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Monoclonal Antibodies Against Sugarcane Mosaic Virus

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ABSTRACT

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By using purified virus preparations of sugarcane mosaic virus strains A, B and D (SCMV-A, -B and -D) as immunogens, 19 stable hybridoma cell lines secreting specific monoclonal antibodies (MABs) were obtained. The MABs were identified to belong to IgA, IgG2a and IgM subtypes. According to antigenic specificity against SCMV-A, -B, -D strains and the B strain of maize dwarf mosaic virus (MDMV-B), they were divided into five serogroups. The serogroup I MABs recognized common epitopes and reacted with all four strains strongly, while serogroup II and III showed different degrees of serological reaction with the strains. Serogroup IV reacted with the three strains of SCMV but not with MDMV-B. Serogroup V was highly specific to SCMV-D and reacted weakly with MDMV-B, but did not react with SCMV-A and -B. The titres of three representative antibodies in the culture supernatant, ascitic fluid or as purified immunoglobulin were measured. The detectable concentration of SCMV in corn and sugarcane leaves was also determined. Application of these monoclonal antibodies to differentiate SCMV strains is discussed.

Key words: sugarcane mosaic virus, maize dwarf mosaic virus, strains, monoclonal antibody.

INTRODUCTION

Sugarcane mosaic virus (SCMV)(16,20) exists as various strains which differ in their ability to cause infection and in the degree of host plant injury they cause (1,13). The different strains usually produce similar symptoms on most current commercial sugarcane varieties. They can, however, be separated by the distinctive symptoms shown on selected indicator plants (1,9,15,19,21).

Several laboratories have attempted to develop serological techniques for differentiating SCMV strains (3,12,17,18). Serogroups have been established by using polyclonal antibodies. But their classification is somewhat different among laboratories, and one serogroup often contains more than one pathological strain.

Monoclonal antibodies (MABs) specific for virus strains have provided a method for identifying strains of plant viruses (2,4,14). In this paper, we report the preparation of MABs against SCMV that can be applied for differentiation of strains of this virus.

MATERIALS AND METHODS

Virus strains and immunization

Three strains of SCMV, SCMV-A, -B, and -D (10), and B strain of maize dwarf mosaic virus (MDMV-B)(5) maintained on a susceptible corn variety Tainan No. 5 by abrasion method were used in the present investigation. The three SCMV strains were purified as described in the previous report (7). They were used to immunize BALB/c mice, one for each strain. Two intraperitonal injections 2 weeks apart were made, each with 100 μg purified SCMV in 0.15 ml saline solution. The immunogen was mixed with the same volume of complete Freund's adjuvant in the first injection, and with incomplete adjuvant in the second one. One week after the second injection, the mice were given a final injection of the same amount of purified virus without adjuvant. Two to four days later, they were killed and their spleens were removed aseptically for cell fusion.

Hybridoma production and immunoglobulin class determination

The cell fusion and hybridoma cloning were performed according to the procedure described by Wu and Su (22). The cell suspensions of spleens of SCMV-immunized mice were mixed with myeloma cell suspensions (NS-1) at the ratio of 5:1 in cell mumber, and centrifuged at 1,000 rpm for 10 min. Cell fusion was achieved by adding dropwise 1 ml of 50% PEG (MW 1,500) in RPMI-1640 medium (R medium) to the pellet in the centrifuge tube in water bath at 37 C over a period of 75 sec. After incubation for 45 sec and dilution with R medium, the suspension was centrifuged. Cells in the pellet were resuspended in HAT medium (R medium supplemented with fetal bovine serum, Na-pyruvate, L-glutamine, penicillin, streptomycin, hypoxanthine, aminopterin and thymidine), distributed in 98-well culture plates, and incubated in CO_2 incubator. One week later, HT medium (HAT medium without aminopterin) was added. Once established, the hybridomas were grown in R-S medium (R medium supplemented with 15% fetal bovine serum, 1 mM Na-pyruvate, 1 mM L-glutamine, 100 units/ml of penicillin and 100 μg/ml of streptomycin). Hybridomas secreting specific antibody were selected by indirect ELISA as described below and cloned by limiting dilution to single cells. Immunoglobulin (Ig) subclass of MABs was also determined by the same method. Monoclonal isotyping kit (Pharmingen) was used as check.

