γ -Herpesvirus-Induced Protection Against Bacterial Infection Is Transient

Eric J. Yager, Frank M. Szaba, Larry W. Kummer, Kathleen G. Lanzer,
Claire E. Burkum, Stephen T. Smiley, and Marcia A. Blackman

Abstract

Herpesviruses are widely disseminated in the population and establish lifelong latency, which is associated with a variety of pathological consequences. A recent report showed that mice latently infected with either murine γ -herpesvirus-68 (γ HV68) or murine cytomegalovirus (mCMV), mouse pathogens genetically similar to the human herpesviruses, Epstein-Barr virus, Kaposi's sarcoma-associated herpesvirus, and cytomegalovirus, had enhanced resistance to subsequent bacterial infection, suggesting protective as well as deleterious effects of latency. Here we confirm that latent γ HV68 infection confers protection against subsequent infection with *Listeria monocytogenes*. However, the effect is transient, lasting only a few months.

Introduction

HERPESVIRUSES ARE LARGE DOUBLE-STRANDED DNA viruses that infect both animals and humans. Although acute herpesvirus infections are rapidly cleared, viral latency is established in the host and then maintained for life. The majority of the world's population is latently infected with multiple herpesviruses and viral reactivation from latency is associated with a variety of human pathologies. The human β -herpesvirus, cytomegalovirus (CMV), is normally controlled in immunocompetent adults, but it can cause severe disease such as hepatitis following reactivation in immunosuppressed individuals. In addition, there is a strong relationship between lifelong CMV infection and the occurrence of oligoclonal T-cell expansions in the elderly that compromise immune function (17). Although latent infection by human γ -herpesviruses, including Epstein-Barr virus (EBV) and Kaposi's sarcoma-associated herpesvirus (KSHV), is usually asymptomatic, γ -herpesvirus latency has been associated with the development of a variety of malignancies including Burkitt's lymphoma, Hodgkin's disease, nasopharyngeal carcinoma, Kaposi's sarcoma, and B-cell lymphoproliferative syndromes (18). Because of these and other health complications associated with herpesvirus infections, the development of successful prophylactic and therapeutic vaccination strategies against these viruses continues to be an important research goal (1).

Murine gammaherpesvirus 68 (γ HV68, MHV-68, or Murid herpesvirus-4) is a natural rodent pathogen that is struc-

turally, biologically, and genetically related to the human γ -herpesviruses (4,15,25). Intranasal infection of mice with γ HV68 causes an acute respiratory infection that is rapidly resolved, followed by the establishment of a lifelong latent infection. Because of its close homology to human γ -herpesviruses, γ HV68 provides a valuable *in vivo* experimental model to study γ -herpesvirus pathology and host immunity.

A recent report has described symbiotic protection against subsequent bacterial challenge in mice harboring latent β - and γ -herpesvirus infections (2). Specifically, it was shown that mice latently infected with either the β -herpesvirus, murine cytomegalovirus (mCMV), or the γ -herpesvirus γ HV68, were protected against lethal challenges by *Listeria monocytogenes* or *Yersinia pestis*. Protection was also manifest by a reduction in the numbers of bacteria recovered from the spleens and livers of latently infected mice following challenge. These protective effects were restricted to the latent phase of infection and induction of protection was virus-specific, as mice previously infected with the α -herpesvirus HSV-1 or Sindbis virus failed to exhibit increased resistance against similar bacterial challenges. Host protection was observed in latently infected mice as far out as 3 mo post-infection, and was shown to be associated with elevated serum levels of inflammatory cytokines including IFN- γ and TNF- α , which are proposed to result in the chronic activation of bactericidal macrophages. While investigating the impact of γ HV68 infections on immunity in aged mice, we discovered that the symbiotic protective effect failed to persist with lifelong latency. This observation prompted us to determine

how long after the establishment of γ HV68 latency this symbiotic protection persisted.

Materials and Methods

Mice

Female C57BL/6 mice were obtained from the Trudeau Institute animal breeding facility. The mice were housed under specific pathogen-free conditions before infection, and in ABSL3 containment after viral infection. The Institutional Animal Care and Use Committee at the Trudeau Institute approved all animal procedures.

Viral infection

γ HV68, clone WUMS (Washington University School of Medicine), was propagated and titered on NIH-3T3 fibroblasts (ATCC CRL1568) (25). Mice between 6 and 8 wk of age were anesthetized with 2,2,2-tribromoethanol and intranasally infected with 400 PFU of γ HV68. Mice were considered latently infected at 30 d after the initial γ HV68 infection. Viral latency was confirmed with quantitative PCR using primers specific for the γ HV68 ORF50 gene on DNA isolated from the spleens of infected mice (24).

