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EDITORIAL COMMENTARY

Deep Lessons From the Uncultured

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(See the major article by Hage et al on pages 1673–83.)

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In the current issue of *The Journal of Infectious Diseases*, Hage et al [1] report on the use of modern DNA sequencing methods in a study of the genetic diversity of human cytomegalovirus (HCMV). Information about HCMV population dynamics provided by this and other studies of HCMV genetic diversity reveal a richer and more subtle biology of the virus than previously documented and suggest that more forms of population diversity remain to be identified. The results provide important guidance to researchers and point to future clinical applications during acute and long-term care of patients suffering from or at risk of HCMV infection.

GOING DEEP INTO THE WILD

Recent advances in nucleotide sequencing have enabled novel insights related to genetic variation in populations of organisms and microbes throughout nature. On slowly changing genetic backdrops that help to define species, a plethora of biological mechanisms have evolved for generating genetic and epigenetic adjustments that enhance biological fitness by enabling rapid responses to changes in the biologic milieu. Methods for laboratory culture of bacteria and fungi were developed in the 1800s, and then for replication of viruses in cultured cells in the middle of the 20th century. Much of what we know about the genetics of microbes is based on study of material propagated in laboratories. Information gained in recent years makes it clear that much can be learned from uncultured, wild microorganisms.

Typical contemporary DNA sequencing methods rely on sequencing large numbers of random fragments generated from target sequences [2]. Accurate sequencing is dependent on determining every position in a sequence tens to hundreds if not thousands of times. An important byproduct of such depth of coverage is reliable detection of sequence heterogeneities that are present even at low frequencies. Use of these methods to sequence virus genomes present in uncultured clinical specimens has led to discovery of novel free and endogenous viruses [3] and provides important information about the extent and nature of inter- and intrahost virus genetic diversity [4–10].

HUMAN CYTOMEGALOVIRUS: A SHAPE-SHIFTING CHAMELEON

Human cytomegalovirus is a pathogen of high societal importance [11]. It is a major cause of morbidity and mortality in immunocompromised patients [12], but its major societal impact is due to transplacental congenital infections that cause a spectrum of effects, including severe, often life-threatening disease in neonates, hearing loss (sometimes delayed) in young children [13,14], and associations with other developmental disorders [15].

Human cytomegalovirus has complex biology that includes a protracted replication cycle that is dependent on multiple virally encoded tools that regulate intrinsic, innate, and adaptive defenses (reviewed in [12,16]). After primary infection, the virus persists for the lifetime of the host, making use of a repository of latently infected cells from which sporadic reactivations of lytic replication produce infectious virions that reseed the latent repository, stimulate the immune system, and are vehicles for cell-to-cell and host-to-host transmission.

The HCMV genome is the largest (approximately 236 kbp) of any known human virus. Of its approximately 170 protein-coding genes, only 45 are required for production of infectious virions in cultured cells. Most of the other genes, referred to as *accessory genes*, play roles in modulating interactions with host immune functions and enabling efficient entry and replication in various cell types. The genome, thus, represents a robustly complex toolbox for evolutionary survival in a host that is well equipped to defend itself.

Much of what is known of HCMV molecular and cell biology is the product of studies that used strains of the virus that were cultured from clinical specimens obtained in the 1950s (reviewed in [17]). In 1996, Cha et al reported that genomes of the 2 most widely used laboratory strains of the virus (AD169 and Towne) lack similar 13–15-kB segments that are present in viruses with less extensive culture passage history [18].

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Subsequent studies made it clear that the HCMV genome changes during replication in cultured cells, with some changes occurring in a specific sequence during adaptation of virus present in fresh clinical specimens to replication in cultured cells [19]. Genomic changes that occur during culture include point mutations and small insertions or deletions (indels) [6] and deletion of large gene blocks, such as described above. Many of these changes affect genes that regulate cell tropism and evasion of intrinsic, innate, and adaptive defenses.

Studies of genes modified or lost upon passage in culture have indicated that HCMV is a shape-shifting chameleon for which replication in 1 cell type results in production of virions more suited to infecting cells of other types [20,21]. This enables infection of a wide range of cell types (eg, fibroblasts, endothelial cells, epithelial cells, monocytes) that populate a wide variety of tissues (eg, lung, intestinal, renal, retinal, nervous system, placental, fetal) that are important sites of HCMV's clinical manifestations.

HUMAN CYTOMEGALOVIRUS GENETIC DIVERSITY AND VIRUS BIOLOGY

In the work by Hage et al [1], modern sequencing methods were used to obtain 57 complete HCMV genome sequences from uncultured clinical specimens obtained from various bodily compartments in renal and stem cell transplant recipients, as well as from congenitally infected children and their mothers. In addition, the authors capitalized on the power of longitudinal specimen collection [22] by collecting specimens at multiple time points. Although only minor population shifts were observed over time in some patients, in several individuals, the major HCMV population in blood switched from 1 genotype to another over the course of the study. Whole-genome genotype switching is consistent with long-standing evidence that individuals can be infected by, and then harbor, multiple strains of virus. Clinically, organ transplant donor–recipient mismatches with respect to HCMV glycoprotein H genotypes are more likely to have adverse outcomes [23].

