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Productive Persistent Infection of Hematopoietic Cells by Human Foamy Virus

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Human foamy virus can establish persistent infections in human hematopoietic cell lines, such as H92.1.7 (erythroblastoid cells), Jurkat ($CD4^+$ T cells), and U937 (myeloid-monocytic cells). The infection is characterized by constant production of infectious viruses (for >2 1/2 years) with no cytopathic effects on the host cells. Electron microscopy of the infected cells showed a viral morphology similar to that observed for particles produced after acute infection. We have detected, in addition to the full-length form of *bel1*, a previously described deletion in the *bel1* gene of the proviral DNA in these cells. RNA containing this 301-bp deletion, which mapped to the splice donor and acceptor sites of the intron of the *bet* gene, was also found in encapsidated virion RNA. However, the presence of this defective provirus harboring the deletion in *bel1* does not prevent productive persistence in these chronically infected cells, since the virus titer does not decrease during cultivation.

Foamy viruses are complex retroviruses with a genomic organization resembling those of human immunodeficiency virus and human T-lymphotropic virus. These viruses have been isolated from a variety of animal species, including primates, cats, and bovines (10). Although there have been several reports of foamy virus associated with patients suffering from various diseases (1, 5, 12, 24, 27), a recent study by Schweizer et al. (23) has convincingly concluded that foamy virus is not prevalent in humans. Thus far, the strain of human foamy virus (HFV) that was originally recovered from a patient with nasopharyngeal carcinoma in 1971 by Achong et al. (1) has remained the only isolate available for research.

The genome of HFV contains *gag*, *pol*, and *env*, as well as several other genes located between *env* and the 3′ long terminal repeat (8). These genes, including the *bel1*, *bet*, and *bel3* genes, are all derived from singly and/or multiply spliced mRNA (18). Among them, only the *bel1* gene, which encodes a potent transcriptional transactivator, is required for viral replication (14). When fibroblastic cells are infected in vitro, foamy virus is highly cytopathic and rapidly induces multinucleated syncytia with a vacuolated foamy appearance in the cytoplasm, eventually killing the cells (4, 10). This characteristic cytopathic effect has enabled foamy virus to be isolated from various tissues of infected hosts (25), including peripheral blood lymphocytes (2, 7, 17, 19). However, it is puzzling that the rapid cytopathicity caused by acute virus infection in vitro is usually not found in in vivo animal models, where infection is usually persistent and asymptomatic and accompanied by the presence of circulating neutralizing antibody (4, 10).

Tissue-specific tropism and characteristics of HFV gene expression in vivo are largely unexplored. Little is known about the ability of foamy virus to replicate in different cell types other than fibroblasts. It is known that epithelial cells do not support active replication of foamy virus (15); yet, it remains unclear if the resistance of epithelial cells is due to a lack of specific receptors or to the absence of intracellular factors. Several latently infected cultures have been established from epithelial cells (6, 11, 22) and lymphoblastoid cells (20); failure of HFV replication in these cultures has been suggested to be caused by methylation of proviral DNA (11, 22). In these cases, very little or no infectious virus was produced and only proviral DNA was detected.

In this study, we examined HFV replication in hematopoietic cells, which not only are the main source for isolating viruses from infected hosts (1, 2, 5, 7, 12, 17, 19, 24, 27) but also are the primary target cells for many other retroviruses. Infec-

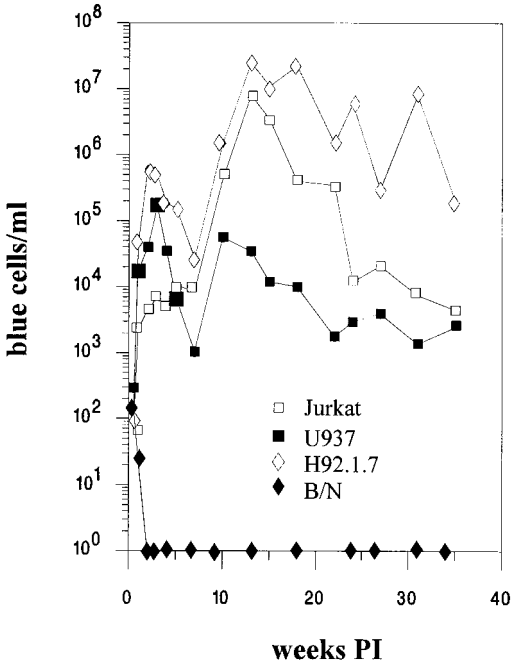

FIG. 1. Long-term growth of wild-type HFV in human hematopoietic cells. The infected suspension cells were subcultured every 5 to 7 days, and an aliquot of culture medium including cells was periodically removed and assayed for virus titers by the FAB assay (28). The experiments have been repeated several times with similar results, although there are slight differences in the growth curves seen for individual experiments. PI, postinfection.

