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

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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 atelie, 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 glareolus) 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., 1990a, 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 $4 \times 10^5$ 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...