J. Stanislus, and A. Stein for providing CMV isolates from their collections.

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