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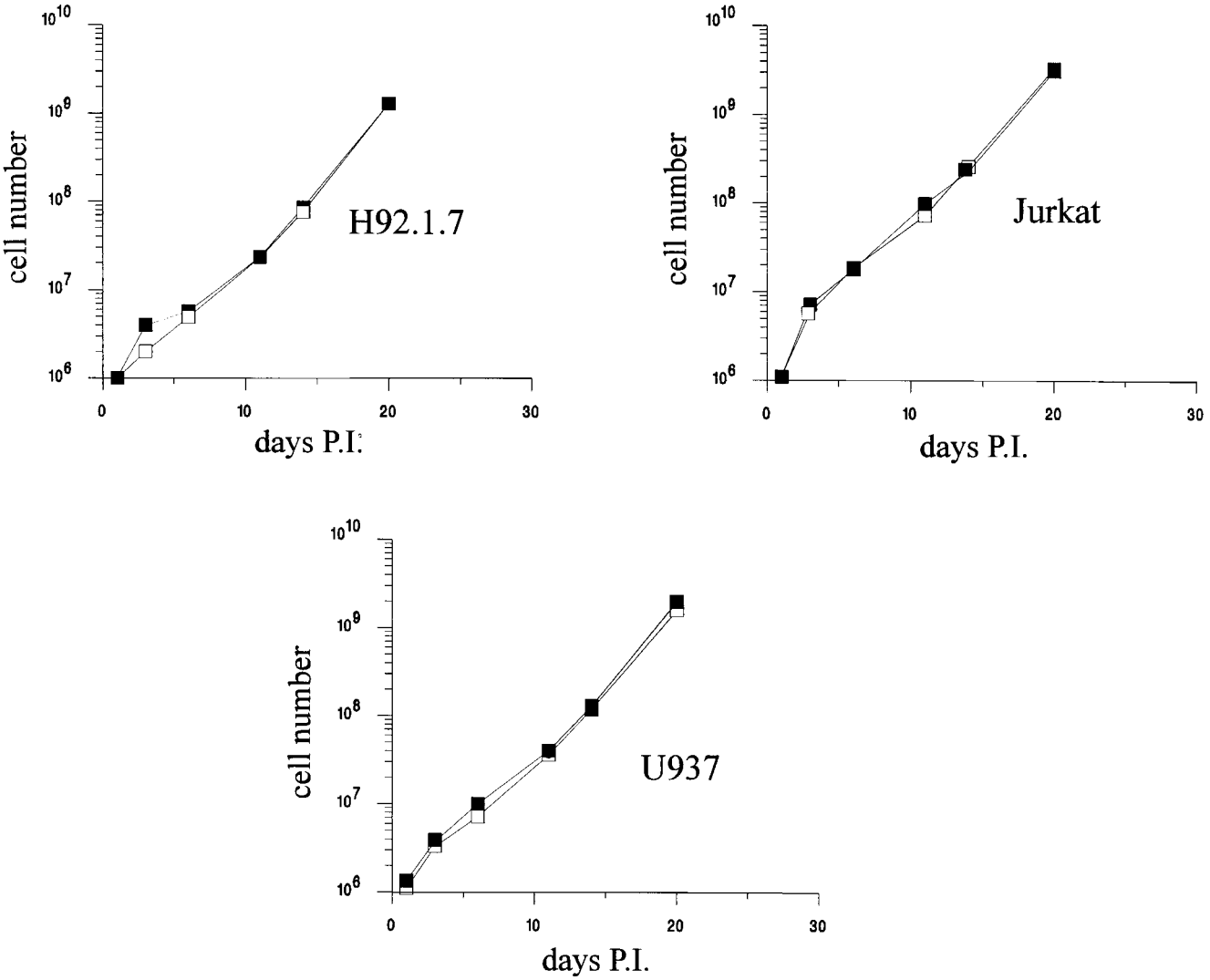

