Infection of neonates with murine gammaherpesvirus 68 results in enhanced viral persistence in lungs and absence of infectious mononucleosis syndrome

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We used the murine gammaherpesvirus 68 (γHV-68), which serves as a model for human gammaherpesvirus infection, to determine whether age at infection altered the pattern of gammaherpesvirus pathogenesis. We infected mice intranasally at 8 days old (pups) and 6 weeks old (adults) to investigate differences in γHV-68 pathogenesis. There was no difference between adults or pups in acute infection in the lungs at 6 days post-infection (p.i.). However, mice infected as pups exhibited a more disseminated viral infection with viral DNA detected in the spleen, liver and heart as measured by quantitative PCR (Q-PCR). In addition, viral DNA was detected in the lungs of mice infected as pups until 60 days p.i. Three viral transcripts (M2, M3 and M9) were expressed at both 30 and 60 days p.i. In contrast, no viral DNA or mRNA expression was detected in lungs of mice infected as adults at 30 or 60 days p.i. Mice infected as adults experienced a peak in latent infection in the spleen at 16 days p.i., corresponding with an increase in splenic weight and expansion of the Vβ4+ CD8+ T-cell population, similar to infectious mononucleosis observed following infection of young adults with Epstein–Barr virus. However, the increase in splenic weight of infected pups was not as pronounced and no significant increase in Vβ4+ CD8+ T-cell expansion was observed in infected pups. Together, these data suggest that the pathogenesis of murine gammaherpesvirus γHV-68 is age-dependent.

INTRODUCTION

The two human gammaherpesviruses, Epstein–Barr virus (EBV) and Kaposi’s sarcoma-associated herpesvirus (KSHV), are associated with a number of malignancies including Burkitt’s lymphoma and Kaposi’s sarcoma. These cancers are endemic in Africa where primary infection with human gammaherpesviruses occurs early in life (Biggar et al., 1978; Mbulaiteye et al., 2004). Primary infection during childhood is thought to be asymptomatic but little is known as to whether infection at an early age affects the establishment and maintenance of viral persistence. With regard to EBV, infection early in life is thought to be a risk factor for endemic Burkitt’s lymphoma (de-The, 1977) and delay of primary infection until adolescence results in infectious mononucleosis (IM), suggesting that there are age-dependent differences in disease outcomes.

Because EBV and KSHV are strict human pathogens, murine gammaherpesvirus-68 (γHV-68) serves as a model for studying human gammaherpesvirus pathogenesis. Following intranasal infection of adult mice, γHV-68 replicates in the lungs and is cleared by 10–13 days post-infection (p.i.) (Cardin et al., 1996; Ehtisham et al., 1993).

Concurrent with the acute infection, lifelong latency is established in the germinal centre and memory B cells of the spleen (Flano et al., 2002; Marques et al., 2003). Latent viral load peaks at 14–16 days p.i., along with a transient splenomegaly similar to that seen during IM in humans (Tripp et al., 1997). By 30 days p.i., viral load in the spleen decreases and is maintained at a constant level. Nearly all studies examining the pathogenesis of γHV-68 have been done in adult mice. One early study which was conducted in 5- and 10-day-old mice found that infection at these young ages led to greater mortality than infection of 21-day-old mice (Rajcani et al., 1985). Additionally, mice infected at 3–4 weeks old had detectable levels of virus in the heart, kidney, adrenal gland and spleen early in infection (Sunil-Chandra et al., 1992a). A more recent study found that infection of newborn BALB/c mice with γHV-68 led to myocarditis and an increased viral load in the heart and blood (Hausler et al., 2007). These studies suggest that there are age-dependent differences in the pathogenesis of γHV-68.