The authors also detected minor virus subpopulations that harbored markers for antiviral resistance, providing the virus the ability to rapidly respond to therapeutic intervention. A patient with HCMV retinitis had distinct virus populations in the vitreous humor compared with blood. Other recent studies of HCMV diversity have also shown that major populations of the virus can differ between blood and urine in congenitally infected children and that population diversity can shrink dramatically (a genetic bottleneck) as infection proceeds from 1 compartment to another [9,10]. In addition, the diversity of HCMV populations undergoes a substantial genetic bottleneck at the time of human-to-human transmission [10], providing evidence that HCMV transmission can be initiated by a very small number of infectious virions.

The level of diversity seen for HCMV in uncultured specimens is in the range of diversities seen for RNA viruses [24], which have historically been thought to have greater genetic diversity than DNA viruses. In contrast to RNA viruses, wild populations of HCMV exhibit extensive interstrain recombination that appears to maintain core replicative functions while generating diversity in genes that facilitate immune escape [4,6,9]. These recombinational genotypic shifts are loosely analogous to segment-shuffling of influenza viruses.

Somewhat surprisingly, accessory genes with long evolutionary histories are inactivated in some wild lineages [25]. This can occur through small changes, such as point mutations or indels that can inactivate genes by mechanisms such as translational frameshifting, without discarding all of the information gained during their evolution. Under subsequent selective pressure, relatively small changes would be sufficient for reactivation of these genes at much lower biological cost than waiting for de novo evolutionary emergence of a tool that serves the same purpose. Interestingly, no evidence for virus gene inactivation was seen in a study of uncultured HCMV genomes present in malignant glioma specimens, suggesting that these genomes might represent replication competent virus [26].

DEEP LESSONS FROM THE UNCULTURED

It is important to conduct research with viruses that are genetically similar to uncultured wild virus and to understand the genotypic limits of whatever strain is used. For animal models that use viruses such as murine cytomegalovirus, guinea pig cytomegalovirus, and rhesus cytomegalovirus, it is important to understand how the strain studied compares with uncultured virus from the same host. In studies of humans, assays for endpoints such as stimulation of protective immunity need to be evaluated against collections of strains that reflect the diversity of the target population.

Nomenclature matters. As suggested by Wilkinson et al [17], it seems reasonable to apply the term *clinical strain* to virus present in clinical specimens that has not been passaged in cultured cells. Descriptions of strains termed *low-passage* should be accompanied by details of their provenance, passage number, nature of passage (through infected cells or cell-free virus), and the cell types used for passage.

The form of virus diversity in the wild changes in response to environmental change, including changes due to human behavior. For HCMV, such changes include immune suppression in the context of organ and stem cell transplantation and antiviral therapy. Herpesviruses of farmed animals provide excellent examples of the speed with which related viruses evolve in response to environmental factors such as crowding, lifespan changes, and vaccination [27–29]. We anticipate changes in HCMV populations in response to increased use of daycare and look forward to the problem

of monitoring HCMV in vaccinated populations.