FIG. 2. Multiplication of cells infected by HFV. H92.1.7, Jurkat, and U937 cells were either mock infected (open squares) or infected by HFV (closed squares). The number of viable cells was determined by trypan blue staining and is presented as cumulative cell number (corrected for the number of cells discarded during cultivation). P.I., postinfection.

tion of hematopoietic cells grown in suspension cultures with HFV was performed as described previously by using a cocultivation method (28). These cells were cocultivated for 24 h with mitomycin-treated human embryonic lung fibroblastic (HEL) cells acutely infected with HFV, in which virus yield usually peaks at 72 h after infection with a rapid development of cytopathic effects and cell killing. The suspension cells were then washed and subcultured every 5 to 7 days and maintained in RPMI medium containing 5% fetal bovine serum. An aliquot of culture medium including cells was periodically removed and assayed for infectious virus titers by the FAB assay, in which transactivation of β-galactosidase driven by the HFV long terminal repeat promoter required expression of functional Bel1 proteins (28).

Our results showed that, while medium- to high-titer viruses were continually produced in HFV-infected Jurkat (a $CD4^+$ T-cell line), U937 (a histiocytic lymphoblastoid cell line), and H92.1.7 (an erythroblastoid cell line originally called HEL) cells (16) during 8 months of cultivation (Fig. 1), no decrease in the cell survival rate or in the presence of cytopathic effects was observed. In fact, there was no or little difference in the multiplication rate between mock-infected and HFV-infected cells, even immediately following infection (Fig. 2). These infected cells have been maintained in culture for more than 2 years, and they are still releasing infectious viruses. In contrast, no or very little viral production was detected when B/N cells (an Epstein-Barr virus-transformed B-lymphocytic cell line) (28) were infected (Fig. 1). The same results were obtained with several other B-lymphocytic cell lines, such as Manca, Raji, and Ramos (data not shown). Genomic DNA obtained from cells at the 12th and 30th weeks after infection was analyzed by PCR or Southern blot hybridization. We found that HFV proviral DNA was present in persistently infected Jurkat, U937, and H92.1.7 cells but could not be detected in the infected B/N cells (data not shown). Interestingly, a very low virus titer was detected when a cell line which was a hybrid between T and B lymphocytes, 174xCEM (NIH AIDS Reagent Program catalog no. 272), was infected (data not shown). However, we do not know if the inability to infect B-lymphotropic cells is due to a lack of specific receptors or viral cofactors or