Bacterial infection and measurement of bacterial loads

Stocks of *L. monocytogenes* (strain EGD, supplied by Robert North, Trudeau Institute) were prepared after passage

through C57BL/6 mice (12). The mice were challenged with 2.5×10^6 cfu of bacteria via intraperitoneal injection, and spleens and livers were removed from infected mice 3 d later. The numbers of viable bacteria in each tissue type was determined by plating tissue homogenates on BHI-agar plates.

Measurement of cytokine levels

Cytokines were detected in whole sera taken from γ HV68-infected mice at the indicated times post-infection using the mouse inflammation Cytometric Bead Array (BD Biosciences, San Jose, CA) according to the manufacturer's instructions.

Data analysis

Statistical significance was calculated using the nonparametric Mann-Whitney rank test using Prism 4 software (GraphPad Software, La Jolla, CA). *p* Values <0.05 were considered significant.

Results

γ -Herpesvirus latency-mediated protection against bacterial infection is short-lived

Previous studies have described a symbiotic protection from bacterial infection in mice that harbor a latent β - or γ -herpesvirus infection that persisted for up to 3 mo post-in-

FIG. 1. Herpesvirus latency-mediated protection against subsequent bacterial infection is finite. Mice latently infected with γ HV68 for the indicated lengths of time (indicated by “+” and open circles) and uninfected age-matched control mice (indicated by “–” and closed circles) were challenged with *L. monocytogenes*. The number of viable bacteria detected in the spleens (A) and livers (B) of mice 3 d after challenge are shown. In addition, the amount of latent γ HV68 present in the above groups of mice was assessed using quantitative real-time PCR, and the number of copies of viral ORF50 gene detected in DNA isolated from splenic tissue (C) are shown. Each symbol represents data obtained from an individual mouse and bars indicate the medians calculated from the data. n.d., not detected; NS, not significant; ***p* < 0.05 as determined using the Mann-Whitney rank test (two-tailed, 95% CI).

fection (2). To determine how long after infection this protection was maintained, we challenged mice latently infected with γ HV68 with *L. monocytogenes* at periods between 2 and 19 mo after the initial viral infection. Consistent with the previous findings we observed that during the first 5 mo following γ HV68 infection, latently-infected mice exhibited greatly reduced viable bacteria counts in spleen and liver tissue as compared to age-matched uninfected control mice (Fig. 1A and B). However, this protective effect was completely lost in mice latently infected with γ HV68 after more than 5 mo, in that as early as 6.5 mo after infection mice were found to have viable bacteria counts similar to age-matched uninfected controls, indicating that latency-induced host protection waned with time. Importantly, the viral load assessed in terms of genome copy was constant between the 5- and 6.5-mo time points (Fig. 1C). These results indicate that the enhanced resistance against bacterial infection conferred to the host by γ -herpesvirus latency is finite and not maintained throughout lifelong viral latency.

Transient elevation in the levels of inflammatory cytokines following the establishment of γ -herpesvirus latency

It was proposed that the enhanced resistance to bacterial pathogens observed in latently infected mice was due to a systemic activation of macrophages and heightened innate immunity, likely due to a prolonged and elevated production of inflammatory cytokines (2). Consistent with this notion, the authors found that levels of TNF- α and IFN- γ remained elevated in the serum of latently infected mice for up to 41 d following γ HV68 infection. To determine how long IFN- γ and TNF- α cytokine levels remained elevated in the serum of latently infected mice, we measured the cytokine levels in sera of γ HV68-infected mice at extended times following the initial infection. Consistent with the original report (2), elevated levels of IFN- γ (Fig. 2A) and TNF- α (Fig. 2B) were detected in the sera of mice 30 d following γ HV68 infection. However, the levels of these two cytokines returned to baseline in latently infected mice at 2 mo post-infection, where they remained for as long as 8 mo post-infection. These results indicate that γ HV68 infection increases host production of cytokines known to influence macrophage activation and pathogen resistance; however, this effect is apparently lost shortly after the establishment of latency.

Discussion

Our results confirm the findings of a previous study that demonstrated that herpesvirus latency conferred host protection against subsequent bacterial infection (2). The authors speculated that latent herpesvirus infection represented a symbiotic relationship in which heightened activation of the innate immune system protects the host from ensuing infections. These data raised the possibility that latent herpesvirus infection may in fact be beneficial to the host, and suggested that vaccines to prevent or control latency may have unintended negative consequences for host immunity. Here we confirm that beneficial effects do exist, but this protection is transient, lasting only as long as 5 mo following acute γ HV68 infection, despite a stable viral load. Furthermore, elevated serum levels of IFN- γ and TNF- α , which were associated with the protective effect, declined even more quickly and could no longer be detected at 2 mo after the

FIG. 2. Temporary elevation in the levels of inflammatory cytokines following the establishment of γ -herpesvirus latency. The levels of IFN- γ (A) and TNF- α (B) in the sera of uninfected control (C) mice and mice latently infected with γ HV68 for the indicated lengths of time were assessed using a cytometric bead-based immunoassay. Each symbol represents data obtained from an individual mouse, and bars indicate the medians calculated from the data. NS, not significant; ** $p < 0.05$ as determined using the Mann-Whitney rank test (two-tailed, 95% CI).

initial infection. Thus, rather than representing symbiosis, latency-mediated protection is likely a consequence of transient changes in the activation status and host immunity responsible for the initial reduction of the number of virus-infected cells and establishment of latency.

