

温州萎縮ウイルス(SDV)に対するモノクローナル抗体の作成

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Production of Monoclonal Antibodies to Satsuma Dwarf Virus

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Key words : monoclonal antibody, satsuma dwarf virus, citrus mosaic virus, ELISA.

Since the initial description by Köhler and Milstein¹⁾, monoclonal antibodies have been used to study the antigenic properties not only of animal viruses but also of plant viruses^{2,3)}. Progress in plant virology has been reviewed by Sander and Dietzgen⁴⁾. Monoclonal antibodies, which permit rapid and accurate diagnosis of viral infection, may be useful especially in fruit trees with great advantage, as the large scale diagnosis of young trees are required.

Enzyme-linked immunosorbent assay (ELISA) has been performed to detect citrus viruses, such as satsuma dwarf virus (SDV)⁵⁾ and citrus mosaic virus (CiMV)⁶⁾. Because they were antigenically closely related, more specific and sensitive assay methods were required using monoclonal antibodies. Then we attempted to produce monoclonal antibodies to SDV.

SDV particles purified by the methods of Usugi *et al.*⁷⁾ were mixed with an equal volume of Freund's complete adjuvant, and injected into peritoneal cavity of 8-weeks old BALB/c mice (ca. 100 μ g SDV per mouse). After 4 weeks the mice were boosted intravenously with purified virus particles (ca. 100 μ g per mouse), and then three days later, the spleen was removed. The serum titre of the mouse was 1 : 320 using interfacial ring tests. Cell fusion was done according to widely reported protocols. Briefly, myeloma cells, P3-X63-Ag8-U1 (P3U1), were grown in the RPMI medium, which was consisted of RPMI-1640 solution (Flow Laboratories Inc. USA) supplemented with glutamine (Nakarai), sodium pyruvate (Sigma), Kanamycin (Meiji Seika), Penicillin-streptomycin (Flow) and Fungizone (Flow), plus 15% FCS (fetal calf serum, Gibco). The spleen cells (5×10^7) and myeloma cells (5×10^6) were mixed and washed with the RPMI

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medium. After removing the medium completely, the mixed cells were fused by gently adding 1 ml of 50% of polyethylene glycol (Merck 4,000 GK)⁸⁾ solution in the RPMI medium containing 15% of dimethylsulfoxide (Wako) prewarmed at 37 C. Then, after 30 sec, with gently rolling the tube, 1 ml of the RPMI medium was added every 30 sec for 5 min. The fusion was stopped by adding 1 ml of FCS. The cells were pelleted and resuspended in 40 ml of HAT selective medium, which was consisted of the RPMI medium plus 15% FCS and hypoxanthine-aminopterin-thymidine (50x HAT solution, Flow), and were distributed in 0.1 ml amounts to the wells of 96-well microculture plates containing 1-2 day old peritoneal macrophage cultures (4,000 cells/0.1 ml HAT selective medium). The plates were incubated at 37 C in humidified 5% CO₂. The hybridoma cells were fed every 2-3 days by changing half of the medium replaced with HAT selective medium for 14 days. Among 450 wells, 72 wells contained visible cell colonies, and tested for secreted antibody to SDV. An aliquot (0.1 ml) of the culture medium was removed from each well, and mixed with small amounts of SDV particle (40 ng) in a tube and incubated at 37 C for 1 hr. The antigenic reactivity of SDV particles disappears, if the culture medium contains anti-SDV antibodies. This was detected by ELISA developed by Usugi *et al.*⁵⁾.

Two hybridomas, 1F6 and 7F9, showed to secrete anti-SDV antibodies. The desired hybridoma cells were then cloned by the limiting dilution method in a 96-well microculture plate with peritoneal macrophages as feeder cells. Several cell lines originated from 1F6 and 7F9 were obtained, and frozen at a cell density of 3×10^6 /ml in 1 ml of the RPMI medium plus 20% FCS and 10% dimethylsulfoxide at -70 C and then stored in liquid nitrogen. Ascites fluid was obtained by intraperitoneal injection of BALB/c

mice, primed 4 weeks earlier by intraperitoneal injection of Pristane (Aldrich), with about 10^7 hybridoma cells in 0.5 ml of the RPMI medium. At 5 to 7 days after inoculation, the ascites fluid was collected by puncturing the mouse with a 21 gauge needle, and supernatant was tested after centrifugation.

The cell lines, derived from 1F6 and 7F9 were tested for the antigenic specificity using the culture medium by the interfacial ring tests as shown in Table 1. The results demonstrate that the cell line 1F6 reacted only with SDV, while 7F9 reacted with both SDV and CiMV. The specificity of the cell line 1F6 was further proved using ELISA developed by Usugi *et al.*⁶⁾ based on the modified method of Clark and Adams⁹⁾, in

Table 1. The reactivity of the culture medium of each hybridoma cells against SDV and CiMV by the interfacial ring test

Cell lines	Antigen			
	SDV CiMV ×5 ^{a)}		SDV CiMV ×2 ^{a)}	
1F6.F11.H 9	+	-	+	-
1F6.F11.C10	+	-	+	-
1F6.F11.G 9	+	-	+	-
7F9.A10.A 1	+	+	n.t ^{b)}	n.t
7F9.A10.E 2	+	+	n.t	n.t
7F9.A10.B 3	+	+	n.t	n.t
7F9.A10.B 6	+	+	n.t	n.t
7F9.E10.D 9	+	+	+	+
7F9.E10.C10	+	+	n.t	n.t
7F9.E10.E12	+	-	+	+

a) dilution of the culture medium.

b) Not tested.

