A CELL LINE DEFECTIVE IN MURINE CORONAVIRUS INTERNALIZATION

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Murine coronavirus (MHV) infections of continuous cell lines show a wide spectrum of cell-virus interactions. Cell lines have been identified that can be lytically infected, persistently infected, or non-infectable (1,2,3). At present tne mechanisms involved in determining the outcome of the infectious process are not clearly understood. In previous work a cell line, the rat C6 glial line, was identified that was resistant to infection by both the MHV_3 and JHM strains of MHV (1). Studies have been initiated to examine the reasons for this restriction in order to understand mechanisms whereby cells can be refractory to coronavirus infections and to understand the replication strategy of these agents.

Adsorption of MHV_3, JHM, or A59 strains of MHV over a range of multiplities from 0.2 to 10 to the C6 cells occurred with similar kinetics and saturation levels as to the totally permissive host murine L2 cells, thus indicating that this stage of the infectious process was not altered. To examine whether the C6 cells could internalize the virus, MHV-adsorbed cells were warmed to 37^{o} for various periods of time and internalized virus measured as described in the legend to Figure 1. As shown in Figure 1, MHV internalization did not occur in the C6 cells with either JHM or MHV_3 strains but readily occurred when L2 cells were used as host. Vesicular stomatitis virus (VSV) was internalized by the C6 cells. Similar results were obtained when assays were carried out for internalized infectious virus. C6 cells contained no internalized infectious virus whereas, infectious virus could be recovered from L cells. These studies indicate that C6 cells are unable to internalize MHV.

It is possible that several stages of the MHV replicative cycle may be blocked in the C6 cells. To examine this MHV (strains JHM or MHV) were introduced into C6 cells by polyethylene glycol fusion. Under such conditions MHV can be shown to replicate in the C6 cells in that the cells scored as infectious centers (varying between 0.1 to 3% depending upon the initial multiplicity of infection), progeny virus was released (from 10^2 to 10^4 pfu/ml) over several days, viral antigen was detected by immunofluorescent labelling, and viral proteins were detected by pulse labelling with ^{35}S methionine.

Taken together these results indicate that the C6 cells are restrictive to murine coronavirus replication by preventing the penetration stage of the viral replicative cycle and may be useful in examining this stage.

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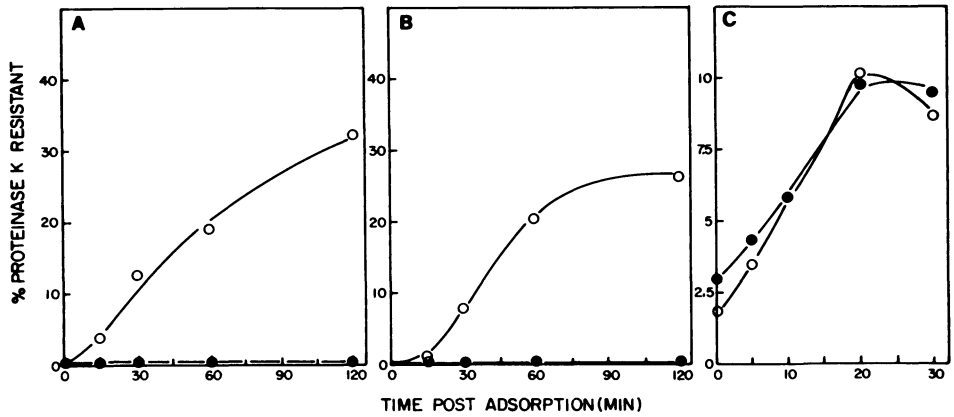

Figure 1. Internalization of viruses to mouse L2 and rat C6 cells. Cultures of MHV-adsorbed (panels A,B) or VSV-adsorbed (panel C) L (○) or C6(●) cells were warmed at 37° for various periods of time, treated with proteinase k to remove external virus, and assayed for internalized virus by the infectious center assay. (A) MHV_3 at moi of 5; (B) JHM at moi of 2; (C) VSV at moi of 0.5.

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