The neonatal immune system is not fully developed and may not be able to clear viral infection as efficiently as the...
adult immune system. Neonates exhibit a decreased ability to mount the pro-inflammatory response that is often needed to deal with viral infection. Neonatal T cells are skewed toward a T helper cell type 2 (Th2), or anti-inflammatory, response and produce high levels of interleukin (IL)-4 but low levels of interferon (IFN)-γ and IL-2 after T-cell receptor stimulation (Adkins et al., 1993; Adkins & Hamilton, 1992). This is most likely to be due to suboptimal stimulation by dendritic cells (DCs), as well as to low IL-12 production by DCs (Goriely et al., 2004). Differences between adult and neonatal immune responses are further exaggerated in the mucosal environment of the lung. While the T cells in the neonatal lungs are able to overcome the suppressed T helper cell type 1 (Th1) response when removed from the lungs, the neonatal lung environment is not capable of supporting an inflammatory response (Garvy & Qureshi, 2000). It is possible that the differences in neonatal immune responses affect viral infection, especially in the mucosal environment.

In this study, we compared γHV-68 infection of neonatal mice to infection of adult mice. We found that mice infected as pups (8 days old) had disseminated acute infection and high-level long-term persistence of the virus from the lungs. In addition, pups did not experience the same degree of transient splenomegaly and expansion of Vβ4+CD8+ T cells associated with the IM-like syndrome seen in adult mice. This suggests that age of infection influences γHV-68 pathogenesis.

**METHODS**

**Cell lines and virus preparation.** All cell culture reagents were purchased from Cellgro (Mediatech). Murine embryonic fibroblast (MEF) cells were grown in Dulbecco’s modified Eagle's medium (DMEM) supplemented with 4.5 g glucose l−1, 1 mM sodium pyruvate, 10% fetal bovine serum (FBS; Mediatech), 2 mM L-glutamine, 100 U penicillin ml−1 and 100 μg streptomycin ml−1. Owl monkey kidney (OMK) cells were grown in RPMI supplemented with 10% FBS, L-glutamine, penicillin and streptomycin. γHV-68 virus stocks were grown as described elsewhere (Cardin et al., 1996).

**Mice and infections.** BALB/c mice were purchased from the Jackson Laboratories and bred in-house under specific-pathogen-free conditions. Earlier studies have suggested a dose-dependent mortality following infection of neonatal mice with γHV-68 (Rajcani et al., 1985). To determine at what age and with what dose to infect mice, BALB/c pups were infected intranasally at 1, 4, 8 and 12 days old with varying doses (40, 400 or 4000 p.f.u.). Pups were infected with a final volume of 5 μl virus diluted in Hanks’ balanced salt solution (HBSS; Mediatech). All mice infected at 8 and 12 days survived infection with all doses tested. The final age and dose for all subsequent experiments with pups was 8 days old and 400 p.f.u. Adult mice were infected when 6–8 weeks old with 4 × 10^6 p.f.u. with a final volume of 50 μl. For all experiments, at least four mice were used per group.

**Tissue preparation.** Tissues were removed from mice and either processed immediately or frozen at −80 °C. Mouse lungs processed immediately were perfused with 10 ml HBSS containing 10 mM EDTA before removal. Lung tissues were then processed into single-cell suspensions as described elsewhere (Garvy & Qureshi, 2000). Briefly, lungs were minced and digested in RPMI supplemented with 5% FBS, 2 mg collagenase D ml−1 and 20 μg DNase I ml−1 for 90 min at 37 °C with constant rocking. Cells were then filtered through a 70 μm cell strainer and red blood cells were lysed in a hypotonic solution. Cells were resuspended in 2 ml HBSS and overlaid onto 3 ml Ficol-Paque PLUS (Amersham Biosciences), then centrifuged at 800 g for 20 min at room temperature. Cells were harvested from the interface and resuspended in DMEM containing 2.5% FBS, L-glutamine, penicillin and streptomycin for limiting dilution assay (LDA). Spleen cells were isolated as described elsewhere and resuspended in PBS containing 0.3% BSA (Fisher Scientific) and 10 mM HEPES buffer (Mediatech) for flow cytometry (Weck et al., 1996).

**Viral titres of infected tissues.** MEFs were seeded in 2 ml volumes in 12-well plates at a concentration of 1 × 10^5 cells ml−1 in DMEM. Cells were allowed to adhere overnight. Tissue samples were thawed and weighed, then homogenized in 2 ml complete DMEM. Homogenates were diluted 1:10 for the highest concentration and 1:10 serial dilutions were made. Remaining homogenates were stored at −80 °C. For the plaque assay, supernatants were aspirated off MEF monolayers and homogenate dilutions were plated in 1 ml complete DMEM. Plates were incubated for 2 h at 37 °C, and the plaque assay procedure was continued as described elsewhere (Weinberg et al., 2004). Titres are expressed as p.f.u. g−1 tissue.

