

RESTRICTED REPLICATION OF A TEMPERATURE SENSITIVE MUTANT OF MHV-A59 IN MOUSE BRAIN ASTROCYTES

Mario van Berlo, Guus Wolswijk, Jero Calafat*, Marian Horzinek and Ben van der Zeijst

Institute of Virology, State University Utrecht, The Netherlands and *The Netherlands Cancer Institute, Antoni van Leeuwenhoekhuis, The Netherlands

Temperature-sensitive (ts) mutants of mouse hepatitis virus (MHV-A59) are drastically attenuated in their pathogenic properties: intracerebral inoculation of mice with mutants results in more prolonged infection of the central nervous system without clinical signs (Koolen et al., 1983, *Virology* 125, 393-402). This was surprising in the case of mutant ts-342 which was "leaky" and grew well in tissue culture cells at 37°C. We have studied the replication of mutant ts-342 in primary cultures of mouse brain

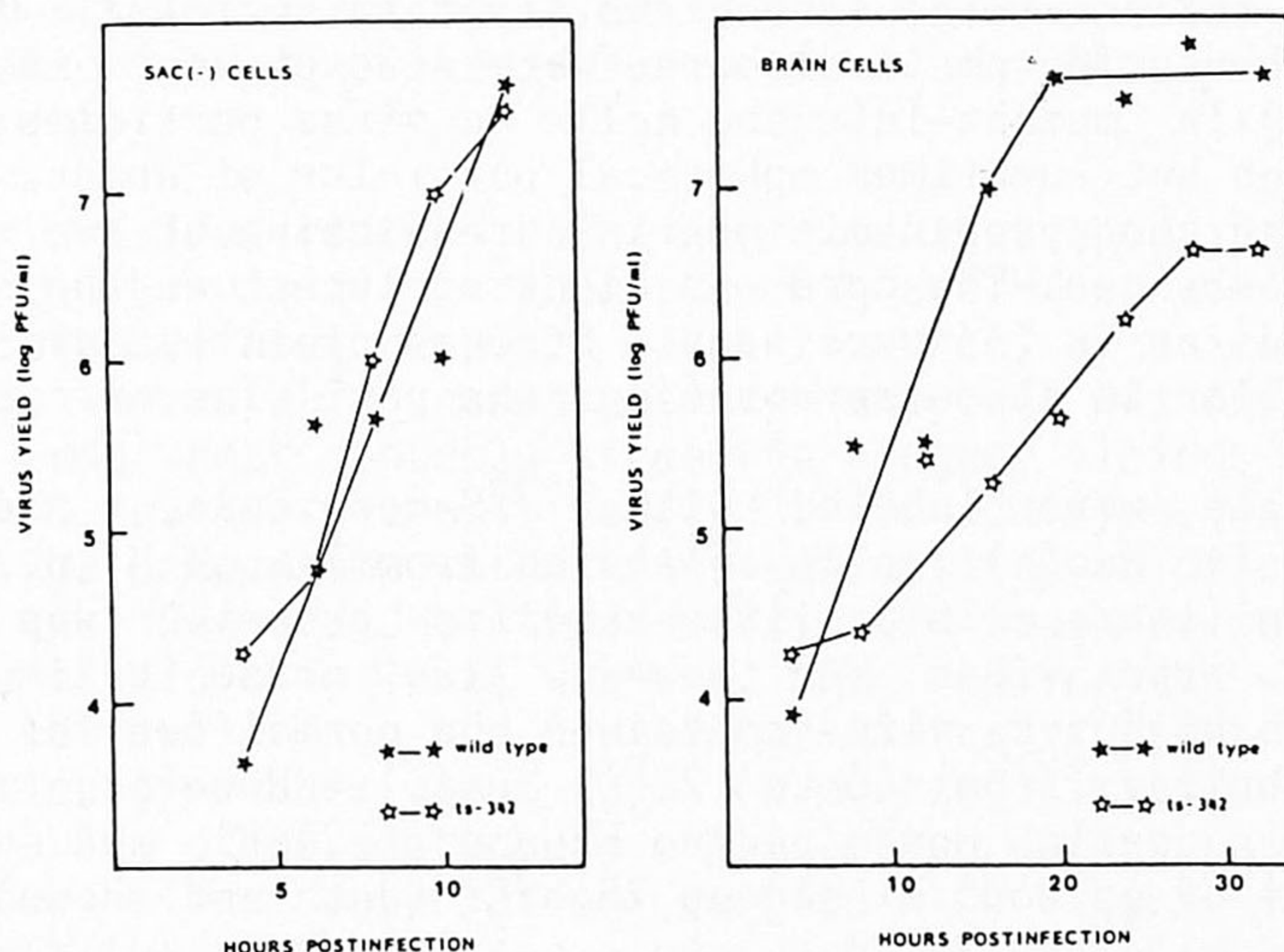


Fig.1. Growth kinetics of MHV-A59 and its mutant ts-342 in Sac(-) cells (A) or in mouse brain cells (B). The cells were infected with 50 PFU/cell.

cells, derived from approximately 14-day-old Balb/c mouse embryos. Cultures became confluent after seven days. The cells were identified as astrocytes since they contained glial fibrillary acidic protein (GFAP).

