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Importance of Antibody in Virus Infection and Vaccine-Mediated Protection by a Latency-Deficient Recombinant Murine γ-Herpesvirus-68

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The human γ-herpesviruses EBV and Kaposi’s sarcoma-associated herpesvirus establish lifelong latent infections, can reactivate in immunocompromised individuals, and are associated with the development of malignancies. Murine γ-herpesvirus-68 (γHV68), a rodent pathogen related to EBV and Kaposi’s sarcoma-associated herpesvirus, provides an important model to dissect mechanisms of immune control and investigate vaccine strategies. Infection of mice with γHV68 elicits robust antiviral immunity, and long-term protection from wild-type γHV68 infection that lasts for at least 10 mo. In this report, we examine the immune correlates of AC-RTA-mediated protection and show that sterilizing immunity requires both T cells and Ab. Importantly, Ab was also critical for mitigating viral infection in the brain, and in the absence of Ab-mediated control, amplification of the AC-RTA virus in the brain resulted in fatality. Our results highlight important considerations in the development of vaccination strategies based on live-attenuated viruses. The Journal of Immunology, 2012, 188: 000–000.

The human γ-herpesviruses EBV and Kaposi’s sarcoma-associated herpesvirus (KSHV) are widely disseminated pathogens that establish lifelong latent infections requiring constant immune surveillance. Latent infection is largely benign in the majority of infected individuals, and it has been demonstrated that γ herpesvirus latency can be beneficial to the host by enhancing resistance to bacterial infections (1). However, the viruses have oncogenic potential, and infection is associated with the development of malignancies in immunosuppressed individuals and specific high-risk groups. For example, posttransplant lymphoproliferative diseases are a well-known consequence of EBV in immunosuppressed transplant recipients, and both KSHV and EBV cause a high rate of malignancy in AIDS patients. In addition, regions of Southeast Asia have a 20-fold higher than normal incidence of EBV-associated nasopharyngeal carcinoma, and EBV is associated with a high frequency of Burkitt’s lymphoma in areas of holoendemic malaria in equatorial Africa (2, 3). Thus, the development of vaccines capable of preventing the establishment of γ-herpesvirus latency will lower the risk of associated malignancies. Because of the species specificity of the γ-herpesviruses, EBV and KSHV cannot be directly used in a mouse model. However, the mouse γ-herpesvirus-68 (γHV68) is highly homologous to EBV and KSHV, and mechanisms of immune control are similar (4–6). Importantly, γHV68 provides a natural in vivo infection model in which host immune evasion mechanisms are preserved.

Many important principles have been established by vaccination studies using the mouse γHV68 model. Early vaccination strategies that focused on subunit and inactivated viral vaccines met with limited success. For example, similar to results in human trials (7), strategies targeting γHV68 gp150 (the homolog of EBV gp350) were shown to lessen infectious mononucleosis-like symptoms, but failed to impact the establishment of long-term latency (8). In addition, dominant lytic and latent epitopes that have been identified in the mouse model were used in targeted vaccination strategies. Although vaccination with well-characterized lytic T cell epitopes reduced the extent of lytic infection upon challenge, this strategy failed to prevent the establishment of latency and did not substantially lower the long-term latent viral load (9, 10). This is consistent with accumulating data showing that latency can be established independent of the lytic infection (11–14), which explains why targeting the lytic phase of infection does not significantly impact the establishment of latency. Subunit vaccines that directly target the establishment of latency were also unsuccessful, as vaccination with a defined latency epitope yielded only transient protection (15, 16). Recent vaccination studies have shown only partial protection with live attenuated mutant viruses incapable of replication or reactivation (14, 17–19). Because of the oncogenic nature of the γ-herpesviruses, engineered viruses that are incapable of establishing latency hold the greatest promise for successful and safe vaccine candidates (20–22).