Preparation of immunoglobulin

To produce antibodies in ascitic fluid, BALB/c mice were primed intraperitoneally with Pristane (2,6,10, 14-tetramethyl-pentadecane) one week before intraperitoneal injections of 10^7 cells of hybridoma. The ascitic fluid was removed within 10–14 days, and centrifuged at 3,400 rpm for 10 min. For purification of Ig from cell culture supernatant or ascitic fluid, the ammonium sulphate precipitation method (11) was applied. DEAE-sephacel column chromatography of the Ig was performed according to the method described by Garvey et al. (8). The protein yield was calculated spectrophotometrically assuming an A_{280nm} of 1.40 for 1 mg/ml concentrations and the antibody activity was determined by indirect ELISA.

Indirect ELISA

Indirect ELISA was used to detect antibody-producing hybridomas and antibody acitvity. The leaves of corn plants inoculated with SCMV-A, -B, -D or MDMV-B were homogenized with extraction buffer (0.01 M potassium phosphate buffer containing 0.3% ascorbic acid, 0.01 M Na-DIECA, and 0.3% 2-mercaptoethanol, pH 7.4) in the amount of 0.1 g/ml. After centrifugation of 3,400 rpm for 5 min, the supernatant was collected as antigen. The healthy tissue was treated in the same way and used as a healthy control. Antigen coating was carried out by adding 50 μl of antigen preparation to each of the 96-well plates. After incubation overnight at 4 C or at 37 C for 2 hr, the plates were rinsed 5 times with PBS-Tween (127 mM NaCl, 2.6 mM KCl, 1.1 mM KH_2PO_4, 8.5 mM Na_2HPO_4 and 0.05% Tween 20, pH 7.3) and 200 μl of 0.1% bovine serum albumin (BSA) in 0.05 M bicarbonate buffer (pH 9.6) was then added to each well. After incubation for 1 hr at 37 C, the plates were rinsed as before. For detecting antibody production by hybridomas, 50 μl supernatant of hybridoma culture was added to a well. Diluted antiserum was used as positive control and supernatant of myeloma cell culture as negative control. After incubation at 37 C for 2 hr and rinsed as before, 50 μl of alkaline phosphatase-labelled goat anti-mouse IgG and IgM (Zymed Labs.) diluted (1,500x) in PBS-Tween supplemented with 0.1% BSA was added to each well and the plates were incubated at 37 C for 2 hr. Following final rinsing the wells were treated with 200 μl of 0.2% p-nitrophenyl phosphate in a solution containing 0.02% NaN_3 and 9.7% diethanolamine, pH 9.8 for 1 hr at 37 C. The A_{405nm} of each well was measured with a Bio-Tek EIA plate reader.

RESULTS

Production of monoclonal antibodies

The fusion of spleen and myeloma cells yielded a total of more than 200 wells with actively growing primary hybridomas. Media from such wells were tested against the extracts from healthy and infected corn tissue in indirect ELISA. Finally, 3, 10 and 10 primary hybridoma lines showing positive reaction to SCMV-A, -B and -D, respectively, and with a high ELISA value were selected. Some of them were cloned by limiting dilution to single cell, and 3, 13 and 3, respectively, monoclones with the ability to produce antibodies were obtained. These MABs were identified as IgA, IgG2a and IgM, respectively (Table 1).

Specificity of antibody

All of the MABs were tested for their specificity against SCMV-A, -B, -D and MDMV-B. As shown in Table 2, they can be divided into 5 serogroups according to their reactions to different antigens. The serogroup I recognizing common epitopes reacted with 4 strains strongly. The serogroups II and III showed reactions with the 4 strains in different degrees. The former reacted more strongly with SCMV strains than with MDMV-B, while the latter group reacted more strongly with MDMV-B. The 3 strains of SCMV all strongly reacted with serogroup IV which, however, showed no reaction with MDMV-B. The MABs of

serogroup V reacted strongly with SCMV-D and weakly with MDMV-B, but failed to react with SCMV-A and -B.

