マレック病ウイルス(MDV)及び七面鳥ヘルペスウイルス(HVT)に対する単クローン抗体の樹立

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Preparation of Monoclonal Antibodies against Marek's Disease Virus and Herpesvirus of Turkeys

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ABSTRACT. Fifty-two monoclonal antibodies specific for Marek's disease virus (MDV) and herpesvirus of turkeys (HVT) were established. Twenty-two of these antibodies were specific for three different serotypes. Of the remaining antibodies, 22 cross-reacted with all combinations of the three serotypes (1·2·3, 1·2, 1·3 and 2·3). Using the serotype-specific antibodies, 10 presumable serotyped MDV and HVT and 7 field isolates could be serotyped by indirect immunofluorescence test. One of 7 field isolates was identified to a serotype 2 MDV which has been isolated for the first time in Japan.—KEY WORDS: HVT, MDV, monoclonal antibody.

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Marek's disease (MD) is a lymphoproliferative disease in the chicken caused by MD virus (MDV). A herpesvirus of turkeys (HVT) was shown to be antigenically related to MDV. An avian herpesvirus antigenically related to MDV has been subdivided into three serotypes based upon virus neutralization, agar-gel precipitation and immunofluorescence tests [2, 3, 4]. Serotype 1 includes pathogenic strains of MDV and the attenuated viruses of these strains. The serotype 2 includes the naturally occurring nononcogenic MDV, while the serotype 3 includes the nononcogenic HVT. The validity of this classification has recently been confirmed by Lee *et al.* [13] through the use of type-specific monoclonal antibodies. Several workers have also developed monoclonal antibodies which in an immunofluorescence assay recognized both serotype-common and serotype-specific antigens of MDV and HVT [6, 19, 22]. These monoclonal antibodies have been applied to biochemical analyses of virus polypeptides by using immunoprecipitation techniques [7, 8, 9, 10, 19, 22].

In Japan, there is no report yet which describes the isolation of the MDV strains belonging to the serotype 2 MDV and the establishment of the monoclonal antibodies specific for serotype 2 MDV.

The presnet study was designed to obtain the monoclonal antibodies specific to the three different serotypes and to isolate the serotype 2 MDV from field chickens. This report presents the result of development of many monoclonal antibodies specific for serotype-specific an serotype-common antigens of MDV and HVT and identification of a serotype 2 MDV from some field isolates.

MDV strains used included pathogenic strains, GA [5], BC-1 [20] and RB-1B [18], attenuated strains, C2 [12] and CVI-988 [16], and nonpathogenic strains, SB-1 [17] and HPRS-24 [1]. HVT strains, FC-126 [21], O1 [15] and TK/A (Marivax THV, a Well-

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come company's vaccine strain), were also used. Of these virus strains, GA (serotype 1), SB-1 (serotype 2) and FC-126 (serotype 3) strains were used as immunizing antigens for the preparation of hybridomas in this study.

Somatic cell fusions were performed by a modification of basic method described by Galfre *et al.* [6]. Spleen cells were prepared by mechanical disruption of spleens removed from female BALB/c mice immunized with sonicated cell suspension from MDV- or HVT-infected chick embryo fibroblasts (CEF). The schedule for immunization were according to that of the report described by Lee *et al.* [13]. Spleen cells from immunized animals were fused with SP2/0-Ag 14 myeloma cells. The resulting hybridomas were selected with HAT medium. The details were described previously [14]. Cultures producing antibody positive for virus antigens were screened by the indirect immunofluorescence test using FITC-conjugated anti-mouse (IgG+IgM). The MDV- or HVT-infected CEF cells on coverslips were fixed with cold acetone for 15 min and used as virus antigens for the test. After cloning of positive hybridomas in limiting dilution, 52 hybridomas were established. Some of those were inoculated into pristane-primed BALB/c mice for ascitic fluids from each hybridoma were used in the present study.

Specificities against the immunizing virus strains of monoclonal antibodies from the established hybridomas are summerized in Table 1. Of the 52 monoclonal antibodies specific for the immunizing GA, SB-1 and FC-126 strains, 22 were each specific for the three different viruses, and 22 were cross-reactive between the three different strains. Though the remaining 8 monoclonal antibodies were reactive against SB-1-infected CEF, these antibodies were lost from our minor negligence before the examination of reactivity with the other infected CEF. The 22 cross-reactive monoclonal antibodies consisted of antibodies to all combinations of the three virus strains (1·2·3, 1·2, 1·3 and 2·3). It has been recently proved that some of 64 monoclonal antibodies to MDV and HVT obtained by Ikuta *et al.* [7] also consisted of antibodies to all combinations of the three different serotypes (personal comunication, manuscript in preparation). The presence of th cross-reactive antibodies to all combinations of the three different serotypes indicates that the three kinds of serotypes closely relate to each other serotype. Furthermore, the fact that sera from MDV-infected convalescent chickens generally have cross-reactivity to the three different serotypes [21] may support the presence of our cross-reactive monoclonal antibodies.

