Equine gammaherpesvirus 2 (EHV2) is latent in B lymphocytes

H. E. Drummer, G. H. Reubel, and M. J. Studdert

Centre for Equine Virology, School of Veterinary Science, The University of Melbourne, Parkville, Victoria, Australia

Accepted November 22, 1995

Summary. Peripheral blood leukocytes were collected from 5 Thoroughbred horses and examined for the presence of EHV2 in sub-populations of mononuclear cells. Peripheral blood mononuclear cells were separated on Percoll gradients and then enriched for plastic adherent cells (predominantly monocytes), surface immunoglobulin positive (sIg+) B lymphocytes and T lymphocytes, using panning techniques. The purity of each cell population was assessed by fluorescence activated cell scanning. In an infectious centre assay, each cell population was inoculated onto equine foetal kidney monolayer cell cultures which are fully permissive for the replication of EHV2. Only enrichment for sIg+ B lymphocytes resulted in a marked increase in the number of infectious centres, indicating that EHV2 is present in B lymphocytes. Freeze-thawing of sIg+ B lymphocytes, prior to inoculation onto EFK monolayer cell cultures, resulted in the complete abrogation of infectious centre formation, confirming that EHV2 is latent in B lymphocytes i.e., infectious free virus was not present in the cells. The number of EHV2 infected B lymphocytes varied considerably between horses from 4 to 780 per 10⁶ cells. Evidence was also obtained that direct cell to cell contact between the epithelial cells and sIg+ B lymphocytes was necessary for the production of infectious centres. The data indicate that EHV2, like other members of the Gammaherpesvirinae, is latent within B lymphocytes.

Introduction

Based on biological properties, herpesviruses are classified in three subfamilies, Alphaherpesvirinae, Betaherpesvirinae, and Gammaherpesvirinae [21]. Viruses in the subfamily Gammaherpesvirinae are defined for their lymphotropism and are associated with aberrations of these cells in vivo including transient and chronic lymphoproliferative disorders and in vitro, with the immortalisation of B lymphocytes. Gammaherpesviruses generally have a narrow host range, slow replication cycles, and establish latency in B and/or T lymphocytes. Although the viral genome is replicated in lymphoblastoid cells, infection is frequently
non-productive. Some gammaherpesviruses are known to productively infect epithelial cells. Gammaherpesviruses are divided into two genera, the \( \gamma_1 \) and \( \gamma_2 \) herpesviruses based on genetic criteria that include GC content and genomic organisation. \( \gamma_1 \)-herpesviruses include Epstein Barr virus (EBV), the cause of infectious mononucleosis/glandular fever and several lymphoproliferative disorders of humans, and is tropic for B lymphocytes. \( \gamma_2 \)-herpesviruses include herpesvirus saimiri (HVS2) and are associated with lymphoproliferative disorders in lower primates. \( \gamma_2 \)-herpesviruses are typically associated with tropism for T cells, although murid herpesvirus strain 68 (MHV68) is tropic for B cells [25] and herpesvirus sylvilagus is tropic for both B and T cells [10].

Equine herpesvirus 2 (EHV2) is a recently classified member of the Gammaherpesvirinae [27] and the genome of 184 kbp has been completely sequenced [26]. EHV2 is a slowly cytopathic, highly cell associated virus and can be isolated from buffy coat cells or nasal secretions of up to 90% of adult horses [7, 20]. EHV2 has a relatively broad host-range in vitro in that it is known to replicate in rabbit, guinea pig, feline and ovine kidney cell cultures as well as equine cell cultures of kidney, thyroid, brain, lung, testis, spleen, lymph node, bone marrow, dermis and leukocytes (see [1]). The exact role of EHV2 in producing disease is not fully defined. However, it has been associated with immunosuppression, upper respiratory tract disease, conjunctivitis, general malaise and poor performance [1–3, 6, 17, 23, 24, 28]. It has also been suggested that EHV2 may play a role in transactivation and reactivation of latent alphaherpesviruses, EHV1 (equine abortion virus) and EHV4 (equine rhinopneumonitis virus) [30].

While EHV2 has been linked to a number of disease syndromes in the horse, little is known about the pathogenesis of the virus in causing disease. The reclassification of EHV2 as a gammaherpesvirus suggests new approaches to investigating the role of this virus in various diseases. In this study, we investigated the site and nature of latency of EHV2 and the mechanism of infection of permissive epithelial cells from latently infected B lymphocytes. We show that like EBV and some of the lower primate gammaherpesviruses, EHV2 is latent in B lymphocytes.

**Materials and methods**

**Virus and cells**

Equine foetal kidney (EFK) monolayer cell cultures are permissive for the growth of EHV2 and were used in infectious centre assays at passage 5. EFK cells were grown in minimal essential medium (MEM) containing Earle’s salts, L-glutamine and non-essential amino acids and supplemented with 10% v/v foetal calf serum (FCS; Gibco BRL), 0.5% v/v lactalbumin hydrolysate (Sigma) and 0.006 M NaHCO\(_3\). For infectious centre assays, EFK cells were maintained in MEM supplemented with 1% v/v FCS, 50 \( \mu \)g/ml ampicillin, 0.013 M NaHCO\(_3\) and 0.015 M HEPES (Sigma) at pH 7.4 (maintenance medium; MM). A semi-solid overlay medium consisted of MM with 1% carboxy methyl cellulose.

EHV2 strain 94/01, isolated from peripheral blood mononuclear cells (PBMC) of a horse was propagated in EFK monolayer cell cultures for use as a positive control in Millicell (Millipore) experiments (see below) and was used at passage 2.