IFN- γ was shown to be critical for the symbiotic protection against bacterial infection, with elevated levels in the serum for as long as 41 d post-infection, and latently-infected IFN- γ -deficient mice were not protected from bacterial infection (2). IFN- γ has been shown to block latent γ HV68 reactivation in a cell type-specific manner via regulation of viral latent gene expression (21,22). Both CD4 and CD8 T cells produce IFN- γ during γ HV68 latency (3). In addition, it was recently shown that the γ HV68 M1 gene product induces persistent activation of CD8 T cells bearing a V β 4 T-cell receptor, and that these T cells appear capable of suppressing

virus reactivation via long-term IFN- γ production (6). Although IFN- γ is produced at high levels following the initiation of the host antiviral immune response, it is not critical for control of the initial γ HV68 infection (19). However, IFN- γ appears to be critical for long-term control of γ HV68 infection and its symptoms, since infected IFN- γ -receptor-deficient mice show multiple organ fibrosis and enhanced virus reactivation (5,7,14,23). Why then are the elevated levels of IFN- γ seen early after γ HV68 infection not maintained during latency? We speculate that as the level of latent virus equilibrates, the amount of viral antigen is reduced, and as a consequence fewer virus-specific T cells are induced to produce IFN- γ and other cytokines. Thus, sufficient levels of IFN- γ are still being produced to control virus reactivation, but not enough to maintain protection against ensuing bacterial challenges.

Although γ -herpesvirus infections may provide the human host a temporary benefit of protection against subsequent infections as observed in the mouse, the transient protection afforded by herpesvirus latency is unlikely to outweigh the deleterious consequences of infection. Although the pathogens are widely distributed in the human population, generally there is low incidence of disease. Nevertheless, there are specific susceptibility groups with high risk for pathological consequences. For example, there is a high incidence of endemic Burkitt's lymphoma in equatorial Africa (9,13), and there are regions of Southeast Asia with a 20-fold higher than normal incidence of EBV-associated nasopharyngeal carcinoma (11). Immunosuppression as a consequence of disease (AIDS) or post-transplant therapy also can lead to increases in EBV-associated lymphoproliferative syndromes and lymphomas, as well as KSHV-associated Kaposi's sarcoma (16,20). Finally, there is an elevated incidence of infectious mononucleosis in developed countries, which has been associated with the development of Hodgkin's lymphoma and multiple sclerosis (8,10). Thus the development of prophylactic and therapeutic vaccination strategies against human γ -herpesviruses remains an important goal.

Conclusion

These data confirm the previous report of Barton *et al.* (2) that early γ HV68 latency confers protection against bacterial infection, but show that the effect is transient. Therefore, there is no evidence that γ -herpesvirus latency provides a lifelong benefit to the host.

Disclosure Statement

No conflicting financial interests exist.

Acknowledgements

We would like to thank Drs. David Woodland and Mike Freeman for helpful discussions and comments on the manuscript. This work was supported by National Institutes of Health grants AG21600, AI51602, and AI42927 to M.A.B., and AI61577 to S.T.S. and the Trudeau Institute.