We have recently generated a live-attenuated virus, termed AC-replication and transcription activator (RTA), that fails to establish latency and elicits strong and long-lasting protection against infection with wild-type (WT) virus (23). A dual strategy was used to generate AC-RTA. First, the γHV68 genome was modified to allow...
constitutive overexpression of the viral RTA, which drives the virus preferentially into the lytic cycle. Second, viral sequences necessary for the establishment of latency and oncogenicity were deleted, including ORF73 (a latency-associated nuclear Ag, LANA, homolog), ORF72 (v-cyclin), and M11 (a viral bcl-2 homolog). AC-RTA exhibits enhanced lytic replication demonstrated by larger plaque size in vitro, higher peak viral titers in vivo, and faster clearance from immunocompetent mice (23). We previously showed that infection of BALB/c mice with AC-RTA generated a strong lytic infection in the lung that was efficiently cleared by the immune system. Although the virus failed to establish latency, strong protective immunity was generated that prevented subsequent infection with WT virus (23); both virus-specific T cells and Abs were induced (23, 24). In the current studies, we examined the vaccination efficacy of AC-RTA in C57BL/6 mice and determined the immune correlates of protection. Consistent with the finding that both humoral and cellular immunity are required to control latent γHV68 infection, we found that both Ab and T cells contribute to protective immunity induced by AC-RTA. Unexpectedly, however, we found that Ab-deficient mice failed to control AC-RTA infection and died with high viral titers in the brain.

Materials and Methods

Mice and virus infections

Female 6- to 12-wk-old C57BL/6, activation-induced cytidine deaminase (AID)/−/−, secretory μ-chain (μs)/−/−, AID/μs double-knockout mice (AID/−/−; μs/−/−), B6.129S2-H2Ab1−/− (H2 class-deficient [μMT]), and B6.129S-H2Ab1−/−MHC class II−/− mice were obtained from the Trudeau Institute animal facility and maintained in specific-pathogen-free conditions. Mice were infected intranasally (i.n.) with 400 PFU or i.p. with 104 PFU γHV68 (strain WUMS) or AC-RTA (23). All animal studies were approved by the Institutional Animal Care and Use Committee of the Trudeau Institute.

Plaque assays

Plaque assays were performed as described previously (25). In short, spleens, lungs, and brains were homogenized, serially diluted, and plated on NIH3T3 cell monolayers for 1.5 h. Homogenates were then removed and overlaid with 0.75% carboxymethylcellulose in media. Plates were incubated for 6 d at 37°C, 10% CO2. Cells were then fixed with methanol, stained with Giemsa violet stain, and plaques counted.

Flow cytometry

Splenocytes were treated with Fc Block, then stained with Ab against CD8α (BD Biosciences) and allophycocyanin-conjugated MHC class I tetramers specific for γHV68 epitopes H-2Dp/RF6s2-6 (AGPH2DM2EI) or H-2Kb/ ORF61524-531 (TSINFVKI) (obtained from the Trudeau Institute Molecular Biology Core Facility). Samples were collected on a BD FACSCanto II cytometer (BD Biosciences) and analyzed using FlowJo software (Tree Star).

ELISA

ELISAs for virus-specific IgG were performed as described previously (26). In short, plates were coated with γHV68 virions (0.5 μg/well), incubated with 4-fold serially diluted sera from experimental or control mice, and then incubated with appropriate alkaline phosphatase-conjugated goat anti-mouse Ab (Southern Biotechnology Associates). Plates were then washed and incubated with p-nitrophenyl phosphate substrate. OD was then measured at 405 nm; OD values higher than twice the naive serum samples were considered a positive reaction.

Serum transfers

Serum was harvested from whole blood collected from mice at 1 or 4 mo after γHV68 infection or AC-RTA vaccination. To test the protective efficacy of immune serum, naive mice were treated i.p. with 250 μl serum 1 d prior to infection with γHV68. Lungs were harvested at 3 and 7 d postinfection (p.i.) and analyzed by plaque assays.

In vitro neutralizing Ab assay

The neutralizing activity of serum Ab was assessed in vitro as described previously, with slight modifications (27). Briefly, heat-inactivated serum samples were serially diluted 2-fold in media and mixed 1:1 with 103 PFU/ml γHV68 for 1.5 h at 37°C, then overlaid on NIH3T3 cell monolayers for plaque assays.

In vivo T cell depletion with Ab

T cell subsets were depleted from naive or AC-RTA-vaccinated mice by treatment with mAbs to CD4 (GK1.5), CD8 (2.43), or anti-Thy1.2 (30H12) (BioXCell). For each group, 250 μg Ab was administered i.p. every 2 to 3 d beginning 2 d prior to γHV68 challenge. Lungs and spleens were harvested at 7 d p.i. and analyzed by plaque assays.

Quantitative real-time PCR

DNA was isolated from homogenized brain tissue using the DNeasy Mini kit (Qiagen) and quantified with a Nanodrop ND-1000 spectrophotometer (Thermo Fisher Scientific). The copy number of the γHV68 ORF50 gene in 200 ng DNA was determined by quantitative real-time PCR essentially as described (16), using a standard curve quantitation method on an Applied Biosystems 7500 Real-Time PCR System (Applied Biosystems), using Taqman Gene Expression master mix (Applied Biosystems). Primers, probes, and reaction cycles used are as described in Usherwood et al. (16).