**DNA extraction and real-time quantitative PCR.** All DNA extraction procedures were done from homogenized tissue lysates using the QIAamp DNA Mini kit (Qiagen). Real-time quantitative (Q)-PCR was performed using primers and probe against γHV-68 viral DNA as described elsewhere (Weinberg et al., 2004). Mouse β-actin primers and probes have been described elsewhere (Schuetze et al., 2005). Reactions were performed using iQ Supermix (Bio-Rad Laboratories) according to the manufacturer’s protocol.

**LDA.** MEFs were seeded in 200 μl volumes in 96-well plates at a concentration of 5 × 10^3 cells ml−1 in DMEM containing 2.5% FBS (Invitrogen), L-glutamine, penicillin and streptomycin. Lung cells were resuspended at a concentration of 10^6 cells ml−1, and LDA was performed as described elsewhere (van Dyk et al., 2000; Weck et al., 1996) with the exception that threefold serial dilutions were made. To determine the levels of preformed lytic virus, a duplicate aliquot of cells was subjected to three rounds of freeze-thawing and lysates were plated in 100 μl volumes onto MEF monolayers. The percentage of wells exhibiting cytopathic effect after 21 days was calculated.

**RNA extraction and RNase protection assay (RPA).** RNA was extracted from whole tissue as described elsewhere (Chomczynski & Sacchi, 1987; Rochford et al., 2001). Viral gene expression was determined by using a riboprobe recognizing both lytic (K3, Rta, M8, DNA polymerase and gB) and latent (M2, M3, M9, M11, ORF73 and ORF74) γHV-68 genes (Rochford et al., 2001). The RPA and quantification were done as described elsewhere (Hobbs et al., 1993; Rochford et al., 2001).

**Flow cytometry.** Single-cell suspensions were prepared as described above, and all reagents and methods have been described elsewhere (Hobbs et al., 1993), with the exception that cells were first blocked using Fc/III/II receptor (BD Biosciences) on ice. Anti-CD8 and -Vp/V4 monoclonal antibodies were obtained from BD Biosciences. Cells were collected on an LSRII flow cytometer (Becton Dickinson) and live cells were analysed by gating on forward-side scatter characteristics. Data were acquired using FACS DIVA software (BD Biosciences) and analysed using FlowJo software (Tree Star).
RESULTS

γHV-68 pathogenesis differs at acute and latent stages following infection of pups and adult mice

Intranasal infection of adult mice with γHV-68 can be broadly divided into three stages: acute infection in the lung, early latent infection in the spleen and long-term latent infection in the spleen (Tibbetts et al., 2003). However, it is unknown whether infection of very young mice affects γHV-68 pathogenesis. To answer this question, we infected pups (8 days old) and adult mice (6–8 weeks old) and determined the viral load in the lungs and spleen at time points representative of acute (6 days p.i.), early latent (15 days p.i.) and long-term latent (30 days p.i.) infection. DNA was extracted from lung and spleen tissue and Q-PCR was used to measure viral load (Fig. 1). When we examined viral load in lungs at 6 days p.i., we observed no significant difference between infected pups and adults, suggesting that acute infection of lungs was age-dependent. However, at both 15 and 30 days p.i., viral DNA was readily detected in lungs of mice infected as pups but not in the lungs of mice infected as adults.

We next examined viral load in the spleen. Following infection of adult mice, no viral load was detected in the spleen at 6 days p.i. but by 15 days p.i., a high viral load was observed (>1 x 10^6 copies per 100 ng DNA) (Fig. 1). By 30 days p.i., the viral load had decreased by over 1 log but was still almost 1 x 10^6 copies per 100 ng DNA. This pattern of viral DNA detection in the spleens of mice infected as adults was similar to previous observations (Sunil-Chandra et al., 1992b; Tripp et al., 1997; Usherwood et al., 1996). In contrast, following infection of pups, viral load was readily detected in the spleens at 6 days p.i. Viral loads dropped at 15 days p.i. and were not significantly different between 15 and 30 days p.i.

