MONOCLONAL ANTIBODIES TO THE THREE CLASSES OF MOUSE HEPATITIS VIRUS STRAIN A59 PROTEINS

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Hybridoma cell lines producing monoclonal antibodies to mouse hepatitis virus strain A59 (MHV-A59) have been established by fusion of spleen cells of immunized mice with P3X63Ag8.653 mouse plasmacytoma cells. Culture fluids were screened for their ability to immunoprecipitate virus-specific proteins from ^{35}S-methionine-labeled infected cells. Eleven clones were obtained which fell into three classes (Figure 1).

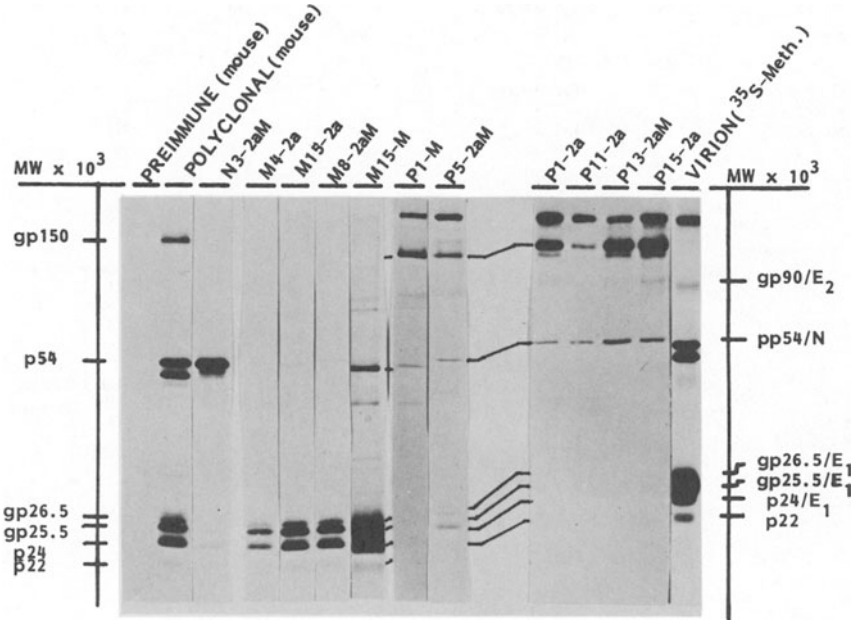

Figure 1

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One clone reacted with the nucleocapsid protein (N). Four clones reacted with the matrix protein (E1) which is present, both in infected cells and in virions, as the unglycosylated form gp24/E1 and two glycosylated forms gp25.5/E1 and gp26.5/E1. All three modifications of E1 were precipitated which is in agreement with earlier fingerprinting data for MHV strain JHM (1). The third class consisted of six clones specific for E2. The hybridomas produced IgG2a and/or IgM antibodies. The presence of two Ig species was not due to insufficient cloning, since the results remained the same after additional cloning. Possibly these cell lines carry out the IgM/IgG switch. Monoclonal antibodies against the viral glycoproteins, E1 and E2 recognized viral proteins on the surface of infected L929 cells but not on Sac(-) cells. Four out of six of the anti-E2 but none of the anti-E1 clones were able to neutralize the virus in the absence of complement. On the addition of complement a slight increase in neutralization by three anti-E1 clones was observed (Table).

Table 1. SUMMARY OF MONOCLONAL ANTIBODIES

HYBRIDOMA CELL LINES	POLYPEPTIDE SPECIFICITY	IMMUNOGLOBULIN ISOTYPE*	% NEUTRALIZATION AT ANTIBODY DILUTIONS☆ NO COMPLEMENT ADDED			COMPLEMENT ADDED		
			1/10	1/100	1/500	1/10	1/100	1/500
N3-2aM	N	IgG2a/IgM	12	5	10	1	1	7
P1-2a	E_2	IgG2a	100	83	49	100	79	54
P11-2a	E_2	IgG2a	42	7	7	44	11	-10
P13-2aM	E_2	IgG2a/IgM	4	12	12	31	8	-17
P15-2a	E_2	IgG2a	100	90	60	100	89	60
P1-M	E_2	IgM	17	2	10	17	8	-3
P5-2aM	E_2	IgG2a/IgM	99	97	90	100	97	30
M4-2a	E_1	IgG2a	2	-6	1	22	18	0
M15-2a	E_1	IgG2a	4	-6	-2	12	10	-4
M8-2aM	E_1	IgG2a/IgM	4	-1	4	8	-8	-2
M15-M	E_1	IgM	6	-8	-10	15	37	24

*As determined by Ouchterlony immuno diffusion test.

☆Monoclonal antibodies were adjusted to an initial concentration of 5 mg/ml of the active immunoglobulin isotype.

Further studies are in progress to elucidate the relationship between the various species of E2 with monoclonal antibodies directed against gp150.

REFERENCE

1. Siddell, S.G., J.gen.Virol. 62:259-269 (1982).