Statistical analysis

Data were analyzed using the Student t test, one-way ANOVA, or log-rank (Mantel–Cox) test for survival curves. All analyses were performed using Prism 5 software (GraphPad).

Results

AC-RTA confers long-lasting protection following vaccination

Our previous study examined protection from WT γHV68 challenge in BALB/c mice up to 3 mo after vaccination with AC-RTA (23). To determine if AC-RTA vaccination induced long-lasting protection in C57BL/6 mice, we i.n. vaccinated mice with AC-RTA, challenged them i.n. with WT γHV68 either 1 or 6 mo postvaccination, and examined viral titers in the lungs 7 d after challenge. As shown in Fig. 1A, AC-RTA vaccination of C57BL/6 mice was highly protective against WT virus challenge, with protection lasting for at least 6 mo.

We next examined the cellular and humoral immunity elicited by AC-RTA vaccination compared with WT virus infection (Fig. 1B). We have previously examined virus-specific CD8 T cell responses in the spleen p.i. with AC-RTA and WT virus. As we previously reported, numbers of tetramer-positive cells for two different epitopes, ORF6487/Db (ORF6) and ORF61524/Kb (ORF61), were comparable 12 d p.i. with either virus, and 1 mo p.i., ORF61-specific cells, but not ORF6-specific cells, were reduced in the absence of latency amplification (24). In this study, we examined tetramer levels 7 mo after WT infection or AC-RTA vaccination (Fig. 1B). Although ORF61-specific cells are normally maintained long-term at a higher level than ORF6-specific cells after WT virus infection, in the absence of latency, both pools of epitope-specific T cells were maintained comparably. This shift in immunodominance is similar to a previous report using a different latency-deficient recombinant virus (28) and implicates latency or reactivation from latency in the preferential maintenance of ORF61-specific T cells.

We have previously shown that the levels of virus-specific IgG elicited by WT virus infection of C57BL/6 mice are maintained throughout the long-term (22 mo), but neutralizing titers progressively decline (29). Therefore, we examined virus-specific Ab responses and found that both WT virus and AC-RTA vaccination elicited robust class-switched Ab responses that were sustained as long as 6 mo p.i. as measured by ELISA, although AC-RTA vaccination elicited lower Ab titers than WT γHV68 infection (Fig. 1C).

Together, the data show that vaccination with AC-RTA provides long-lasting protection from WT viral challenge in C57BL/6 mice and induces sustained cellular and humoral immunity.
Both virus-specific T cells and Ab contribute to protection

To determine the relative roles of antiviral CD4 and CD8 T cells in AC-RTA vaccine-mediated protection, mice were depleted of CD4 T cells, CD8 T cells, or both by mAb administration 30 d after vaccination with AC-RTA, beginning 2 d prior to WT virus challenge. Protection was assessed by monitoring the presence of WT γHV68 in the lung 7 d after challenge (Fig. 2A). Depletion of T cells resulted in reduced vaccine-mediated protection, with indications of a greater role for CD4 T cells than CD8 T cells (two out of five fully protected after anti-CD4 treatment versus four out of five fully protected after anti-CD8 treatment), although there was variation between individual mice. However, even total T cell depletion did not completely abrogate protection, suggesting a role for Ab. Challenge of mice depleted of T cells 4 or 8 mo after AC-RTA vaccination again resulted in partial loss of protection (Fig. 2B), consistent with a role for both T cells and Ab in long-term protection.

Because Ab was playing a role in protection, we addressed the contribution of antiviral Ab in two ways. First, we passively transferred WT- or AC-RTA–immune serum into naive mice, then challenged with WT virus (Fig. 3A). Sera from both WT- or AC-RTA–infected mice were protective compared with naive serum. A previous report has shown that the class-switched and neutralizing Ab response to γHV68 rises rapidly the first 3 wk p.i. and continues to increase over several months (27). Consistent with that report, we found that serum from WT-immune mice 4 mo p.i. provided greater protection than serum from WT-immune mice 30 d p.i., especially when protection was measured 7 d after challenge. AC-RTA–immune serum did not exhibit the same increase in protective efficacy over time after vaccination: AC-RTA–immune serum 4 mo p.i. elicited significantly less protection 7 d after challenge than WT-immune serum from mice 4 mo p.i. (p = 0.0415, Student t test), consistent with the lower overall antiviral IgG after AC-RTA vaccination compared with WT γHV68 infection (Fig. 1C). Second, we compared the ability of serum obtained from WT- and AC-RTA–infected mice 4 mo p.i. to neutralize virus in an in vitro assay (Fig. 3B). At each dilution, serum from WT-immune mice 4 mo p.i. had more neutralizing activity than serum from AC-RTA–vaccinated mice, although both sera neutralized γHV68 more effectively than naive serum. The enhanced protective efficacy of serum from mice infected with WT virus raises the possibility that the presence of latent virus drives maturation of the neutralizing response. The possibility that the Ab-specific response generated after vaccination by vectors that do not induce latency is deficient may have important implications for vaccination efficacy in cases of declining T cell function.