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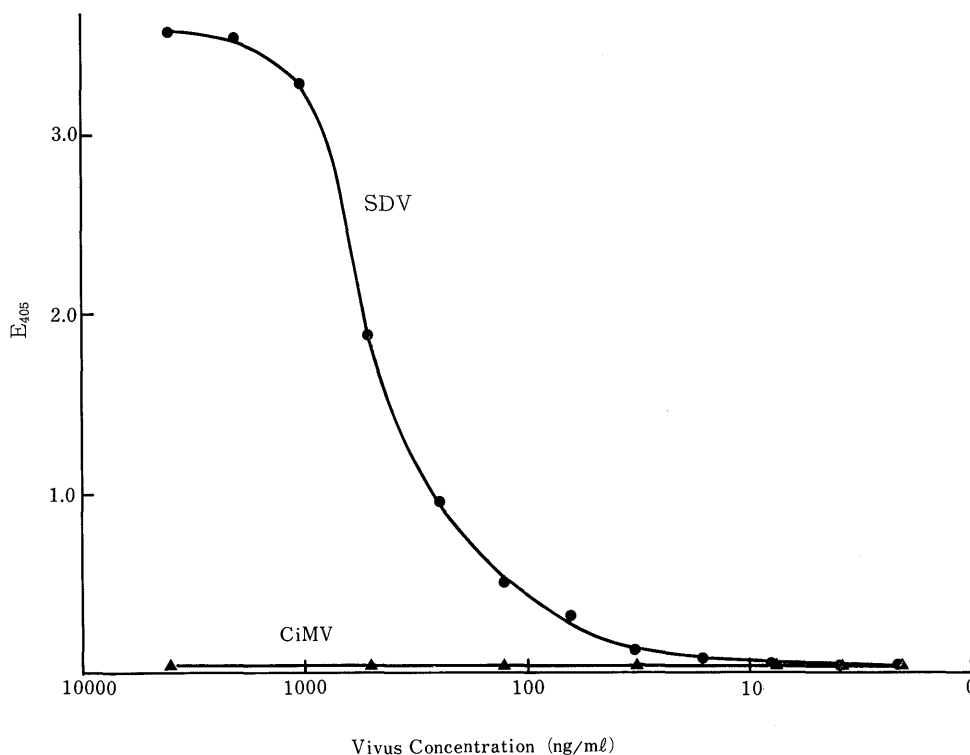


Fig. 1. ELISA tests on SDV and CiMV with monoclonal antibodies. Gamma-globulin was prepared from the ascites fluid of the cell line 1F6F11H9.

which gamma-globulin prepared from the ascites fluid of the cell line 1F6F11H9 was used. As shown in Fig. 1, the antibody specifically reacted with SDV, not with CiMV, while the antibodies to SDV prepared from the immunized rabbit also reacted with CiMV above 100 ng/ml at virus concentrations (data not shown).

In this paper, we showed that hybridoma cells could be developed to produce the monoclonal antibodies that react with specifically one type of viruses. Therefore, monoclonal antibodies specific for CiMV will be obtained from mice immunized with CiMV as well. In addition, we succeeded in obtaining the antibodies with a titre of approximately 1:10,000 by complement fixation test from the ascites fluid of the cell line 1F6F11H9. This also shows the potential usefulness of monoclonal antibodies in viral diagnosis capable of performing a large quantity of assays.

In this study, we developed two positive clones. The technique for screening we applied was easy for the testing. However, we may obtain more positive clones by improving the assay system e.g., ELISA or reverse passive hemagglutination inhibition test as described by Inoue *et al.*³⁾.

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和 文 摘 要

野津祐三・宇杉富雄・西森 敬：温州萎縮ウイルス（SDV）に対するモノクローナル抗体の作成

純化した SDV で、BALB/C マウスを免疫し、得られた脾細胞をマウス骨髄腫細胞と融合させた。SDV 粒子に対する抗体産生細胞をスクリーニングするため、培養上清を SDV 粒子と反応させ、抗原の中和の有無を、ELISA 法で検定した。その結果 2 株が抗体産生を示した。そのうち 7F9 由来株は、SDV とカンキツモザイクウイルス（CiMV）の両者に反応する抗体を産生し、1F6 由来株は、SDV のみに反応する抗体を産生した。それぞれ単クローン化した後、マウス腹腔内に接種した。1F6 由来株接種により得られた腹水は、約 10,000 倍の抗体価（補体結合法）を示した。この腹水より γ -グロブリンを精製し、ELISA 法を行い、CiMV とは反応せず、SDV にのみ高い特異性を示す抗体が得られたことを確かめた。

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