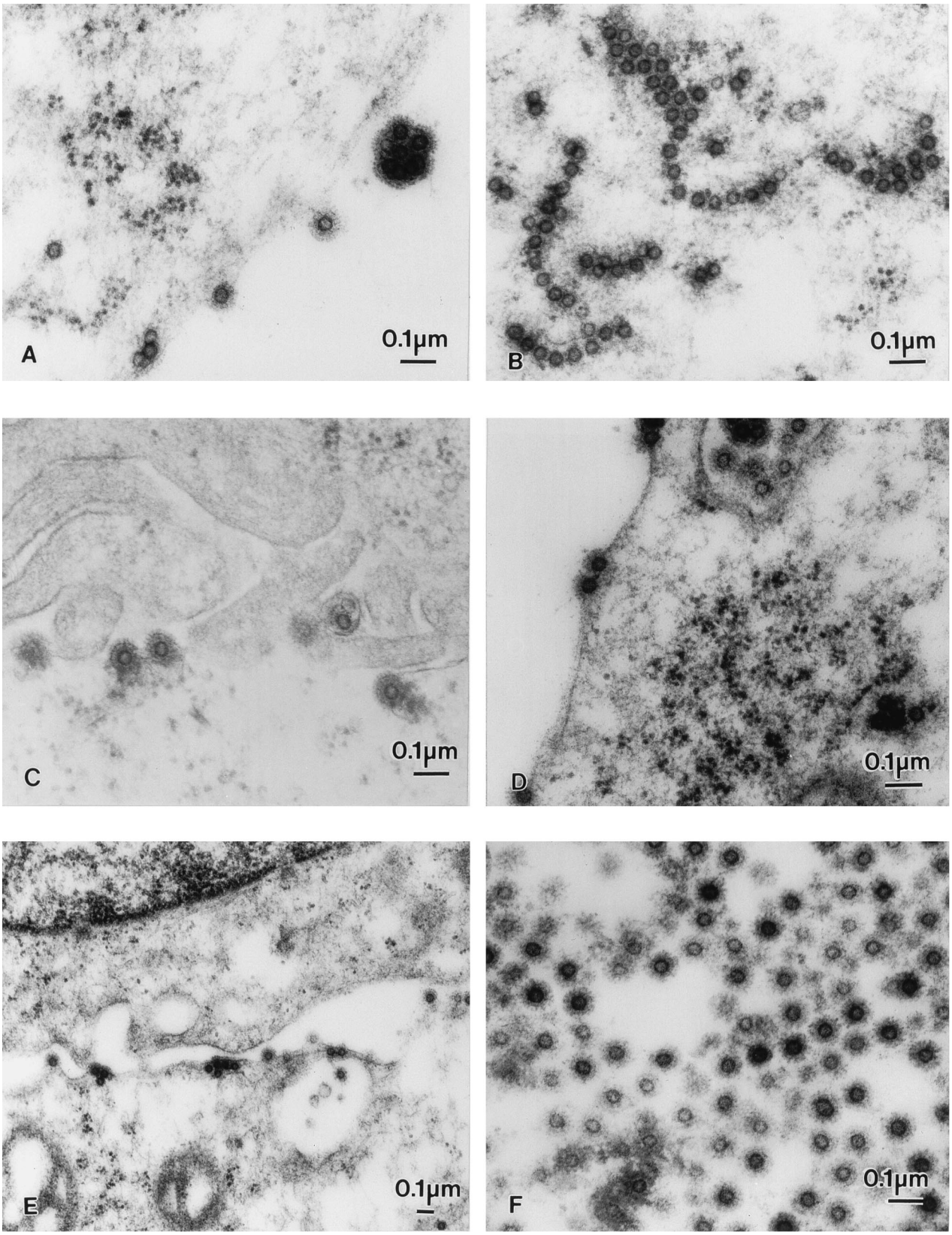

FIG. 3. Electron micrographs of HFV. Persistently infected H92.1.7 cells (5×10^6) (A, B, and D to F) or Jurkat cells (C) were centrifuged for 5 min at 1,000 × *g*. The pellets were fixed in half-strength Karnovsky's fixative and postfixed in Collidine-buffered osmium tetroxide. The samples were dehydrated at graded ethanol and propylene oxide before being embedded in Polybed A12. Sections were then stained with half-saturated uranyl acetate and lead tartrate and examined by JEOL100 Sx transmission electron microscopy. Budding of virus particles through cell membrane or plasma membrane is shown (A and C to E). Virus particles are also present in large numbers in the cytoplasm (B) and outside the cells (F).

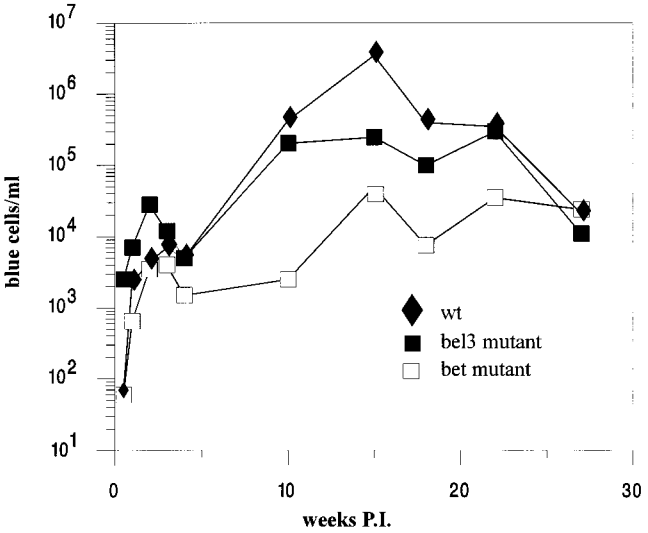

FIG. 4. Chronic infection of Jurkat cells with wild-type (wt) or mutant HFV defective in either the *bet* or *bel3* gene (28). The infected cells were subcultured every 5 to 7 days, and an aliquot of culture medium including cells was periodically removed and assayed for virus titers by the FAB assay (28). P.I., postinfection.

to the presence of certain cellular inhibitors that block infection of B cells. Since it has been shown that simian foamy virus type 11 can infect Raji B cells in vitro (17), the difference in cell tropism between simian foamy virus and HFV is intriguing since they are closely related.