Taken together, our data show that T cells and Ab are both influenced by the presence of latent virus. Importantly, each contributes to the long-lasting protection afforded by AC-RTA vaccination.

Ab-deficient mice succumb to AC-RTA vaccination

To further investigate the role for Ab in AC-RTA vaccine-mediated protection in vivo, we infected Ab-deficient AID−/− mice with γHV68.
AC-RTA (30, 31). These mice are deficient in class-switching, somatic hypermutation, and IgM secretion, thereby lacking circulating Abs while still retaining expression of Ig on the B cell surface. These mice maintain a complete B cell compartment, with the exception of plasma cells (30). Because B cells are the major reservoir of latent virus (32, 33), these mice are excellent models for assessing the importance of humoral immunity in AC-RTA–mediated protection. To this end, C57BL/6 and AID−/− mice were infected with WT and AC-RTA. Unexpectedly, at ~3 wk, the AID−/− mice succumbed to AC-RTA infection (Fig. 4A). To determine if lethality was a characteristic unique to this particular strain of mouse, we infected two additional strains of Ab-deficient mice, MHC class II−/− mice (Fig. 4B) and μMT mice (Fig. 4C). All three strains succumbed to AC-RTA infection with similar kinetics, confirming a requirement for Ab in survival following AC-RTA infection.

Because AID−/− mice cannot undergo class switching or secrete IgM, we were unsure whether circulating IgM could prolong survival of Ab-deficient mice after AC-RTA infection. We infected AID−/− mice, which lack class-switched Ab but can still secrete IgM (34), and μMT mice, which cannot secrete IgM, but can still generate and secrete other Ab classes (35), with AC-RTA. AID−/− mice succumbed to AC-RTA infection with similar kinetics as above (Fig. 5A), whereas μMT mice survived infection (Fig. 5B). These data show that circulating IgM is not sufficient to allow survival of AC-RTA–infected mice lacking class-switched Abs.

AC-RTA is biased toward lytic rather than latent infection because it has an ectopic copy of RTA driven by a strong hybrid promoter (23). Consistent with enhanced lytic replication, AC-RTA generates larger plaques and enhanced viral titers in multiple-step growth experiments in vitro (23). In addition, we have previously shown that AC-RTA infection of C57BL/6 mice results in higher and more rapid titers in the lung compared with WT infection (24). Therefore, we asked whether elevated AC-RTA titers could account for the death of AID−/− mice. Whereas C57BL/6 mice clear both WT and AC-RTA virus by day 10 after i.n. infection (24), analysis of viral clearance in the lungs of AID−/− mice showed impaired clearance of both viruses in the absence of Ab. Interestingly, AC-RTA infection was cleared with faster kinetics than WT virus in AID−/− mice (Fig. 6A), ruling out uncontrolled virus replication in the lungs as a cause of death in the mice. Mice that succumbed to AC-RTA infection were moribund, exhibiting severe weight loss, ataxia, and hind-limb paralysis, consistent with the mice undergoing encephalitis. Therefore, we measured the lytic viral load in the brains of C57BL/6 and AID−/− mice following infection with WT γHV68 or AC-RTA (Fig. 6B). Remarkably, high titers of lytic virus were found in all the brains of AID−/− mice on days 15 and 21 post-AC-RTA infection, whereas only one out of nine WT γHV68-infected AID−/− mice had a detectable, but still very low, amount of virus. At no time did we detect lytic WT γHV68 in the brains of C57BL/6 mice. In Ab-deficient mice, WT γHV68 infection produced high levels of viral DNA in the brain, indicating that Ab plays a major role in preventing viral spread to the brain, but the virus in the brain is mostly maintained in a latent state (Fig. 6C). Regardless of Ab, AC-RTA infection led to significant levels of viral DNA in the brain, consistent with the highly lytic nature of
AC-RTA; once AC-RTA reaches the brain, it undergoes lytic replication that is mitigated by Ab. Taken together, in both WT γHV68 and AC-RTA infections, Ab is essential for controlling viral invasion of the brain, and the importance of this Ab-mediated control is heightened by highly lytic AC-RTA infection, which causes Ab-deficient mice to die.