Dissemination of γHV-68 following infection of pups

The early detection of viral DNA in the spleens of pups at 6 days p.i. suggested that viral infection might not be restricted to the lungs during the acute phase as reported previously following infection of adult mice (Cardin et al., 1996; Ehtisham et al., 1993). To test whether γHV-68 could establish a disseminated infection in pups, we infected pups and adult mice and harvested various tissues at 6 and 30 days p.i. Tissues were homogenized, DNA was extracted from total tissue lysates and viral load was determined by Q-PCR. We detected high levels of viral DNA in liver, heart, mediastinal lymph node (MLN), spleen and lung in pups at 6 days p.i. (Table 1). In contrast, viral DNA was only detected in the lung and MLN of adults at 6 days p.i. By 30 days p.i., viral DNA was detected in MLN, spleen, lung, kidneys and adrenal glands in mice infected as pups. Viral DNA was only detected in MLN and spleen in adults at 30 days p.i. These data suggest that infection of pups led to a more disseminated viral infection.

Pups exhibit delayed clearance of infectious virus from the lungs following acute infection

Because we observed a high viral load in the lungs of pups at 15 and 30 days p.i. (Fig. 1), we wanted to examine the kinetics of infection more closely and determine if infectious virus was detected at later time points than is typically observed following infection of adult mice. To this end, pups were infected with γHV-68 and the viral load in lungs was determined every 3 days from 6 to 30 days p.i. by Q-PCR. The viral load peaked at 9 days p.i. (Fig. 2a) and began to decline by 12 days p.i. The levels remained fairly constant from 15 to 30 days p.i. We next determined whether infectious virus could be detected in the lungs of pups at selected times p.i. Plaque assays were performed on lung homogenates and the results are shown in Fig. 2(b). We observed infectious virus in the lungs from 3 to 15 days p.i., although by 12 days p.i. infectious virus was not detected in all infected mice. By 18 days p.i. no infectious virus was detected in the lungs of infected pups. This suggests that the viral load observed from 18 to 30 days p.i. represents a high-level latent infection in the lungs of pups.
Latent virus in the lungs persists in infected pups but not adults

Since we observed a high viral load maintained in the lungs of pups infected with \(\gamma\text{HV}-68\), but were unable to detect infectious virus, we wanted to confirm that the elevated viral load was due to a latent infection. To do this, pups and adults were infected with \(\gamma\text{HV}-68\) and at 30 days p.i., single-cell suspensions of lungs were generated by collagenase digestion. LDA was done to detect the frequency of latently infected cells that could reactivate. Because the LDA is more sensitive than a plaque assay in detecting infectious virus, cell lysates were also prepared to determine whether any preformed virus could be detected (Weck et al., 1996). A low frequency of latently infected cells was found in the lungs of mice infected as pups, but no latently infected cells were detected in adult mice (Fig. 3a). No preformed virus was detected in lungs of pups or adults at 30 days p.i.

We next compared the pattern of viral gene expression in lungs of mice infected at both ages at 30 days p.i. (Fig. 3b). While no viral gene expression was detected in adult mice at 30 days p.i., three viral genes associated with latent infection, M3, M9 and M2, were expressed in the lungs of pups at 30 days p.i. To determine if the virus was eventually cleared, we also examined viral gene expression in lungs of mice infected as pups at 60 days p.i. (Fig. 3b). Interestingly, M2, M3 and M9 transcripts were still readily detectable and the levels of transcripts were similar between 30 and 60 days p.i. Q-PCR was used to detect viral DNA in the lungs of pups at 60 days p.i. and no significant difference was found compared to 30 days p.i. (data not shown). These data suggest that virus was maintained in a latent state in lungs of mice infected as pups.

