CHARACTERIZATION OF MHV-A59 PERSISTENTLY INFECTED CELLS

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The aim of our work is to characterize MHV-A59 persistently infected cloned 17Cl1 cell lines on a molecular level. These cell lines were obtained by three consecutive infections of 17Cl1 cells (2 PFU/cell) and cloned three times by limited dilution in the presence of conditioned medium which contained 30 percent ultrafiltrated supernatant from 17Cl1 cells.

The obtained cell lines showed fusion area to a different extent and a reduced growth rate. None of them produced plaquing virus except the uncloned lines at an early passage number. At this stage small plaque mutants could be isolated. After cloning no intracellular particles could be detected by electron microscopy.

Dot blot hybridization with reverse transcribed viral cDNA showed the presence of virus specific RNAs. No hybridization was obtained with the DNA fraction.

All cell lines were resistant to MHV-A59 reinfection. Only the cloned lines were also resistant to infection with VSV.

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The presence of virus specific proteins was shown by immunofluorescence using a mouse antiserum highly enriched for anti E1 antibodies or monoclonal anti E1. Unlike during lytic infections the detected antigens were located in small clusters outside the golgi area. (see Fig.1). From the sizes and location these positively stained organelles might constitute lysosomes.

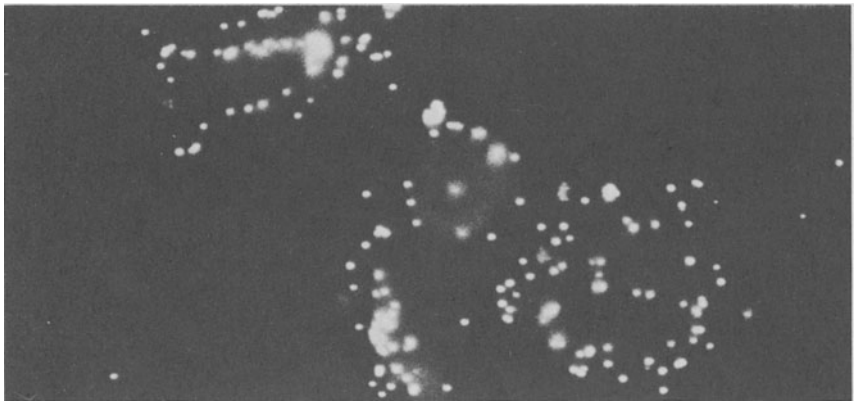

Fig.1. Intracellular clusters of viral antigens in persistently infected cloned cells (3iC2). Cells were incubated with mouse anti E1 and stained with FITC goat anti mouse. In a similar experiment a rabbit anti golgi serum kindly provided by Brian Burke, EMBL Heidelberg, FRG was added in a second step.

Table 1 shows a summary of the results:

TABLE I

CHARACTERIZATION OF THE CELL LINES

	Persistently infected cells uncloned	3iC1 cloned	3iC2 cloned	3iC3 cloned
Cell passage time (days)	7	7	7	7
Cell dilution at passsage	1:10	1:10	1:3	1:3
Need of conditioned medium	-	only during cloning	+	+
Cell fusion area	(+)	+	+++	++
Number of lytic crises (=CPE after passage)	2	-	-	-
Virus specific RNA	+	+	+	+
Virus specific DNA (tested in hybridization with reverse transcribed viral cDNA)	-	-	-	-
Expression of viral antigens (immunofluorescence)	+	+	++	++
Production of small plaque mutants	+ (at passage 12 earlier passages were not tested)	-	-	-
Intracellular virus particles	not tested	-	-	-
Resistance to MHV-A59 reinfection	+	+	+	+
Resistance to infection with VSV	-	-	+	+