We next examined whether the infiltration and expansion of AC-RTA in the brain of AID−/−μs−/− mice was a consequence of the route of inoculation. It has been shown that following i.n. administered AC-RTA that a high concentration of virus can be detected at the site of inoculation (23), raising the possibility that this facilitates localization to and infection of the brain. To address this, we i.p. infected AID−/−μs−/− mice (Fig. 7A) or MHC class II−/− mice (Fig. 7B) with AC-RTA and measured survival. AID−/−μs−/− mice infected with AC-RTA i.p. succumbed to infection with faster kinetics than i.n. infected mice (compare Fig. 7A with Fig. 4A; median survival: i.p. 15 d, i.n. 21 d; p = 0.0139), whereas MHC class II−/− mice succumbed with similar kinetics (compare Fig. 7B with Fig. 4B; median survival: i.p. 25 d, i.n. 23.5 d; p = 0.6177). In either case, AC-RTA resulted in death in both strains following i.p. infection, conclusively demonstrating that AC-RTA is lethal to Ab-deficient mice regardless of the route of infection.

We next measured lytic viral titers in the lungs, spleens, and brains of AID−/−μs−/− mice following i.n. or i.p. infection with AC-RTA to determine if i.p. infection resulted in increased viral loads in the brain (Fig. 7C). Interestingly, i.p. infection led to accelerated dissemination to the spleen compared with i.n. infection at 7 d p.i., but lytic virus could not be detected in the brain until 14 d p.i. The reasons for the delayed kinetics of virus in the brain compared with the spleen remain unclear.

Taken together, our data emphasize the importance of Ab responses in a recombinant viral vaccine strategy. In immunocompetent animals, AC-RTA vaccination leads to long-lasting...
lead to infection in the brains of immunocompromised mice, including neonatal BALB/c mice (38) and adult mice deficient in type I IFN receptor (36). These observations are also consistent with a study using an HSV vaccination model in which it was shown that Ab and T cells elicited by a mutant replication-deficient vaccinating virus contributed to protection of the host at distinct stages of challenge infection, with Ab preventing encephalitic infection of the CNS (39). Therefore, our studies validate the importance of the mouse model for examining vaccine strategies and understanding correlates of immune protection and raise a caution that live vaccines that are attenuated and protective in an immunocompetent host may have unanticipated virulence in immunocompromised individuals.

It has long been known that Ab participates in immunity to γHV68 (17, 26, 40). Two separate questions are raised by these studies: what is the role of Ab in protection elicited by vaccination with AC-RTA, a latency-deficient virus, and what is the role of Ab in control of the initial virus infection?

The observation that T cell depletion lowered the protective efficacy following vaccination is similar to previous observations using a different live attenuated virus that was capable of establishing latency but was incapable of reactivation. In agreement with our findings, it was shown that protection could be partially reconstituted by passive transfer of Ab (17). These earlier studies also suggested that Ab-mediated protection was not mediated by the simple blocking of the initial infection because vaccination was effective against high challenge doses (17). This conclusion is consistent with our data showing that passively transferred sera had only modest protection in terms of viral titers at day 3 (and thus did not prevent initial infection), but showed greater than one log reduced viral titers at day 7 (Fig. 3A).

Our data show that passive transfer of serum from WT mice obtained 4 mo p.i. is more protective than Ab obtained from mice 30 d p.i. (Fig. 3A). In contrast, serum from AC-RTA–vaccinated mice is equally protective over time, consistent with the possibility that Ab elicited by AC-RTA infection failed to undergo some aspects of Ab maturation. It is possible that differences in protective efficacy reflect differences in neutralizing activity, as assessed in an in vitro assay (Fig. 3B). However, the contribution of virus neutralizing Ab in protection is poorly understood. The observation that the protective efficacy of serum matures over time in WT γHV68-infected but not vaccinated mice raises the interesting possibility that latency or reactivation is necessary to drive this unknown aspect of Ab maturation, as AC-RTA vaccination fails to establish latency. The differences in protective Ab are not simply a consequence of class switching, as our analysis failed to reveal any statistically significant differences in isotype profile as late as 6 mo p.i. (data not shown). Differences in protection may reflect a change in specificity of virus-specific Abs associated with different phases of infection, as complex serological patterns of Abs specific for different viral proteins have been described during EBV latency, following reactivation, and associated with tumors (41).

