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SHORT COMMUNICATION

MONOCLONAL ANTIBODIES SPECIFIC TO AND CROSS-REACTIVE WITH MAREK'S DISEASE VIRUS AND HERPESVIRUS OF TURKEYS

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In order to identify the specific and cross-reactive antigens to Marek's disease virus (MDV) and herpesvirus of turkeys (HVT), we prepared hybridomas producing monoclonal antibodies against these viruses. These monoclonal antibodies were screened by the indirect immunofluorescence method. The purified virus-specific antigens appeared to be more effective immunogens than unpurified virus-infected cell homogenates. Totals of 50 and 14 hybridoma clones were found to produce antibodies specific to MDV and HVT, respectively. Of these, 14 MDV clones and 5 HVT clones produced antibodies that recognized antigenic sites common to both viruses.

Marek's disease virus (MDV) is the etiological agent of Marek's disease (MD), a highly contagious lymphoma of chickens. A herpesvirus of turkeys (HVT) was shown to be serologically related to MDV (Witter et al., 1970), and has been used to protect chickens from MD (Okazaki et al., 1970). At present, this is the only available vaccine against tumor formation. Thus, analysis of serological cross-reactive proteins of MDV and HVT is important for understanding the vaccinal immunity of HVT. Recently, 46 distinct polypeptides specific to MDV were identified by immunoprecipitation using chicken antibodies against MDV purified by affinity chromatography (Ikuta et al., 1981). We found that most virus-specific polypeptides in cells productively infected with MDV or HVT possess cross-reactive antigenic determinants (Ikuta, 1981). In spite of these antigenic similarities, previous studies on the extent of DNA homology between MDV and HVT revealed that the two viruses has very little DNA homology, probably less than 5% (Hirai et al., 1979; Lee et al., 1979; Kaschka-Dierich et al., 1979). Therefore, further studies on the extent of antigenic cross-reactivity between MDV and HVT are required for a

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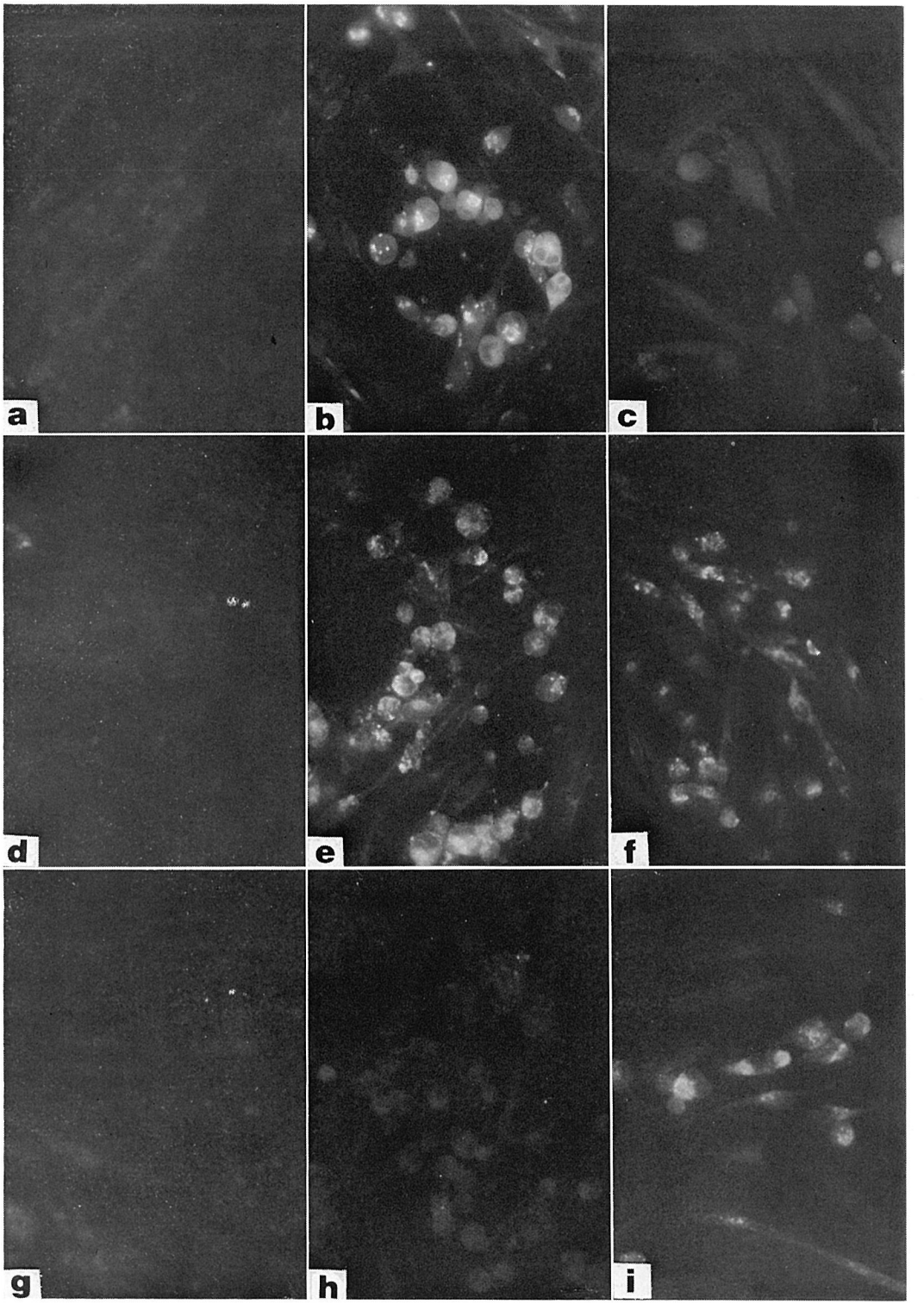
FIGURE 1. MDV and HVT-specific and cross-reactive antigens detected by the immunofluorescence test using monoclonal antibodies. Uninfected CEF (a, d, g) and virus-infected CEF (MDV-infected: b, e, h; HVT-infected: c, f, i) at 48 h postinfection were fixed and treated with antibodies secreted from hybridoma clones. After 30 min, the cells were stained with FITC-conjugated anti-mouse IgG. The hybridoma clones for immunofluorescence staining were the M6 clone producing MDV-specific monoclonal antibody (a, b, c), M18 clone producing monoclonal antibody reactive with both MDV and HVT (d, e, f) and H14 clone producing HVT-specific antibody (g, h, i). These hybridoma cells were obtained by fusion of myeloma cells with spleen cells isolated from mice immunized with MDV antigen (M6 and M18) or HVT antigen (H14) as described in the text.