Pups do not get IM-like syndrome

We observed that infected pups did not have a peak in viral load in the spleen at 15 days p.i., which is a characteristic of an IM-like syndrome that occurs following infection of adult mice (Fig. 1) (Tripp et al., 1997). As we only examined pups at 15 days p.i., we wanted to determine if there was a difference in the kinetics of infection that might result in a delay in the peak of viral load in the spleen following infection of pups. Pups were infected with \(\gamma\text{HV}-68\) and spleens were harvested every 3 days from 6 to 30 days p.i., DNA was extracted from homogenates and viral load was determined by Q-PCR. Viral DNA was

Table 1. Dissemination of \(\gamma\text{HV}-68\) in pups and adults

<table>
<thead>
<tr>
<th>Study group</th>
<th>Salivary gland</th>
<th>Lung</th>
<th>MLN*</th>
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*Mediastinal lymph node.

Fig. 2. Infected pups do not clear \(\gamma\text{HV}-68\) from the lungs. Eight-day-old mice were infected with 400 p.f.u. \(\gamma\text{HV}-68\). Every 3 days a group of mice was sacrificed, the lungs were weighed and lysates were made by using a tissue homogenizer. DNA was extracted from total lysates and viral load was measured by Q-PCR (a), while lytic virus was measured by plaque assay (b). Each group consisted of 3–4 mice. Viral load is represented as log viral copy number per 100 ng DNA. Lytic virus is measured as log p.f.u. g\(^{-1}\) tissue. Each point represents a single mouse, and each bar represents the mean for that day.
detected at all time points in mice infected as pups (Fig. 4a). The level of viral DNA in the spleen was fairly constant between time points, indicating that there was not a later peak in viral load.

Two other hallmarks of the IM-like syndrome in infected adult mice are splenomegaly and expansion of Vβ4+ CD8+ T cells (Tripp et al., 1997; Usherwood et al., 1996). Therefore, we examined whether these characteristic features were also observed in infected pups. To test whether Vβ4+ CD8+ T cells were expanded in infected pups, single-cell suspensions were isolated from spleens of pups and adults at 16 days p.i. and age-matched mock-infected controls. Cells were stained with phycoerythrin-conjugated anti-CD8 mAb and fluorescein isothiocyanate-conjugated anti-Vβ4 mAb and analysed by flow cytometry. We measured Vβ4+ CD8+ T-cell expansion at 16, 21, 28 and 42 days p.i. (Fig. 4b). While we did find a significant expansion of this cell population in adult mice by flow cytometry at 16 (P<0.01) and 21 days p.i. (P<0.05), no significant expansion was observed in the spleens of pups at any time point.

The other characteristic of the IM-like syndrome is an increase in weight of the spleen (Tripp et al., 1997). Pups and adults were infected with γHV-68 and splenic weights were measured at 16 days p.i. We found that infected pups do not display the same degree of splenomegaly at 16 days p.i. as adult mice. Infected adult mice at 16 days p.i.
spleens with a weight 2.3-fold greater than spleens of age-
matched mock-infected mice, whereas infected pups have 
spleens that are only 1.5-fold greater in weight than those 
of mock-infected pups (Fig. 4c). To ensure that we were 
not missing the peak in splenomegaly, we also looked at 
splenic weights in pups at 14, 15 and 18 days p.i. and age-
matched uninfected controls, and found that the slight 
increase in splenic weight at 16 days p.i. was the maximum 
crease (data not shown).

DISCUSSION

Age-dependent differences in the pathogenesis of many 
viruses have been widely reported (Imperial, 1999; 
Piedimonte, 2002), although it is likely that the underlying 
mechanisms are different. In this study, we observed that, 
in contrast to infection of adult mice, there was 
disseminated acute infection, a delayed clearance of 
fibers from the lungs, an extended persistence in 
the lungs and no significant IM-like syndrome following 
fection of 8 day old pups with γHV-68. Thus, the 
pathogenesis of the murine gammaherpesvirus γHV-68 is 
also age-dependent.

