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Hemagglutination by Murine Hepatitis Viruses

Absence of Detectable Activity in Strains 3, A59, and S Grown on DBT Cells

Pierre J. Talbot

Virology Research Center, Institut Armand-Frappier, Université du Québec, Laval, Québec, Canada

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Summary. Erythrocytes from twelve mammalian and avian sources in ten different buffers at three incubation temperatures could not be hemagglutinated with murine hepatitis virus (MHV) strains 3, A59, or S grown on DBT cells. Viral antigen preparation in the absence of fetal calf serum, partial virus purification, or various concentrations of red blood cells still failed to yield detectable hemagglutinating activity. Thus, the newly described MHV-DVIM remains the only hemagglutinating strain of murine coronavirus.

Hemagglutination represents a useful model for virus adsorption to cells, a very convenient assay for the presence of virus in biological samples, and may be relevant to viral pathogenesis. Several members of the Coronaviridae family of enveloped RNA viruses were shown to possess such a hemagglutinating activity: namely bovine coronavirus, hemagglutinating encephalomyelitis virus of pigs, human coronavirus strain OC43, rabbit enteric coronavirus, and avian infectious bronchitis virus [1]. Recently, a hemagglutinin was demonstrated on transmissible gastroenteritis virus [2]. However, hemagglutination by murine coronavirus had not been reported until Sugiyama and Amano [3] identified a new enteric strain of murine hepatitis virus (MHV): the diarrhea virus of infant mice (DVIM). Like bovine coronavirus [4] and strain OC43 of human coronavirus [5], the hemagglutinating activity of MHV-DVIM appeared to correlate with the presence of a structural glycoprotein of 140 kD, comprised of 2 subunits of 65–69 kD linked by disulfide bridges [6]. This presumed hemagglutinin appears distinct from the peplomer glycoprotein (named E2, S, or P) of 180–190 kD [5, 6] and its proteolytically cleaved monomers of 100–120 kD [4, 5]. On

Address inquiries to: Dr. Pierre J. Talbot, Virology Research Center, Institut Armand-Frappier, Université du Québec, 531, bd des Prairies, Laval, Québec (Canada), H7N 4Z3

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Table 1. Hemagglutinating titers of MHV antigens with erythrocytes from several sources in various buffers and temperatures

Antigen	Buffer[a]	Erythrocytes[b]						
		rabbit	human O	guinea pig	horse	cattle	sheep	hamster
MHV-3[c]	1, 5	<2	–[d]	–	–	–	–	<2
	3	<2	<2	–	–	–	–	<2
MHV-A59[e]	1–4	<2	<2	<2	–	–	<2	–
	5, 6	<2	<2	<2	<2	<2	<2	–
	7–10	<4	<4	–	–	–	–	–
MHV-S[f]	1–4	<8	<8	<8	–	–	<8	–
	5, 6	<8	<8	<8	<8	<8	–	–
	7–10	<8	<8	–	–	–	<8	–

[a] Buffers as described in the text and incubation at 4, 22, or 37 °. The same result was obtained at any of these temperatures.

[b] Prepared as described in the text and used at 0.3 or 0.5% (v/v). The same result was obtained at any of these concentrations.

[c] Grown in serum-free medium and concentrated 90-fold with polyethylene glycol.

[d] Not done.

[e] Grown with or without serum and used as clarified medium.

[f] Semipurified, as described in the text.

the other hand, the hemagglutinating activity of infectious bronchitis virus [7, 8] and transmissible gastroenteritis virus [2] was localized on the unique peplomer glycoprotein. Thus, the preliminary report of hemagglutination by another strain of MHV (MHV-3; grown in murine liver and semipurified on sucrose gradient) [9], which lacks structural proteins of 140 and 65–69 kD [10–13; PJT, unpubl. data], would suggest that MHV-3 also possesses a hemagglutinin as the only peplomer glycoprotein. The study reported here sought to detect the presence of a hemagglutinating activity on MHV-3 grown on DBT cells and determine if it could also be demonstrated on other strains of MHV. Of particular interest was MHV-S which appears to contain an additional structural protein of approximately 65 kD, as revealed in reducing gels [6, 12; PJT, unpubl. data].