Note

Potential conflicts of interest. P. E. P. reports a gift from the HHV-6 Foundation for activities not related to the submitted work. In addition, P. E. P. has a patent on reagents used in US Food and Drug Administration–approved kits for type-specific serodiagnosis of herpes simplex virus infections, with royalties paid to the US Centers for Disease Control and Prevention. The author has submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Hage E, Wilkie GS, Linnenweber-Held S, et al. Characterization of human cytomegalovirus genome diversity in immunocompromised hosts by whole genomic sequencing directly from clinical specimens. J Infect Dis **2017**;215:1673–83.
2. Houldcroft CJ, Beale MA, Breuer J. Clinical and biological insights from viral genome sequencing. Nat Rev Microbiol **2017**; 15:183–92.
3. Houldcroft CJ, Breuer J. Tales from the crypt and coral reef: the successes and challenges of identifying new herpesviruses using metagenomics. Front Microbiol **2015**; 6:188.
4. Lassalle F, Depledge DP, Reeves MB, et al. Islands of linkage in an ocean of recombination reveals two-speed evolution of human cytomegalovirus genomes. Virus Evolution **2017**; 2:1–14.
5. Renzette N, Kowalik TF, Jensen JD. On the relative roles of background selection and genetic hitchhiking in shaping human cytomegalovirus genetic diversity. Mol Ecol **2016**; 25:403–13.
6. Sijmons S, Thys K, Mbong NM, et al. High-throughput analysis of human cytomegalovirus genome diversity highlights the widespread occurrence of gene-disrupting mutations and pervasive recombination. J Virol **2015**; 89:7673–95.
7. Sijmons S, Thys K, Corthout M, et al. A method enabling high-throughput sequencing of human cytomegalovirus complete genomes from clinical isolates. PLoS One **2014**; 9:e95501.
8. Pokalyuk C, Renzette N, Irwin KK, et al. Characterizing human cytomegalovirus reinfection in congenitally infected infants: an evolutionary perspective. Mol Ecol **2016**; 26:1980–90.
9. Renzette N, Pokalyuk C, Gibson L, et al. Limits and patterns of cytomegalovirus genomic diversity in humans. Proc Natl Acad Sci U S A **2015**; 112:E4120–8.
10. Renzette N, Gibson L, Bhattacharjee B, et al. Rapid intrahost evolution of human cytomegalovirus is shaped by demography and positive selection. PLoS Genet **2013**; 9:e1003735.
11. Institute of Medicine. Vaccines for the 21st century: a tool for decisionmaking. In: Stratton KR, Durch JS, Lawrence RS, eds. Washington DC: National Academy Press, **1999**:165–77.
12. Griffiths P, Baraniak I, Reeves M. The pathogenesis of human cytomegalovirus. J Pathol **2015**; 235:288–97.
13. Ogawa H, Suzutani T, Baba Y, et al. Etiology of severe sensorineural hearing loss in children: independent impact of congenital cytomegalovirus infection and GJB2 mutations. J Infect Dis **2007**; 195:782–8.
14. Rosenthal LS, Fowler KB, Boppana SB, et al. Cytomegalovirus shedding and delayed sensorineural hearing loss: results from longitudinal follow-up of children with congenital infection. Pediatr Infect Dis J **2009**; 28:515–20.
15. Borghi E, Pagani E, Mancuso R, et al. Detection of herpesvirus-6A in a case of subacute cerebellitis and myoclonic dystonia. J Med Virol **2005**; 75:427–9.
16. Gardner TJ, Tortorella D. Virion glycoprotein-mediated immune evasion by human cytomegalovirus: a sticky virus makes a slick getaway. Microbiol Mol Biol Rev **2016**; 80:663–77.
17. Wilkinson GW, Davison AJ, Tomasec P, et al. Human cytomegalovirus: taking the strain. Med Microbiol Immunol **2015**; 204:273–84.
18. Cha TA, Tom E, Kemble GW, Duke GM, Mocarski ES, Spaete RR. Human cytomegalovirus clinical isolates carry at least 19 genes not found in laboratory strains. J Virol **1996**; 70:78–83.
19. Dargan DJ, Douglas E, Cunningham C, et al. Sequential mutations associated with adaptation of human cytomegalovirus to growth in cell culture. J Gen Virol **2010**; 91:1535–46.
20. Scrivano L, Sinzger C, Nitschko H, Koszinowski UH, Adler B. HCMV spread and cell tropism are determined by distinct virus populations. PLoS Pathog **2011**; 7:e1001256.
21. Li G, Kamil JP. Viral regulation of cell tropism in human cytomegalovirus. J Virol **2015**; 90:626–9.
22. Pellett PE. Indictment by association: once is not enough. J Infect Dis **2015**; 212:509–12.
23. Ishibashi K, Tokumoto T, Tanabe K, et al. Association of the outcome of renal transplantation with antibody response to cytomegalovirus strain-specific glycoprotein H epitopes. Clin Infect Dis **2007**; 45:60–7.
24. Renzette N, Bhattacharjee B, Jensen JD, Gibson L, Kowalik TF. Extensive genome-wide variability of human cytomegalovirus in congenitally infected infants. PLoS Pathog **2011**; 7:e1001344.
25. Cunningham C, Gatherer D, Hilfrich B, et al. Sequences of complete human cytomegalovirus genomes from infected cell cultures and clinical specimens. J Gen Virol **2010**; 91:605–15.
26. Bhattacharjee B, Renzette N, Kowalik TF. Genetic analysis of cytomegalovirus in malignant gliomas. J Virol **2012**; 86:6815–24.
27. Atkins KE, Read AF, Savill NJ, et al. Vaccination and reduced cohort duration can drive virulence evolution: Marek's disease virus and industrialized agriculture. Evolution **2013**; 67:851–60.
28. Aoki T, Hirono I, Kurokawa K, et al. Genome sequences of three koi herpesvirus isolates representing the expanding distribution of an emerging disease threatening koi and common carp worldwide. J Virol **2007**; 81:5058–65.
29. Burioli EA, Prearo M, Riina MV, et al. Ostreid herpesvirus type 1 genomic diversity in wild populations of Pacific oyster *Crassostrea gigas* from Italian coasts. J Invertebr Pathol **2016**; 137:71–83.