Of the cell lines tested, infected H92.1.7 cells consistently produced the highest virus titer, with a peak of 10^7 infectious units per ml (Fig. 1). Twenty-four weeks after infection, only about 20 to 30% of the infected H92.1.7 cells were positive for both Bel1 and Gag proteins, as measured by immunofluorescence with specific antibodies (data not shown). These persistently infected H92.1.7 cells were positive for hemoglobin production after hemin treatment (data not shown), indicating that HFV infection does not block differentiation of these cells along the erythroid lineage (16). The virions obtained from these infected H92.1.7 cells showed typical foamy virus morphology, with electron-lucent cores before (Fig. 3B) and after (Fig. 3F) release from the cells. Virus budding occurred either through cell membrane or into endoplasmic reticulum, with entire cores acquiring envelope glycoprotein from the membrane as budding took place (Fig. 3A and C to E). Thin sections of persistently infected Jurkat cells also showed a similar pattern of viral morphology (Fig. 3C). Interestingly, cell-free viruses isolated from infected H92.1.7 cultures could easily infect naive H92.1.7 cells, while cocultivation was required for infection of the erythroid cells when the virus stock was obtained from acutely infected HEL cells. Further investigation is required to determine if the virus produced from these persistently infected cells has acquired morphological alterations or permanent genetic changes.

Infection of hematopoietic cells was also carried out with HFV mutants defective in either the *bet* or *bel3* gene (see Fig. 5A), in which the reading frames were disrupted by insertion of translational termination codons as previously described (28). In this study, we found that the *bel3* mutant could easily establish a long-term persistent infection in Jurkat, U937, and H92.1.7 cells (Fig. 4). We and others have demonstrated previously that in acute infection of fibroblastic cells, mutations in the *bel3* gene do not affect viral replication (3, 13, 28), although it has been suggested recently that the *bel3* gene may possess superantigen activity and thus could have a role in vivo (26). Bet is the most abundant viral protein found in the cytoplasm of infected cells (9, 14). Yet, mutations in the *bet* gene only slightly affect viral replication in acute infection of fibroblasts (28). We found that Jurkat cells infected with the *bet* mutant also remained persistently infected, as judged by both production of infectious viruses (Fig. 4) and presence of proviral DNA (data not shown). We have also examined the virion RNA