clear understanding of these unexpected results. Monoclonal antibodies have proved useful in analyzing the nature of the antigenic determinants within MDV and HVT antigens. In this paper, we report an improved method for obtaining mouse monoclonal antibodies, and studies on the cross-reactivity between MDV and HVT by the immunofluorescence test.

The MDV BC-1 strain, which was isolated from the MD lymphoma-derived cell line MDCC-MSB1 (Akiyama et al., 1974), and the HVT O1 strain (Ono et al., 1974) were used. The BC-1 strain was used at the 10th to 25th passage, and the O1 strain at the 25th to 35th passage. Chick embryo fibroblasts (CEF) were prepared from 10-day-old embryos of specific pathogen-free (SPF) chickens and grown in minimum essential medium (MEM) supplemented with 5% calf serum and 5% tryptose phosphate broth.

For immunization of mice, two procedures were employed. The antigens for procedure A were purified from extracts of virus-infected CEF by affinity chromatography on Sepharose 4B coupled with infected chicken serum IgG. For this, antisera to MDV or HVT were obtained from SPF chickens immunized with virus-infected CEF. The IgG fraction of these antisera was conjugated to cyanogen bromide-activated Sepharose 4B (Pharmacia Fine Chemicals). The mixture was equilibrated with the starting buffer (0.5% NP40, 0.5 *M* NaCl, 0.1 *M* sodium phosphate buffer, pH 7.0), and then virus-infected CEF extract was applied to the homologous virus antibody-coupled column. The virus-infected CEF extract was prepared by sonicating the virus-infected CEF in the starting buffer and then centrifuging the mixture at 8000 rpm in a Hitachi RPR15 rotor for 10 min at 4 C. The adsorbed antigens were eluted with 0.1 *M* glycine-HCl buffer, pH 2.5 and then concentrated and dialyzed against phosphate-buffered saline (PBS), pH 7.0. The antigens for procedure B were homogenates of virus-infected CEF. The purified antigens (about 200 μg of protein/mouse) and unpurified homogenates were each mixed with an equal volume of Freund's complete adjuvant for intraperitoneal inoculation into BALB/c mice of 6–8 weeks old. After 4 to 8 weeks, two intraperitoneal inoculations of a homogenate of virus-infected CEF in MEM without adjuvant, prepared by sonication, were given with a 7-day interval between inoculations. After 3 days, the spleens were removed for hybridization.

