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Phylogenetic studies of frequently diagnostically sampled herpesviruses – Possibilities for clinical applications?

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ABSTRACT

With the modern sequencing technologies and the capability to sequence large viral genomes virtually overnight, full-genome sequencing of large DNA viruses and correlating any sequence variation to specific clinical manifestations is now eminently feasible – but is it worth doing, i.e. can such large-scale viral genomic analyses be clinically useful? A few clinical conditions, such as organ and bone marrow transplant recipients, require the regular diagnostic sampling and testing for certain viruses over an extended period of time. Such frequent sampling leads to the routine archiving of multiple samples from the same patient over an extended time period and it is the purpose of this mini-review to explore whether such routinely collected samples can be utilized for viral sequencing studies to produce clinically useful outcomes. Specific examples of this include the monitoring of Epstein-Barr virus (EBV) and cytomegalovirus (CMV) levels in transplant recipients, as rising levels of these viruses in these patients can cause serious and sometimes fatal disease during the post-transplant period when their immune system is gradually recovering from the transplant process. Other herpesviruses, such as herpes simplex virus (HSV) and varicella zoster virus (VZV), may also reactivate and cause disease in post-transplant and other types of immunocompromised patients, though the specific complications may differ between patients. Although the natural mutation rate for these human herpes DNA viruses are very low, all of these viruses are capable of developing specific drug resistance within a few weeks of therapy, demonstrating that these viruses do have the ability to adapt rapidly to their local environment if the need arises. Specific problems with analyzing these large DNA virus genomes include the selection of the appropriate gene target (unless a full-genome analysis is the aim), and how to deal with overlapping and inverted reading frames, which are discussed in this mini-review.

1. Introduction

Modern advances in chemotherapy and organ transplantation have resulted in an ever-increasing burden from opportunistic viral infections. A large proportion of such viral infections arise from the reactivation of double-stranded (ds) DNA herpesviruses, many of which have been acquired asymptomatically during childhood. These viruses have remained latent within these individuals until their immune system has been compromised by disease and/or various forms of therapeutic interventions. Reactivation of these previously, relatively benign, viruses in the immunocompromised state can cause numerous complications requiring frequent admissions to hospital and treatment with toxic antiviral drugs, with significant morbidity and mortality outcomes in these patient cohorts. Other dsDNA viruses posing considerable problems in transplant recipients include the adenoviruses and the polyomaviruses (JC and BK viruses).

Whilst phylogenetic analysis at a population level has been applied to most of these viruses, routine, weekly surveillance for some of these viruses in transplant recipients (particularly for cytomegalovirus, CMV) provides a potentially valuable, high-density, clinical sample resource for high-resolution, intra-host viral population phylogenetic analyses. Such intra-host studies of CMV, for example, in this particular group of immunocompromised patients, may yield interesting and potentially clinically applicable results – as these patients nearly all suffer from a similar spectrum of post-transplant CMV-related disease.

Abbreviations: HSV, herpes simplex virus; VZV, varicella zoster virus; EBV, Epstein-Barr virus; CMV, cytomegalovirus; HIV, human immunodeficiency virus; CSF, cerebrospinal fluid; BAL, broncho-alveolar lavage; CAEBV, chronic active EBV; NPC, nasopharyngeal carcinoma; PTLD, post-transplant lymphoproliferative disorder.

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This short review article summarizes some of the problems these viruses can cause in the immunocompromised host and how a detailed phylogenetic analysis may be helpful in some cases. Problems and limitations related to such a phylogenetic approach for these relatively large DNA viral genomes are also discussed.

2. Herpes simplex viruses 1 and 2 (HSV-1, 152 kb genome; HSV-2, 155 kb genome)

By adulthood, over 50% of the human population in most developed countries will have been infected by HSV-1, which usually causes asymptomatic infection during childhood (Smith and Robinson, 2002). The virus becomes latent in the trigeminal ganglia (i.e. clusters of nerve cells around the top of the spine and brainstem) and reactivates from time to time to cause painful cold sores and oral ulcers, and occasional viral meningitis and encephalitis (Tyler, 2004). In immunocompromised patients, these ulcers can be large and painful and become secondarily infected with bacteria. For transplant recipients, low-level doses of the antiviral, acyclovir can be given prophylactically, to pre-empt any such reactivation during the period of most severe immunocompromise (Glenny et al., 2009; Ljungman, 2001; Slifkin et al., 2004). Any breakthrough infection can be treated with higher doses of acyclovir and often the infection resolves as the immunosuppression is reduced, if engraftment is successful. However, in some patients, prolonged therapy also selects for drug resistance viruses, which may be difficult to treat as second-line agents (e.g. foscarnet or ganciclovir) have much greater toxicity which may not be tolerated by some patients (Eisen et al., 2003). Drug resistance to acyclovir is otherwise lower in the general, immunocompetent population (Piret and Boivin, 2011; Stránská et al., 2005), but can reach relatively higher levels in immunocompromised transplant patients on long-term therapy (Piret and Boivin, 2011; Stránská et al., 2004), including in those infected with HIV (Levin et al., 2004; Piret and Boivin, 2011; Ziyaeyan et al., 2007). Screening for acyclovir resistance is not routine in many diagnostic laboratories, though many positive HSV-1 swabs may be archived from such patients as the response to different antivirals are tested. Such samples may be sequenced as part of a research project to examine the evolutionary response of the virus to these changes in *in vivo* selection pressures under different drug treatment environments.

