Letter to the Editor

Herpesvirus Latency and Symbiotic Protection from Bacterial Infection

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We are pleased that Yager *et al.* (15) confirmed our finding that latent infection with murine gammaherpesvirus 68 (MHV-68, also γ -HV68) increases resistance to infection with *Listeria monocytogenes* (2). We are especially interested in their finding that latency-mediated protection, which was waning by 3 mo in our experiments, is still evident at 5 mo, but becomes undetectable by 6.5 mo post-infection with MHV-68. This is an important finding that highlights, along with other reports (5,8), the potential for β - and γ -herpesvirus infection to increase resistance against heterologous infection. We sincerely appreciate the opportunity afforded by Dr. Blackman and colleagues, who kindly shared a copy of their manuscript prior to publication, and by the editors to comment on four questions raised by this article.

Is the Observed Cross-Protection Symbiosis?

Yager *et al.* argue that “transient changes in the activation status and host immunity” during early herpesvirus latency do not represent symbiosis, and that “there is no evidence that γ -herpesvirus latency provides a lifelong benefit to the host.” We respectfully disagree. There is no requirement that the benefits of mutualistic symbiosis be permanent; only that they improve the net fitness of the species. Furthermore, mutualistic symbionts frequently extract evolutionary costs for a subset of host individuals (4). While we do not overlook the toll of Epstein-Barr virus (EBV)-associated malignancies on humans, nor the impact of human cytomegalovirus (CMV) on immunocompromised patients, the vast majority of people infected with these viruses do not succumb to these diseases. The crux of the question, therefore, is whether latent β - and γ -herpesvirus infection is neutral, detrimental, or beneficial for those who do not develop severe disease. We argue that protection from secondary infection may well provide enhanced fitness, particularly in parts of the world where infectious disease remains a predominant cause of childhood mortality. Indeed, the putative symbiotic benefits of herpesvirus latency would occur during childhood, when infection with EBV, human herpesvirus (HHV)-6, HHV-7, and human CMV typically takes place. A virus that increases the odds that its host will survive to a reproductive age, even if the period of enhanced fitness lasts only months, confers

a lifelong benefit and meets the definition of a beneficial symbiont.

Is the Dissociation Between Latent Infection (Which Is Lifelong) and Protection Against Heterologous Infection (Which Lasts Only Months) an Indication that Latency is Not the Cause of the Cross-Protection?

We tested whether latency was necessary for cross-protection using a mutant virus that fails to establish latency, and found that this virus did not confer protection (2). Thus, some aspect of prolonged infection, and not mere acute infection, is required for the observed cross-protection. What then is the explanation for diminished cross-protection over time? The short answer is that we do not know, but we point out that latency changes over the life of the host. Many studies of MHV-68 have demonstrated that reactivation from latency in explanted cells is more efficient at early time points compared to late time points (13,14). A change in the nature of latency over time has been observed for murine CMV as well (1,9,12). After clearance of acute infection, murine CMV DNA is detected for several months in the blood and bone marrow. By 12 mo, the blood is largely clear of viral DNA, while lung tissue persists as a reservoir of virus that is impervious to clearance by CD8⁺ T lymphocytes. There is also substantial evidence for multiple forms of latency with EBV (10). We posit that a similar phenomenon may apply to MHV-68, with earlier forms of latency being more efficient at generating cross-protection.

Is the Observed Cross-Protection of Sufficient Duration to be Physiologically Significant?

One may legitimately ask whether 5 or 6 mo of cross-protection is enough to impact human health. After all, while mice typically live less than a year in the wild, humans live much longer. As described above, the timing of the putative protection in humans is predicted to coincide with life-threatening childhood infections. In addition, the selective advantage conferred by latent infection need not be large to profoundly alter host evolution: a survival advantage conferred to less than 1% of latently-infected individuals would ensure

that latency became the predominant form of herpesvirus-host interaction within a few thousand generations (3).

Furthermore, while our study examined individual herpesvirus infections in mice, children are infected with up to four β - and γ -herpesviruses, potentially extending their period of protection. It remains to be seen whether infection by multiple herpesviruses confers more or less cross-protection than that observed during single infections in mice.

Importantly, we and Yager *et al.* challenged latently infected mice intraperitoneally with a high dose of *L. monocytogenes*. Natural bacterial infections usually involve a lower dose initiated at a mucosal surface, the anatomic location where β - and γ -herpesvirus reactivation is thought to occur, and where virus-specific T-cell infiltration is observed for months after primary EBV infection in humans (6). Future studies should address the possibility that cross-protection from physiologic infectious challenges lasts even longer than the cross-protection observed by Yager *et al.*

Finally, it is worthwhile to point out that the transient immunosuppression caused by measles virus lasts no longer than months. Nevertheless, this period has important implications for human health (7). This effect of measles virus infection, the opposite of the protective effect we and Yager *et al.* observe in mice, has significantly altered the evaluation of measles virus vaccination in humans, and prompted highly relevant analyses of secondary infections in vaccinated individuals. For all these reasons, we argue that herpesvirus-induced cross-protection lasting months may well be clinically relevant.

Do These Studies Have Implications for Human Vaccination?

Yager *et al.* state that "the development of prophylactic and therapeutic vaccination strategies against human γ -herpesviruses remains an important goal." Of course, our experiments and those of Yager *et al.* do not assess any of these issues in humans, and any extension to humans is purely speculative. But we want to clarify our view that the finding of a potential benefit from herpesvirus latency certainly does not argue against the development of prophylactic or therapeutic herpesvirus vaccines. On the contrary, a live attenuated vaccine, such as the varicella-zoster virus (VZV) vaccine, may well confer any potential benefits of latent infection, while simultaneously protecting against primary VZV infection. In contrast, if natural latent infection modulates the human immune system in a manner that is not recapitulated by a vaccine, such as might occur with a non-replicating subunit vaccine, then potential benefits could be lost. Furthermore, there is the potential for herpesvirus-induced immune activation to alter the host response to non-infectious self and environmental antigens (11). We have no desire for our findings to be interpreted as evidence that vaccines are necessarily harmful. Rather, we hope that our findings will stimulate epidemiologic and clinical studies aimed at a better understanding of the precise nature of immune modulation during all phases of herpesvirus latency and all attendant consequences for human health. Such studies could inform the development and testing of vaccines against human herpesviruses such that any potential benefits of natural herpesvirus latency are not overlooked.

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