Virus growth in Sac(-) cells was similar for wild type MHV-A59 and ts-342. In mouse brain cells, however, production of infectious ts-342 virions was only about 5% of wild type virus (Fig.1). Using immunofluorescence no difference was noticed between wild type and ts-342 virus-infected cells.

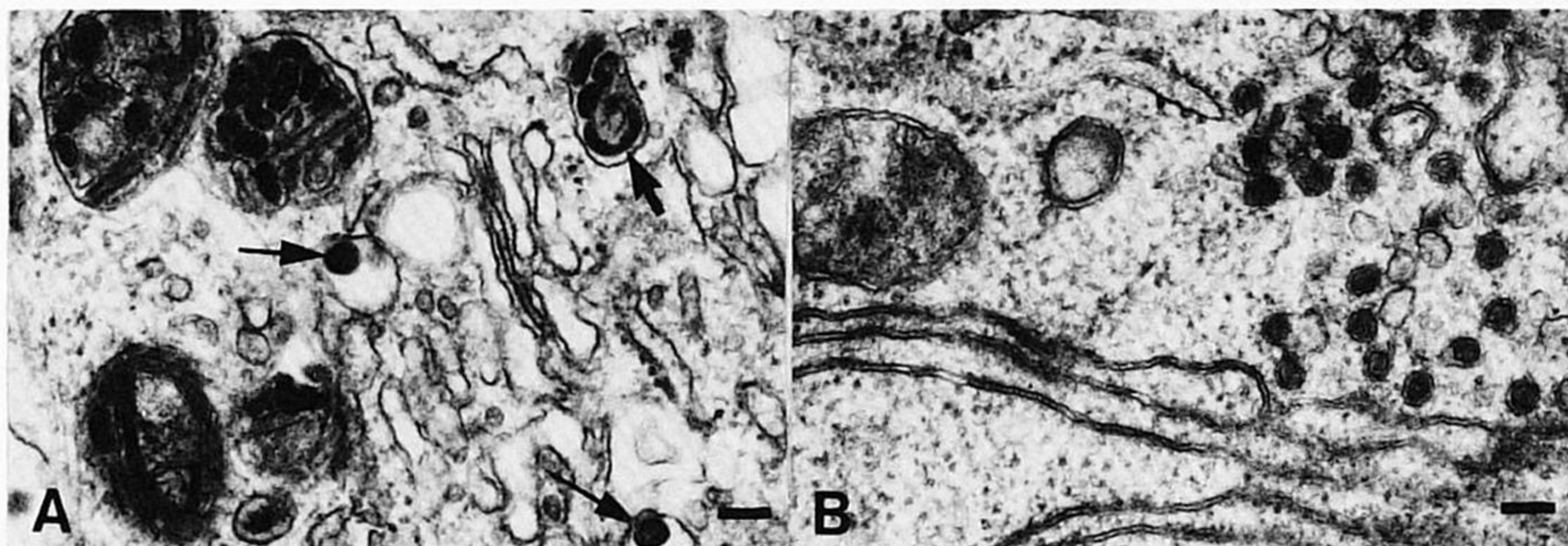


Fig. 2. Electron micrographs of mouse brain astrocytes infected with MHV-A59 (A) and its mutant ts-342 (B). 26 h post infection. Bar = 100 nm

Electron microscopy (Fig.2) showed that in wild type virus-infected cells virus particles were abundant and matured in smooth membrane cisterns closely associated with the Golgi system (A; budding site at thick arrow; free virus at thin arrows). Tubular structures about 30 nm in diameter were also present in these cisterns. (B): In mutant-infected cells no virus particles as in (A) were seen but sometimes spherical particles of about 70 nm were present in the cytoplasmic matrix, consisting of two concentric double membranes. The core was electron-lucent and no nucleocapsid strands as in (A) were seen. Stacked profiles of double membranes similar to those surrounding the particles were present.

Infected cells were labeled with ^{35}S -methionine and ^3H -glucosamine. In Sac(-) cells (labeled from 7 to 9 h p.i.) no difference in intracellular virus-specific proteins was found between wild type virus and ts-342. Also mouse brain cells infected with wild type virus contained the normal set of viral proteins (labeling from 16 to 22 h p.i.). However, ts-342 infected brain cells contained no E2 and pp 24/E1 and only a reduced amount of gp 26.5/E1 and gp 25.5/E1 (data not shown).

The restricted replication of ts-342 in mouse brain cells is probably due to a second site mutation, which affects the host range by causing a reduced level of glycoproteins in astrocytes.