It has been reported that human γ-herpesviruses can infect the CNS. Acute or reactivating infection with EBV in both immunocompromised as well as immunocompetent individuals has been linked to complications such as meningitis and encephalitis (42, 43). In addition, it has been shown that KSHV can be neuroinvasive (44). Neurological complications of KSHV infection are less well defined, but detection of virus in dorsal root ganglia, cerebrospinal fluid, and brain in some patients with CNS complications associated with Kaposi’s sarcoma or AIDS-associated dementia have been described, although the relative contributions of HIV and KSHV to the pathology is controversial (43, 44). It is unclear whether pathogenesis is a consequence of direct virus
invasion of the CNS or whether damage is due to immunological effector mechanisms mediated either by infiltrating CD8 T cells, or deposition of Ag–Ab complexes (43).

Our data show that both the absence of Ab and the enhanced lytic activity of AC-RTA contribute to high viral titers in the brain following AC-RTA infection. Analysis of infectious virus and viral genome copy number in the brain has allowed us to distinguish the relative roles of enhanced lytic activity of AC-RTA and the absence of Ab in the inability of the Ab-deficient mice to control AC-RTA infection within the brain. Whereas AC-RTA infection of both Ab-deficient and WT mice resulted in lytic virus in the brain, the titers rose dramatically with time in the Ab-deficient mice and the mice succumbed to infection, but titers stabilized in the WT mice with no lethality. In contrast, no lytic virus was detected in the brains of either WT or Ab-deficient mice p.i. with WT γHV68, but analysis of genome copy number was consistent with the presence of latent virus in the brain of WT γHV68-infected Ab-deficient mice. Taken together, these data show that both WT γHV68 and AC-RTA can infect the brain in the absence of Ab and that the Ab is essential to control viral infection of the brain. However, due to the highly lytic replication of AC-RTA, which contains an ectopic copy of RTA under the control of a strong and constitutive hybrid promoter to drive the viral lytic cycle, an unchecking AC-RTA infection in the brain of Ab-deficient mice leads to death. How might Ab prevent the robust replication of AC-RTA in the brains of WT mice? One intriguing possibility for Ab control of AC-RTA replication in the brain is through the activity of complement factor C3, as it has been shown that C3 can mediate γHV68 replication during direct CNS infections (45). Alternatively, Ab-neutralizing activity could prevent the spread of lytic virus within the brain, possibly by FcR-mediated engulfment by phagocytes.

How does the virus get to the brain? In the absence of direct infection of the CNS, virus could be transported to the brain via cell-free virus in plasma or in a cell-associated manner. Our data show that both i.n. and i.p. infections lead to dissemination of virus to the brain by 14 d p.i., whereas the lytic titers in the brain are 3 logs higher following i.p. infection. Intriguingly, in vitro AC-RTA infection leads to the overexpression of the gene that encodes the viral regulator of complement activation protein (23, 46), which has recently been shown to enhance virus replication within macrophages (47). Macrophages can acquire virus, especially after i.p. infection (48), suggesting infected macrophages may carry virus to the brain. Enhanced macrophage infection and trafficking of AC-RTA could also explain the higher titers of lytic virus in the brain after i.p. infection compared with i.n. infection. Ab could play a role in preventing this trafficking, but this Ab-dependent prevention might be overcome by the overexpression of regulator of complement activation after AC-RTA infection.

Together, these studies have provided important insight into mechanisms of γ-herpesvirus immune control and immune correlates of protection elicited by an effective vaccine strategy. Both T cells and Ab contribute to effective protection elicited by a highly lytic latency-deficient virus, AC-RTA. Importantly, our study reveals a previously unrecognized role of Abs in controlling viral infection of the brain. However, whereas Abs prevent WT virus from accessing the brain, AC-RTA can readily access the brain regardless of the presence of Abs. AC-RTA was initially constructed to be a nonpersistent virus by replacing the latency locus with an ectopic RTA expression cassette under the control of a strong promoter as a vaccine strategy for γ-herpesviruses. The unanticipated complications associated with AC-RTA infection in Ab-deficient mice underscore the importance of the mouse in vivo infection model for studying vaccination with live-attenuated mutant viruses.
duction of protective immunity against murine gammaherpesvirus 68 infection in the absence of viral latency. J. Virol. 84: 2453–2465.


