The human gammaherpesviruses Kaposi's sarcoma-associated herpesvirus and EBV cause important infections. As pathogenetic studies of the human infections are restricted, murine gammaherpesvirus 68 serves as a model to study gammaherpesvirus pathogenesis. TLRs are a conserved family of receptors detecting microbial molecular patterns. Among the TLRs, TLR9 recognizes unmethylated CpG DNA motifs present in bacterial and viral DNA. The aim of this study was to assess the role of TLR9 in gammaherpesvirus pathogenesis. Upon stimulation with murine gammaherpesvirus 68, Flt3L-cultured bone marrow cells (dendritic cells) from TLR9-/- mice secreted reduced levels of IL-12, IFN-alpha, and IL-6, when compared with dendritic cells from wild-type mice. Intranasal infection of TLR9-/- and wild-type mice did not reveal any differences during lytic and latent infection. In contrast, when infected i.p., TLR9-/- mice showed markedly higher viral loads both during lytic and latent infection. Thus, we show for the first time that TLR9 is involved in gammaherpesvirus pathogenesis and contributes to organ-specific immunity.