Titres of antibodies

By means of indirect ELISA, three representative MABs: 3F1D3D7 to SCMV-A, 5H2G8 to SCMV-B and 8B11A7 to SCMV-D were used to test their titres in three different preparations including culture supernatant fluid, ascitic fluid and purified Ig. The crude extract of corn leaves (0.1 g/ml) infected with SCMV-A, -B or -D was used as antigen. The results obtained were shown in Table 3. The MAB 8B11A7 was confirmed to show monospecificity, only reacted with SCMV-D. The titres of MABs in ascitic fluid were very high, ranged from 1,024,000 to 32,768,000, much higher than those in culture supernatant. The minimum active concentration of purified Ig in the three MABs ranged from 1.25 to 20 μg/ml.

TABLE 1. Isotypes and reaction of the selected hybridoma cell line to their homologous antigen and healthy control in indirect ELISA

Immunogen	Monoclonal hybridoma	ELISA value		Isotype
		D[1]	H	
SCMV-A	3F1B3G11	1.833	−0.016	IgM
	3F1D3D7	1.916	−0.064	IgM
	3F1H6G12	1.802	−0.024	IgM
SCMV-B	5H2G8	1.076	0.025	IgA
	1A8C5	0.708	0.011	IgA
	1A8G4	0.670	0.007	IgA
	1C6A1	0.954	−0.046	IgM
	2A2G7	1.285	0.015	IgA
	2A2H3	1.311	−0.007	IgA
	2B4G1	0.524	−0.038	IgM
	2H7B1	1.018	−0.016	IgM
	2H7G1	0.910	−0.046	IgM
	2H11H	1.169	−0.011	IgG2a
	3C1B6	0.914	−0.014	IgG2a
	3G8F1	1.132	−0.014	IgM
	3G9C7	0.810	−0.035	IgM
SCMV-D	8B11A7	1.732	0.019	IgG2a
	8B11H8	0.869	−0.019	IgG2a
	8B11H10	1.318	−0.077	IgG2a

[1] D: crude extract of corn infected with SCMV-A, -B or -D respectively; H: crude extract of healthy corn.

Detectable dilution of SCMV in diseased tissues

The culture supernatants of the forementioned three MABs were used to determine the detectable dilution end point of SCMV strains in diseased corn and sugarcane leaves. The crude extract (0.1 g/ml) was diluted in a 2-fold series for use in indirect ELISA. The minimum detectable concentrations of corn leaves ranged from 1/2,560 to 1/20,480, while those of sugarcane leaves were 1/160 to 1/20,480 (Table 4).

DISCUSSION

Three strains of SCMV (SCMV-A, -B and -D) and two strains of MDMV (MDMV-A and -B) (6) have been identified in Taiwan. The MDMV strains show no infectivity to sugarcane plants when tested by abrasion method. These virus strains have been previously divided into 3 serogroups with the SCMV strains belonging to a same group (3). In other words, SCMV-A, -B and -D cannot be differentiated by polyclonal antibodies. In the present study, MABs of group V only reacted specifically with SCMV-D and only weakly with MDMV-B. They can, therefore, be used to detect SCMV-D in sugarcane plants.

TABLE 2. Grouping of monoclonal antibodies against SCMV strains

Serogroup	Reaction to				Monoclonal hybridoma
	SCMV-A	SCMV-B	SCMV-D	MDMV-B	
I	++++[1]	+++	++++	++++	3F1B3G11, 3F1D3D7, 3G8F1 3F1H6G12, 2H11H9
II	+++	+++	+++	++	5H2G8, 3C1B6
III	++	++	+++	++++	1A8C5, 2A2G7, 2A2H3
IV	+++	+++	+++	−	1C6A1, 2B4G1, 2H7B10 2H7G1, 3G9C7
V	−	−	++++	++	8B11A7, 8B11H8, 8B11H10

[1] The symbols express ELISA values as follows: −: < 0.100, +: 0.101–0.400, ++: 0.401–0.800, +++: 0.801–1.200, +++: 1.201–1.600, ++++:1.601–2.000.

TABLE 3. Titer end-point of monoclonal antibodies in different preparations[1]

Monoclonal antibody[2]	Antigen		
	SCMV-A	SCMV-B	SCMV-D
3F1D3D7			
CS	4,096	4,096	4,096
AF	32,768,000	2,048,000	2,048,000
PI (μg/ml)	2.5	2.5	2.5
5H2G8			
CS	2,048	1,024	1,024
AF	1,024,000	4,096,000	4,096,000
PI (μg/ml)	20	20	20
8B11A7			
CS	—	—	1,024
AF	—	—	128,000
PI (μg/ml)	—	—	1.25

[1] Expressed in reciprocal of a maximum antibody dilution that yielded positive ELISA value.

