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Murine gammaherpesvirus 68 establishes a latent infection in mouse B lymphocytes *in vivo*

N. P. Sunil-Chandra,* S. Efstathiou† and A. A. Nash

Department of Pathology, University of Cambridge, Tennis Court Road, Cambridge CB2 1QP, U.K.

Murine gammaherpesvirus 68 (MHV-68) is able to persist in spleen cells of infected mice. To determine the cell type harbouring persistent virus, spleen cells from infected animals were separated into immunoglobulin (Ig)-positive (B cell-enriched), Ig-negative (T cell-enriched) and plastic-adherent (macrophage-enriched) fractions. These cells were co-cultivated with permissive BHK-21 cells in an infectious centre assay. The consistent recovery and enrichment of infectious centres in the Ig-positive fraction clearly demonstrates that B cells are a major site of virus persistence/latency. This observation indicates that MHV-68 is biologically similar to Epstein–Barr virus and other members of the B cell lymphotropic gammaherpesvirus 1 subgroup.

Gammaherpesviruses are generally considered to be lymphotropic in nature, with the ability to establish latent infections within lymphocytes (Roizman *et al.*, 1981; Honess, 1984). These viruses can induce a lymphoproliferative disease in the infected host and can efficiently immortalize lymphocytes infected *in vitro*, e.g. Epstein–Barr virus (EBV) and herpesvirus saimiri (HVS) (Shope *et al.*, 1973; Crawford *et al.*, 1982; Fleckenstein & Desrosiers, 1982; Rickinson *et al.*, 1989). On the basis of the available data, gammaherpesviruses have been subdivided into B cell-tropic (gammaherpesvirus 1), characterized by EBV and related viruses of old world monkeys and apes, and T cell-tropic (gammaherpesvirus 2), such as HVS and herpesvirus ateles, both infecting new world monkeys (Honess, 1984). This classification may not hold true for all gammaherpesviruses; for example, herpesvirus sylvilagus can establish a latent infection in both B and T cells of cottontail rabbits (Kramp *et al.*, 1985).

We have been studying murine herpesvirus 68 (MHV-68), a naturally occurring murid herpesvirus originally isolated from bank voles (*Clethrionomys graleolus*) in Czechoslovakia (Blaskovic *et al.*, 1980). Limited sequence analysis of the MHV-68 genome has shown this virus to be closely related to the gammaherpesviruses of primates, EBV and HVS, in terms of both its gene content and organization (Efstathiou *et al.*, 1990*a*, *b*). However, the overall genome structure and G+C content of MHV-68 are most similar to those of the gammaherpesvirus 2 group.

Studies on primary infection of BALB/c mice have shown the lung to be the main tissue productively infected by MHV-68, with virus present in alveolar epithelium and mononuclear cells (Sunil-Chandra *et al.*, 1992). As with the other gammaherpesviruses, the spleen appears to be the major site of virus persistence, with latently infected cells detected by a co-cultivation assay. This technique has been used widely to detect latently infected lymphoid cells taken from animals infected with HVS (Falk *et al.*, 1972; Rabson *et al.*, 1971) and herpesvirus sylvilagus (Kramp *et al.*, 1985; Medveczy *et al.*, 1984). The ability to recover virus by explant culture, but not by direct homogenization of spleen, and the lack of virus antigen expression at this site at late times post-infection (p.i.) (Sunil-Chandra *et al.*, 1992), is taken by us as a definition of virus latency.

The aim of the present study was to identify the lymphocyte population harbouring latent MHV-68. To achieve this, spleen cells were separated into plastic-adherent cells, to enrich for macrophages, immunoglobulin (Ig)-positive cells (B cells) and Ig-negative cells (T cells) using anti-Ig-coated plates. This method offers a rapid and specific means of separating lymphocyte subpopulations (Nash, 1976; Mage *et al.*, 1977; Mason *et al.*, 1987; Wysocki & Sato, 1978). The number of cells harbouring latent virus from each subpopulation was determined by an infectious centre assay (Sunil-Chandra *et al.*, 1992). In three separate experiments, 3- to 4-week-old BALB/c mice (Bantin and Kingman) were infected intranasally with 4×10^5 p.f.u. MHV-68 (Sunil-Chandra *et al.*, 1992) and the spleen was removed on different days p.i. Spleen cells were separated into plastic-adherent, Ig-positive (B cells) and Ig-negative (T cells) fractions, as shown in Fig. 1. Briefly, red blood cells (RBCs) were

† Present address: Division of Medical Virology, Institute of Medical and Veterinary Science, Frome Road, Adelaide, SA 5000, Australia.