The origin and culture of MHV strains 3, A59, and S and of DBT cells were described previously [14]. For use as antigen for hemagglutination assays, MHV-3 was grown in the absence of fetal calf serum (FCS) and harvested from clarified medium by precipitation with 10% (w/v) polyethylene glycol in 0.5 *M* NaCl. After centrifugation at 10,000 *g* for 30 min, the pellet was resuspended and dialyzed against TMEN buffer: 50 mM Tris-HCl (pH 6.2), 0.1 *M* NaCl, 1 m*M* EDTA. MHV-A59, which replicates in vitro more efficiently than any other strain of MHV, was grown with or without 1% (v/v) FCS and used

mouse			day-old chick	rooster
CDI	C57Bl/6	BALB/C		
–	–	–	–	–
–	–	<2	<2	<2
<2	<2	–	<2	<2
<2	<2	<2	–	<2
–	–	<4	–	<4
<8	<8	–	<8	<8
<8	<8	–	<8	<8
<8	<8	–	<8	<8

directly in the form of medium freed of cell debris by centrifugation at 10,000 *g* for 20 min. Finally, MHV-S was grown in medium containing 2% (v/v) FCS, concentrated as described above, and partially purified on a discontinuous 10 and 50% (w/v) Nycodenz® (Nyegaard, Oslo, Norway) gradient centrifuged for 4 h at 25,000 rpm in an SW28 rotor (Beckman Instruments, Fullerton, Calif., USA). The virus band was dialyzed against TMEN buffer. All viral antigens had infectious titers of 6.0–7.3 $\log_{10}$ PFU/ml. Red blood cells from various mammalian and avian sources were collected and washed in Alsever buffer [15] and resuspended in the appropriate buffers. Ten different buffers were tested: (i) TNE (10 m*M* Tris-HCl, pH 7.4, 0.15*M* NaCl, 1m*M* EDTA); (ii) TNB (50 m*M* Tris-HCl, pH 7.2, 0.1 *M* NaCl, 0.1% (w/v) bovine serum albumin); (iii) Auletta (0.15 *M* NaCl, 7 m*M* $CaCl_2$, 4 m*M* $MgSO_4$, pH 7.1) [15], (iv) DGV, pH 7.2 [16]; (v) phosphate-buffered saline, pH 7.4 [17]; and (vi). phosphate-buffered saline with 1% kaolin-treated FCS [18], and (7–10) BABS, pH 5.75, 6.0, 6.2, or 6.4, respectively [16]. The hemagglutination assay was performed as described previously [18], except that various buffers were used, red blood cells were made up to 0.3 or 0.5% (v/v) in the appropriate buffer, and microtiter plates were incubated at 4, 22, or 37°. Control hemagglutinating antigens were either rabbit enteric coronavirus (titer 1/64 with rabbit red blood cells) or pneumonia virus of mice (titer 1/320 with CDI mouse erythrocytes). Both antigens were kindly provided by Dr. Jean-Paul Descôteaux (Institut Armand-Frappier).

As shown in table 1, all tested combinations of buffer, source or concentration of erythrocytes, antigen, or incubation temperatures yielded negative hemagglutination titers. One antigen (MHV-A59) was prepared in the presence or absence of FCS to monitor for the possible presence of hemagglutination inhibitors in serum [16]. However, similar results were obtained with either antigen (table 1). Concentrated or partially purified MHV-3 and MHV-S antigens, respectively, were used to both increase viral infectivity titers and prevent reported inconsistent hemagglutination by crude antigen [9]. Even though not all possible combinations of antigen, erythrocyte, and buffer were tested, the results presented here show that hemagglutination by these three strains of MHV grown on DBT cells is at least not an obvious biological activity, unlike MHV-DVIM [3]. It is possible that the previously reported hemag-

glutinating activity of MHV-3 [9] was dependent on the different host used for virus growth or the virus purification method. Finally, it can be concluded that the 65-kD structural protein observed under reducing conditions in polyacrylamide gels of purified MHV-S [6, 12; PJT, unpubl. data] apparently is not a viral hemagglutinin.

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