[2] CS: culture supernatant; AF: ascitic fluid; PI: purified Ig.

TABLE 4. Detectable dilution end-point of SCMV in corn and sugarcane leaves[a]

Antigen	Monoclonal antibody[1]		
	3F1D3D7	5H2G8	8B11A7
Corn			
SCMV-A	20,480	20,480	—
SCMV-B	5,120	5,120	—
SCMV-D	2,560	5,120	20,480
Sugarcane			
SCMV-A	320	320	—
SCMV-B	20,480	20,480	—
SCMV-D	160	640	640

[1] Expressed in reciprocal of a maximum antigen dilution that yielded positive ELISA reaction.

The MAB 3F1D3D7 had a high titer and showed a strong reaction with the tested strains. It could be a good reagent for detection SCMV and MDMV. Further studies on the reactivity of this MAB toward other potyviruses will be taken in order to know more about its characteristics. The MABs of group IV, which strongly reacted with SCMV strains but not with MDMV-B, could be used to differentiate SCMV from MDMV infection in corn field.

The symptoms of SCMV-A and -B on the differential host CP 31-294 were quite different. However, in the present investigation, all of the 16 MABs obtained using SCMV-A and -B as immunogens recognizing common epitopes of SCMV strains. They were unable to differentiate SCMV-A and -B. Therefore, more cell fusions should be carried out to acquire MABs for use in differentiating these two strains.

The test results of our MABs contained in mouse ascites show the usefulness of these MABs to detect SCMV and MDMV strains in corn and sugarcane leaves by indirect ELISA.

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LITERATURE CITED

1. Abbot, E. V., and Tippett, R. L. 1966. Strains of Sugarcane Mosaic Virus. U. S. Dep. Agric. Tech. Bull. 1340, 25 pp.
2. Briand, J. P., Al Moudallal, Z., and van Regenmortal, M. H. V. 1982. Serological differentiation of tobamoviruses by means of monoclonal antibodies. J. Virol. Methods 5:293-300.
3. Chen, C. T., Deng, T. C., and Chang, C. A. 1987. Sugarcane mosaic in Taiwan III. Serological relationships and coat protein of SCMV strains. Rep. Taiwan Sugar Res. Inst. 118:13-21.
4. De Avila, A. C., Huguenot, C., Resecde, R. de O., Kitajima, E. W., Goldbach, R. W., and Peters, D. 1990. Serological differentiation of 20 isolates of tomato spotted wilt virus. J. Gen. Virol. 71:2801-2807.
5. Deng, T. C. 1985. Identification of maize dwarf mosaic virus B strain and screening for resistance of corn. J. Agric. Res. China 34:195-206.
6. Deng, T. C., and Chen, C. T. 1991. Characterization of maize dwarf mosaic virus strain A isolated from sour grass in Taiwan. Plant Prot. Bull. 32:334-335. (Abstract). (in Chinese)
7. Deng, T. C., and Huang, C. H. 1986. Purification and antiserum preparation of maize dwarf mosaic virus. J. Agric. Res. China 35:350-359. (in Chinese)
8. Garvey, J. S., Cremer, N. E., and Sussdorf, D. H. 1977. "Methods in Immunology," A laboratory Text for Instruction and Research. Benjamin, Reading, Massachusetts. 545 pp.
9. Gillaspie, A. G. Jr., and Mock, R. G. 1984. Sugarcane mosaic virus-A survey of strains from sugarcane with a set of sorghum inbred lines. Sugar Cane 2:1-3.
10. Gillaspie, A. G. Jr., Chen, C. T., Mork, R. G., and Harris, R. W. 1980. Sugarcane mosaic virus strains in Taiwan. Proc. Int. Soc. Sugar Cane Technol. 17:1505-1509.