For isolation of hybridoma cells producing antibodies specific to MDV and HVT, spleens removed from immunized mice were minced, suspended in Dulbecco's modified Eagle medium (DMEM), passed through sterile stainless mesh, and centrifuged at 200 *g* for 10 min. The pellet was treated with hemolyzing solution (0.115 *M* NH_4Cl, 10 m*M* $KHCO_3$, 1 m*M* ethylenediamine-tetraacetate), and washed with DMEM. The spleen cells were mixed at a 5:1 ratio with mouse myeloma cells (SP2/0-Ag14, Shulman et al., 1978) and the mixtures were centrifuged at 200 *g* for 10 min. Then the cells were gently resus-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pended and incubated for 30 sec in 0.5 ml of 50% polyethylene glycol (PEG 4000; Nakarai Chemicals Ltd.). Then ten, 1 ml volumes of prewarmed DMEM were added to the suspension at 30 sec intervals with gentle rolling of the mixture. The cells were pelleted by centrifugation at 120 *g* for 5 min, and the pellet was washed with DMEM containing 15% fetal calf serum (FCS). The final pellet was resuspended very gently in DMEM with 15% FCS containing hypoxanthine-aminopterine-thymidine (HAT medium), and the cells were distributed into the wells of 96-well tissue culture plates (Coster, 2.5×10^5 spleen cells/200 μl/well) and fed every 2 or 3 days with HAT medium. After 2 weeks of selection in HAT medium, the fluids were harvested and screened for anti-MDV and anti-HVT antibodies by the indirect immunofluorescence test. Virus-infected CEF on coverslips were fixed with cold acetone for 10 min, and used as virus antigen for the immunofluorescence test. FITC-conjugated anti-mouse IgG was purchased from Chappel Laboratories. The cells from wells giving a positive reaction were immediately cloned by end-point dilution in 96-well plates with feeder layers of unimmunized normal BALB/c spleen cells. Clones producing virus-specific antibodies were injected intraperitoneally into BALB/c mice. The resulting ascites fluids were harvested, clarified, and tested for the presence of antibodies specific to MDV and HVT.

Figure 1 shows examples of immunofluorescence obtained with monoclonal antibodies specific to, and cross-reactive with MDV and HVT antigens and the results obtained by the two immunization procedures are summarized in Table 1. A total 50 and 14 hybridoma clones were found to produce antibodies specific to MDV and HVT, respectively. Of these, 14 MDV clones and 5 HVT clones produced antibodies that recognized antigens common to MDV and HVT. It is noteworthy that in spite of the low DNA homology between MDV and HVT, a considerable number of the isolated monoclonal antibodies recognized antigens common to MDV and HVT. Most of antibody-producing wells obtained by procedure A gave a positive reaction with infected cells and a negative one with uninfected cells (data not shown). In contrast, a few percent of the antibody-producing wells obtained by procedure B were virus-specific and the rest contained antibodies that reacted with uninfected CEF (data not shown). This could be because MDV and HVT can replicate only in avian cells in vitro and in vivo. Thus, purified virus-specific antigens seem better than unpurified virus-infected CEF homogenates for obtaining virus-specific monoclonal antibodies.

TABLE 1. *Cross-reactivity of monoclonal antibodies in the indirect immunofluorescence test*

Immunogen	Isolated clones	No. of antibodies isolated by procedure: A		B	
		S[a]	C[a]	S[a]	C[a]
MDV	50	26	11	10	3
HVT	14	8	4	1	1

[a] S: MDV or HVT-specific. C: Cross-reactive with both MDV and HVT.

However, the antigens prepared by procedure A could be of a restricted type that is solubilized in the starting buffer used here and is in a form that reacts with coupled chicken sera on affinity chromatography. Therefore, we also used unpurified viral antigens as immunogens to obtain an additional type of antigens (procedure B) (Table 1).

It was not possible to differentiate MDV-infected cells from HVT-infected cells in vitro or in vivo by the immunofluorescence method with infected chicken sera because of the serological cross-reaction between MDV and HVT. In addition, it is difficult to use polyvalent sera to determine the number of virus-specific and cross-reactive determinants in MDV- and HVT-specific proteins. Thus,

the monoclonal antibodies prepared here will be very useful in studies on these problems. However, it is necessary to test these antibodies for their ability to precipitate viral specific proteins. Virus-specific polypeptides are now being investigated in detail by immunoprecipitation with the monoclonal antibodies isolated here.

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