For HSV-2, generally, the seroprevalence in the normal (i.e. low-risk, antenatal, blood donor, heterosexual) population is much less than that of HSV-1 (usually less than 30%), though it can reach as high as 90% in the high-risk (i.e. HIV-positive, commercial sex worker, men-who-have-sex-with-men) population (Smith and Robinson, 2002). Reactivation of this virus is from the sacral ganglia (i.e. clusters of nerve cells around the base of the spine) and, as with HSV-1, may cause tender, painful vesicular skin lesions, as well as meningitis and, more rarely, encephalitis (Tyler, 2004). Prophylactic acyclovir therapy will prevent reactivation in most cases, as for HSV-1, but reactivation can cause large, painful ulcers in the genital region. Rebound infection/reactivation and drug resistance can also be seen with HSV-2.

Various population level phylogenetic surveys have been performed on HSV-1 and HSV-2 mainly for genogrouping/genotyping purposes (Kolb et al., 2011; Norberg et al., 2004, 2007; Schmidt-Chanasit et al., 2009, 2010), but there have been few if any studies examining intra-host evolution of these viruses within immunocompromised hosts. One of the reasons for this is that routine surveillance sampling is not performed routinely for HSV-1 or HSV-2 in immunocompromised patients, as acyclovir prophylaxis is quite successful in controlling this problem in most cases. Such reactivated infections by these viruses are usually investigated as they occur. If the diagnosis of HSV infection is confirmed and the response to higher treatment doses of acyclovir is satisfactory, no further sampling is usually required. Hence, serial sample archives for the same patient for these HSV-1 and HSV-2 viruses may be quite limited.

An exception to this is if acyclovir resistance develops, in which case repeat sampling to test for a treatment response and/or viral clearance may be performed. Sequencing directly from the clinical samples (to avoid any inadvertent viral culture-induced mutations) should be possible in most cases, as the viral loads obtained from such lesions in such immunocompromised patients are usually high (Berrington et al., 2009; Tang et al., 2010). Hence, investigations across a population of immunocompromised patients infected with HSV-1 and HSV-2 in whom these viruses reactivate and then go on to develop drug resistance may be of interest, e.g. do they all take the same time to develop acyclovir drug resistance? At what maintenance dose have they developed drug resistance? Have the same drug resistance mutation patterns developed in all of these patients? Once drug therapy stops what happens to the drug-resistant viral population? Are they archived somewhere in the trigeminal and sacral ganglia? Or do they eventually revert back to wild-type quickly (over days to weeks), or more slowly (over months to years)? Such questions can only be asked (and answered) with large transplant populations, as the incidence of HSV-1 and HSV-2 acyclovir drug resistance is relatively rare.

Another possible angle for a phylogenetic analysis may be to examine the virus isolated from other body compartments, such as blood, cerebrospinal fluid (i.e. the clear fluid surrounding the brain and spinal cord, CSF), saliva, lung secretions (e.g. bronchoalveolar lavages, BALs) and tissue biopsy material from different body sites, which may allow intra-host and inter-host comparisons of viral molecular epidemiology in patients with different patterns of clinical illness, and in primary versus reactivated infections (Rojas et al., 1995; Werdin et al., 2008). It is well-recognized that the virus may be present in the same sample type obtained from the same body site (e.g. CSF or BAL fluids) from two different patients with quite different disease manifestations, i.e. one may have severe disease, whilst the other remains asymptomatic. Host factors will contribute to this, of course, but viral factors may also be present and different viral genotypes may produce a different spectrum of disease in different hosts. One of the ongoing controversies is whether the presence of HSV-1 in the respiratory secretions of critically ill patients on intensive care units is either a cause or a consequence of their severe illness, and whether screening for the presence of HSV-1 in respiratory fluids is warranted, and whether treatment is required (Bouza et al., 2011; Bruynseels et al., 2003; De Vos et al., 2009; Linssen et al., 2008; Ong et al., 2004; Simoons-Smit et al., 2006; van den Brink et al., 2004). A phylogenetic analysis of HSV-1 isolates from a cohort of such patients that are well characterized, clinically, may help to clarify this situation.

One other obstacle to investigating primary or reactivated HSV-1/HSV-2 diversity and evolution, phylogenetically, either within a single host or across multiple hosts, is that the actual number of lesions present during disease are limited – perhaps just 1–2 vesicles, which limits the range of distinct viral isolates that may be present in any single reactivation event. This is in contrast to lesions caused by primary or reactivated varicella zoster virus (VZV) infection, where multiple vesicles are usually present and available for sampling – and in some cases, different lesions within a single patient may contain different strains of the virus (see below), which then suggests some intriguing questions about what differential host selection pressures may be acting upon the virus, or whether co-infection with two different viruses occurred at the outset.

From a phylogenetic viewpoint, the main limitation to examining intra-host evolution is the low natural mutation rate in these