11. Hurrell, G. R. 1982. Monoclonal Hybridoma Antibodies: Techniques and Applications. CRC Press, Florida, 231 pp.
12. Jarjees, M. M., and Uyemoto, J. K. 1984. Serological relationships of strains of maize dwarf mosaic and sugarcane mosaic viruses as determined by microprecipitin and enzymelinked immunosorbent assays. Ann. Appl. Biol. 104:497-501.
13. Koike, H., and Gillaspie, A. G. Jr. 1989. Mosaic. Pages 301-302 *in*: Diseases of Sugarcane - Major Diseases. Ricaud, C., Egan, B. T., Gillaspie, A. G. Jr., and Hughes, C. G. eds. Elsevier, Amsterdam, 399 pp.
14. Permar, T. A., Garnsey, S. M., Gumpf, D. J., and Lee, R. F. 1990. A monoclonal antibody that discriminates strains of citrus tristeza virus. Phytopathology 80:224-228.
15. Persley, D. M., Henzell, R. G., Greber, R. S., Teakle, D. S., and Toler, R. W. 1985. Use of a set of differential sorghum inbred lines to compare isolates of SCMV from sorghum and maize in nine countries. Plant Dis. 69:1046-1049.
16. Pirone, T. P. 1972. Sugarcane mosaic virus. CMI/AAB Description of Plant Viruses. No. 88, 4 pp.
17. Shukla, D. D., Tosic, M., Jilka, J., Ford, R. E., Toler, R. W., and Langham, M. A. C. 1989. Taxonomy of potyviruses infecting maize, sorghum, and sugarcane in Australia and the United States as determined by reactivities of polyclonal antibodies directed towards virus-specific N-terminal of coat proteins. Phytopathology 79:223-229.
18. Snazell, T. E., Bancroft, J. B., and Ullstrapt, A. J. 1971. Purification and serology of maize dwarf mosaic and sugarcane mosaic viruses. Phytopathology 61:1059-1065.
19. Summers, E. M., Brandes, E. W., and Rands, R. D. 1948. Mosaic of Sugarcane in the United States, With Special Reference to the Strains of the Virus. U. S. Dep. Agric. Tech. Bull. 955, 124 pp.
20. Teakle, D. S., Shukla, D. D., and Ford, R. E. 1989. Sugarcane Mosaic Viruse. AAB Descriptions of Plant Viruses. No. 342, 4 pp.
21. Tosic, M., Ford, R. E., Shukla, D. D., and Jilka, J. 1990. Differentiation of sugarcane, maize dwarf, Johnsongrass, and sorghum mosaic viruses based on reactions of oat and some sorghum cultivars. Plant Dis. 74:549-552.
22. Wu, R. Y., and Su, H. J. 1990. Production of monoclonal antibodies against banana bunchy top virus and their use in enzyme-linked immunosorbent assay. J. Phytopathol. 128:203-208.

摘　　要

鄭秋萍[1]、陳主得[2]、鄧汀欽[3]、蘇鴻基[1]. 1993. 甘蔗嵌紋病毒之單元抗體. 植病會刊2:227-231. (1. 台灣大學植物病蟲害學系，2. 台灣糖業研究所植物保護系，3. 台灣省農業試驗所植物病理系)

以純化之甘蔗嵌紋病毒A、B及D等3系統爲免疫原，分別注射小白鼠(BALB/c)，製備單元抗體，結果各選得3株、13株及3株分泌單元抗體之融合瘤細胞單株系。此等單元抗體分屬IgG2a、IgA及IgM等亞型。依其對SCMV-A、B、D及MDMV-B等之抗原特異性，可分爲5個血清群：I群辨認共同抗原基，對4個病毒系統均呈等强反應；II及III群對各系統有反應，但强度有差異；IV群對SCMV之3系統呈强反應，但與MDMV-B不反應；V群只與SCMV-D反應，而不與SCMV-A及-B反應，但與MDMV-B有弱反應。培養上清液、抗腹水抽出液及純化抗體之抗體力價分別爲4,096倍、32,763,000倍及2.5 μg/ml。應用此等單元抗體檢測玉米與甘蔗葉片SCMV濃度之稀釋臨著點可達20,480倍。此外並就如何應用此等單元抗體區分田間SCMV系統加以討論。

關鍵詞：甘蔗嵌紋病毒，玉米矮化嵌紋病毒，系統，單元抗體。