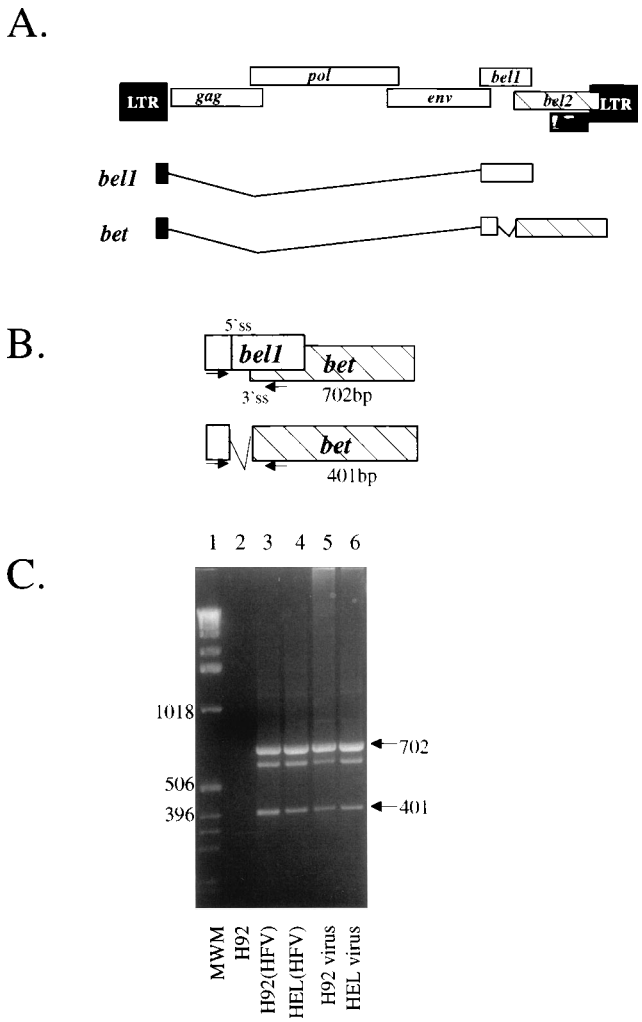

FIG. 5. (A) Schematic diagram of HFV genome and splicing pattern of *bel* genes. (B) Genomic map of *bel1* and *bet* genes. Both 5′ and 3′ splice sites (ss) for *bet* are indicated. The primers used for PCR and RT-PCR analysis are indicated by arrows. (C) PCR and RT-PCR analysis with primers #5′bel1 and #3′bel1 which flank the intron of the *bet* gene. Chromosomal DNA isolated from either mock-infected H92.1.7 cells (lane 2), chronically infected H92.1.7 cells 6 months after infection (lane 3), or acutely infected HEL cells (lane 4) was analyzed by PCR. Lanes 5 and 6 represent RT-PCR analysis of virion RNAs isolated from either chronically infected H92.1.7 cells 6 months after infection (lane 5) or acutely infected HEL cells (lane 6). Viral particles from cell supernatants were pelleted through 20% sucrose cushions, and RNA was isolated from proteinase K-treated virions with 0.2% sodium dodecyl sulfate and phenol-chloroform extraction. PCR products were separated on a 1.5% agarose gel and stained by ethidium bromide. The molecular weight standard (MWM, lane 1) is indicated at the left. The extra band migrating at 600 bp is apparently a heteroduplex between the 400- and 700-bp products. When the purified 600-bp fragment was used in another round of PCR with primers #5′bel1 and #3′bel1, three products of 400, 600, and 700 bp were again amplified (data not shown). Furthermore, only a sequence for either the 400- or the 700-bp product was found when this 600-bp product was cloned and sequenced (data not shown). LTR, long terminal repeat.

derived from either *bet*- or *bel3*-infected Jurkat cells by reverse transcription-PCR (RT-PCR) and DNA sequencing. Our results show that in both cell lines the viruses produced after 6 months in culture still contain the correct mutations in either *bet* or *bel3* (data not shown).

Saïb et al. (21) reported that a latently infected cell line, which was derived from HFV lysis-resistant megakaryocytic cells and did not produce any infectious virus, contained a provirus with a deletion in the *bel1* gene (21). This 301-bp deletion in *bel1* corresponds exactly to the mapped splice donor and acceptor sites of the intron of the *bet* gene and should lead to creation of a *bet* gene without introns and inactivation of the *bel1* gene as shown in Fig. 5B. In the study by Saïb et al., this nonrandom deletion in the proviral DNA was also detected after acute infection of permissive U373MG cells (a human glioblastoma cell line), although the truncated form of *bel1* was more abundant in latently infected cells (21). These authors suggested that the *bel1* deletion derived from reverse transcription of a defective genomic RNA could be responsible for establishment or maintenance of viral persistence and lead to noninfectious virions. We analyzed our persistently infected H92.1.7 cells using PCR to amplify fragments from this region in the chromosomal DNA. PCR was performed with two primers, #5′bel1 (5′GCTGACTATTGCTGAGGA AC3′) and #3′bel1 (5′AAGTTTGGTAGGTTGCTGGA3′), which are expected to generate a fragment of approximately 702 bp in the wild-type *bel1* gene. In addition to the 702-bp fragment, we found an ~400-bp fragment, smaller in size than that reported by Saïb et al. (Fig. 5C, lanes 3 and 4). We confirmed by Southern blotting and DNA sequencing that the shorter fragment of ~400 bp contains a 301-bp deletion in the *bel1* gene (data not shown), consistent with that described by Saïb et al. (21). The *bel1* deletion was also found in the virion RNA isolated from persistently infected H92.1.7 cells as well as from acutely infected HEL cells (Fig. 5C, lanes 5 and 6), indicating that the *bel1*-deleted spliced RNA could also be packaged into virus particles. Since the persistently infected H92.1.7 cells constantly produced high-titer infectious viruses, it seems unlikely that the deletion in the *bel1* gene causes significant interference and loss of infectious virus production in hematopoietic cell types, despite its presence in viral particles. However, we cannot exclude the possibility that the presence of defective provirus plays some important role in HFV biology in vivo.

This report describes the successful establishment of several hematopoietic cell lines persistently infected with HFV. Our results indicate that, in vitro, long-term productive infection of many hematopoietic cell types by HFV can be achieved without cytopathic effects. This prolonged virus production is reminiscent of the persistent infection observed in natural and experimental animal hosts. In animals infected with foamy viruses, hematopoietic cells could be an important reservoir of viral production.

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