References

- Balfour HH Jr.: Epstein-Barr virus vaccine for the prevention of infectious mononucleosis—and what else? *J Infect Dis* 2007;196:1724–1726.
- Barton ES, White DW, Cathelyn JS, Brett-McClellan KA, Engle M, Diamond MS, Miller VL, and Virgin HW: Herpesvirus latency confers symbiotic protection from bacterial infection. *Nature* 2007;447:326–329.
- Christensen JP, Cardin RD, Branum KC, and Doherty PC: CD4+ T cell-mediated control of a gamma-herpesvirus in B cell-deficient mice is mediated by IFN-gamma. *Proc Natl Acad Sci USA* 1999;96:5135–5140.
- Doherty PC, Christensen JP, Belz GT, Stevenson PG, and Sangster MY: Dissecting the host response to a gamma-herpesvirus. *Philos Trans R Soc Lond B Biol Sci* 2001;356:581–593.
- Dutia BM, Clarke CJ, Allen DJ, and Nash AA: Pathological changes in the spleens of gamma interferon receptor-deficient mice infected with murine gammaherpesvirus: a role for CD8 T cells. *J Virol* 1997;71:4278–4283.
- Evans AG, Moser JM, Krug LT, Pozharskaya V, Mora AL, and Speck SH: A gammaherpesvirus-secreted activator of Vbeta4+ CD8+ T cells regulates chronic infection and immunopathology. *J Exp Med* 2008;205:669–684.
- Gangadharan B, Hoeve MA, Allen JE, Ebrahimi B, Rhind SM, Dutia BM, and Nash AA: Murine gammaherpesvirus-induced fibrosis is associated with the development of alternatively activated macrophages. *J Leukoc Biol* 2008;84: 50–58.
- Haahr S, and Hollsberg P: Multiple sclerosis is linked to Epstein-Barr virus infection. *Rev Med Virol* 2006;16:297–310.
- Henle W, and Henle G: Epidemiologic aspects of Epstein-Barr virus (EBV)-associated diseases. *Ann NY Acad Sci* 1980; 354:326–331.
- Hjalgrim H, Smedby KE, Rostgaard K, et al.: Infectious mononucleosis, childhood social environment, and risk of Hodgkin lymphoma. *Cancer Res* 2007;67:2382–2388.
- Ho JH: An epidemiologic and clinical study of nasopharyngeal carcinoma. *Int J Radiat Oncol Biol Phys* 1978;4:182–198.
- Mackaness GB: The influence of immunologically committed lymphoid cells on macrophage activity *in vivo*. *J Exp Med* 1969;129:973–992.
- Moormann AM, Chelimo K, Sumba OP, Lutzke ML, Ploutz-Snyder R, Newton D, Kazura J, and Rochford R: Exposure to holoendemic malaria results in elevated Epstein-Barr virus loads in children. *J Infect Dis* 2005;191:1233–1238.
- Mora AL, Torres-Gonzalez E, Rojas M, et al.: Control of virus reactivation arrests pulmonary herpesvirus-induced fibrosis in IFN-gamma receptor-deficient mice. *Am J Respir Crit Care Med* 2007;175:1139–1150.
- Nash AA, Dutia BM, Stewart JP, and Davison AJ: Natural history of murine gammaherpesvirus infection. *Philos Trans R Soc Lond B Biol Sci* 2001;356:569–579.
- Pagano JS: Epstein-Barr virus: the first human tumor virus and its role in cancer. *Proc Assoc Am Physicians* 1999;111: 573–580.
- Pawelec G, Akbar A, Caruso C, Solana R, Grubeck-Loebenstein B, and Wikby A: Human immunosenescence: is it infectious? *Immunol Rev* 2005;205:257–268.
- Rickinson AB, and Kieff E: Epstein-Barr virus. In: *Fields Virology*. (Knipe PMHDM, ed.). Lippincott Williams & Wilkins, Philadelphia, 2001, pp. 2575–2627.
- Sarawar SR, Cardin RD, Brooks JW, Mehrpooya M, Hamilton-Easton AM, Mo XY, and Doherty PC: Gamma interferon is not essential for recovery from acute infection with murine gammaherpesvirus 68. *J Virol* 1997;71:3916–3921.
- Sarid R, Olsen SJ, and Moore PS: Kaposi's sarcoma-associated herpesvirus: epidemiology, virology, and molecular biology. *Adv Virus Res* 1999;52:139–232.

21. Steed A, Buch T, Waisman A, and Virgin HW: Gamma interferon blocks gammaherpesvirus reactivation from latency in a cell type-specific manner. *J Virol* 2007;81:6134–6140.
22. Steed AL, Barton ES, Tibbetts SA, Popkin DL, Lutzke ML, Rochford R, and Virgin HW: Gamma interferon blocks gammaherpesvirus reactivation from latency. *J Virol* 2006; 80:192–200.
23. Tibbetts S, van Dyk L, Speck S, and Virgin, HW: Immune control of the number and reactivation phenotype of cells latently infected with gammaherpesvirus. *J Virol* 2002;76: 125–132.
24. Usherwood EJ, Ward KA, Blackman MA, Stewart JP, and Woodland DL: Latent antigen vaccination in a model gammaherpesvirus infection. *J Virol* 2001;75:8283–8288.
25. Virgin HW, Latreille P, Wamsley P, Hallsworth K, Weck KE, Dal Canto AJ, and Speck SH: Complete sequence and genomic analysis of murine gammaherpesvirus 68. *J Virol* 1997;71:5894–5904.

Address reprint requests to:
Dr. Marcia A. Blackman
Trudeau Institute
154 Algonquin Ave.
Saranac Lake, New York 12983

E-mail: mblackman@trudeauinstitute.org

Received October 27, 2008; accepted November 10, 2008.