Further specificity of 22 monoclonal anti-

Table 1. Summary of specificity of monoclonal antibodies to immunizing virus strains

Immunizing virus strain	No. of monoclonal antibody	No. of monoclonal antibody specific for						
		GA	SB-1	FC-126	1·2·3[a)]	1·2	1·3	2·3
GA	19	10	0	0	4	3	2	0
SB-1	18[b)]	0	6	0	0	3	0	1
FC-126	15	0	0	6	6	0	3	0
Total	52	10	6	6	10	6	5	1

a) Common antigen between serotypes, 1, 2 and 3.
b) Eight of the 18 monoclonal antibodies were lost.

Table 2. Summary of specificity of monoclonal antibodies specific for the three different serotypes

Monoclonal antibody specific for	No. of monoclonal antibody	Serotype 1					Serotype 2		Serotype 3		
		GA	BC-1	RB-1B	C2	CVI-988	SB-1	HPRS-24	FC-126	O1	TK/A
Serotype 1	8	+	+	+	+	+	−	−	−	−	−
	2	+	−	−	−	−	−	−	−	−	−
Serotype 2	6	−	−	−	−	−	+	+	−	−	−
Serotype 3	3	−	−	−	−	−	−	−	+	+	+
	2	−	−	−	−	−	−	−	+	±	±
	1	−	−	−	−	−	−	−	+	−	−

bodies specific for the three different virus strains was examined using 10 viruses, presumably all member of individual serotypes of MDV and HVT. The result was shown in Table 2. Of the 10 monoclonal antibodies to GA (serotype 1), 8 reacted equally with pathogenic MDV (BC-1 and RB-1B) and attenuated MDV (C2 and CVI-988) but not with nonpathogenic MDV (SB-1 and HPRS-24) and HVT (FC-126, O1 and TK/A), and 2 reacted only GA. Six monoclonal antibodies to SB-1 (serotype 2) reacted with nonpathogenic MDV (HPRS-24) but not with pathogenic and attenuated MDV and HVT. Of 6 monoclonal antibodies to FC-126 (serotype 3), on the other hand, 3 reacted equally with HVT (O1 and TK/A) but not with MDV, 2 reacted strongly with FC-126 but weakly with O1 and TK/A, and the other reacted only with FC-126.

Subsequently, we attempted to isolate and identify serotype 2 MDV from field isolates using our serotype-specific monoclonal antibodies. Peripheral blood leukocytes were separated from the blood of a few normal and MD chickens from some HVT (FC-126)-vaccinated flocks, and were then cocultivated with CEF cells. After the cocultivation, MDV microplaques were observed, and some of them consisted of large plaques of rounded cells of various size. The type of the cytopathic effect was different from that induced by pathogenic MDV such as GA (serotype 1) formed only small plaques, and was similar to that induced by nonpathogenic MDV such as SB-1 (serotype 2) [17]. A large microplaque was cloned by plaque-purification. The cloned isolate was propagated with CEF cells on coverslips, and fixed with cold acetone for serotyping by the indirect immunofluorescence test. The isolate reacted only with monoclonal antibodies to serotype 2 MDV but not with monoclonal antibodies to the other serotypes. Therefore, this isolate seems to belong to serotype 2. On the other hand, 6 isolates formed small plaques were also cloned and serotyped by the above same manner. Five of the 6 isolates reacted only with monoclonal antibodies to serotype 1 but not with monoclonal antibodies to the other serotypes. The remaining one isolate reacted with monoclonal antibodies to serotype 3 and also reacted with a monoclonal antibody intratype-specific for FC-126 strain. Therefore, it is considered that this isolate was a vaccine strain, FC-126, rescued from the vaccinated chicken. Further biological properties of these field isolates are now being investigated in detail.

The data present in this paper strongly supported not only the classification of MDV proposed by Bülow and Biggs [2, 3] but also the result of a panel of type-specific monoclonal antibodies to MDV and HVT

established by Lee *et al.* [13]. Therefore, our type-specific and common-specific monoclonal antibodies will be useful reagents for serotyping MDV and HVT as well as for characterization of the virus polypeptides and of the common antigens.

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要　約

マレック病ウイルス（MDV）及び七面鳥ヘルペスウイルス（HVT）に対する単クローン抗体の樹立：弘瀬秀樹・松田治男・村田昌芳・関屋幸男[1]（広島大学生物生産学部家畜衛生学教室，[1]ゲンコーポ栃木ラボラトリ）——合計52のMDV及びHVTに対する単クローン抗体を得たが，そのうち22はMDV及びHVTの3血清型に特異的であった。型共通抗原に対する抗体は22あって，血清型の全組合わせ（1・2・3，1・2，1・3及び2・3）において交差反応を示した。型特異単クローン抗体を用いて，10株の既知MDV及びHVT並びに7株の野外分離ウイルスが血清型別されたが，野外株のうちのひとつは本邦で初めて分離された2型MDVであった。