Epstein-Barr virus (EBV) is a tumor virus with marked B lymphotropism. After crossing the B-cell membrane, the virus enters cytoplasmic vesicles, where decapsidation takes place to allow transfer of the viral DNA to the cell nucleus. BNRF1 has been characterized as the EBV major tegument protein, but its precise function is unknown. We have constructed a viral mutant that lacks the BNRF1 gene and report here its in vitro phenotype. A recombinant virus devoid of BNRF1 (DeltaBNRF1) showed efficient DNA replication and production of mature viral particles. B cells infected with the DeltaBNRF1 mutant presented viral lytic antigens as efficiently as B cells infected with wild-type or BNRF1 trans-complemented DeltaBNRF1 viruses. Antigen presentation in B cells infected with either wild-type (EBV-wt) or DeltaBNRF1 virus was blocked by leupeptin addition, showing that both viruses reach the endosome/lysosome compartment. These data were confirmed by direct observation of the mutant virus in endosomes of infected B cells by electron microscopy. However, we observed a 20-fold reduction in the number of B cells expressing the nuclear protein EBNA2 after infection with a DeltaBNRF1 virus compared to wild-type infection. Likewise, DeltaBNRF1 viruses transformed primary B cells much less efficiently than EBV-wt or BNRF1
trans-complemented viruses. We conclude from these findings that BNRF1 plays an important role in viral transport from the endosomes to the nucleus.