One of the striking features we observed was the detection 
of latent infection in the lungs of mice infected as pups 
through to 60 days p.i. as well as the expression of 
potentially immunogenic viral proteins, M2, M3 and M9. 
In contrast, we were unable to detect any viral persistence 
in the lungs of adult mice during by 30 days p.i. While 
others have reported very low levels of viral DNA in the 
lungs (Flano et al., 2003), we did not detect any virus by Q-
PCR, LDA plaque assay or RPA. Therefore, any persistence 
in the lungs of adult mice was below the level of detection 
in our assays and compared to the readily detectable viral 
load in the lungs of mice infected as pups suggests that 
there are differences between adults and pups in their 
ability to control latent infection in a mucosal envir-

Long-term latency in the spleen was unaffected by age of 
fection with γHV-68. Interestingly, dose and route of 
fection, whether intranasal or intraperitoneal, also do not 
ffect the splenic latency (Tibbetts et al., 2003). This 
suggests that regulation of systemic (splenic) latency is 
different from the regulation of mucosal latency. Of 
interest is which system, mucosal or systemic, is more 
relevant to long-term persistence of the virus and the 
ability of the virus to transmit between hosts.

While no differences were observed in long-term latency in 
the spleen between mice infected as pups or as adults, we 
found that early latency was different between pups and 
adults, as evidenced by the decrease in IM-like symptoms 
such as splenomegaly and Vβ4+ CD8+ T cells. This is 
similar to the differences in EBV pathogenesis, in which IM 
is rarely observed during primary infection of children but 
is a consistent feature of disease following infection of 
adolescents and young adults (Rickinson & Kieff, 2001). 
Indeed, the age of infection seems to be critical in the 
development of IM-like symptoms, as infection of 3–4-
week-old mice with γHV-68 does lead to splenomegaly 
(Sunil-Chandra et al., 1992a).

Infection during the neonatal period leads to chronic 
persistence in the lungs of mice long after adult mice are 
able to clear the virus. This model represents another 
example of how infection with a virus at a young age can 
lead to a chronic condition. Respiratory syncytial virus 
infection, when first acquired before 1 year of age, 
increases the likelihood of developing long-term airway 
disease (Pullan & Hey, 1982). Other infections that occur 
during childhood that are associated with chronic infec-
tion, such as hepatitis viruses (Cote et al., 2000) and 
cytomegalovirus (Tu et al., 2004) infections, are due to 
differences in the infant and adult immune systems. The 
woodchuck model of hepatitis virus has been used to 
demonstrate that neonatal infection leads to chronic 
persistence, due in part to a decrease in the T-cell response 
along with a decrease in Th1-associated cytokines (Menne 
et al., 2002; Nakamura et al., 2001; Wang et al., 2003). 
Similarly, children infected with cytomegalovirus, a 
betaherpesvirus, exhibit a decreased CD4+ T-cell response 
and decreased IFN-γ production (Tu et al., 2004). Whether 
fection of pups with γHV-68 leads to the same depressed 
response of T cells and cytokines compared to infected 
adult mice is under investigation.

In developing countries, most children are infected with 
EBV by 1 year of age (Biggar et al., 1978) and yet most of 
the studies of EBV pathogenesis are based on cells isolated 
from asymptomatic adults or adults with IM (reviewed by 
Callan, 2003). Our observation that long-term persistence of 
a murine gammaherpesvirus is age-dependent, as is the 
development of an IM-like syndrome, suggests that a 
greater understanding of persistence of gammaherpes-

ves in children is needed. Indeed, we have reported the 
ready detection of EBV DNA and high viral loads in 
peripheral blood in children under the age of four living in
Kenya (Moormann et al., 2005). Whether early age of infection leads to higher viral persistence in mucosal sites in children remains to be determined, but it is relevant to note that endemic Burkitt’s lymphoma only affects children and that the presentation of tumours is primarily in the jaw (de-The, 1977). Early age of infection has also been hypothesized to be a risk factor for development of endemic Burkitt’s lymphoma (de-The, 1977). The results presented here suggest that γHV-68 infection of young mice can be used as a model to study age-dependent persistence of gammaherpesvirus infection at mucosal sites.

ACKNOWLEDGEMENTS

The authors thank Stanley Brunache and Nancy Fiore for excellent technical assistance and Dr Romana Hochreiter for technical guidance. This work was supported in part by an award from the Hendrick’s Foundation, State University of New York (SUNY) Upstate Medical University. This work was presented in part at the International Herpesvirus Workshop in Asheville, NC in July 2007.

REFERENCES


enteritidis and the impact of IL-10 in bone marrow-derived macrophages. *Int Immunol* 17, 649–659.


