Microreview

Human cytomegalovirus persistence

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Summary

Viral persistence is the rule following infection with all herpesviruses. The β-herpesvirus, human cytomegalovirus (HCMV), persists through chronic and latent states of infection. Both of these states of infection contribute to HCMV persistence and to the high HCMV seroprevalence worldwide. The chronic infection is poorly defined mole-

cularly, but clinically manifests as low-level virus shedding over extended periods of time and often in the absence of symptoms. Latency requires long-term maintenance of viral genomes in a reversibly quiescent state in the immunocompetent host. In this review, we focus on recent advances in the biology of HCMV persistence, particularly with respect to the latent mode of persistence. Latently infected individuals harbour HCMV genomes in haematopoietic cells and maintain large subsets of HCMV-specific T-cells. In the last few years, impressive advances have been made in understanding virus–host interactions important to HCMV infection, many of which will profoundly impact HCMV persistence. We discuss these advances and their known or potential impact on viral latency. As herpesviruses are met with similar challenges in achieving latency and often employ conserved strategies to persist, we discuss current and future directions of HCMV persistence in the context of the greater body of knowledge regarding α- and γ-herpesviruses persistence.

Introduction

Mechanisms of viral persistence are among the most poorly understood phenomena in virology. This is due, in part, to the complexity of multiple layered interactions between the virus, the infected cell and the host organism as a whole that contribute to viral persistence. Persistent viral pathogens are well adapted to their host through co-speciation and tend to have reduced transmissibility and overall pathogenesis relative to viruses adopting acute infection strategies (Villarreal et al., 2000). This suggests that viral persistence as a strategy of coexistence comes at the price of moderating viral replication and, therefore, pathogenesis. As such, human cytomegalovirus (HCMV) infection is inapparent in the immunocompetent host, typically causing no overt pathology. Following infection, HCMV coexists for the lifetime of the host through both chronic virus shedding and latency. The individual contributions of the chronic and latent modes of infection to viral persistence are ill defined.

During the chronic infection, virus is persistently shed from restricted sites in the host at low levels and for extended periods of time. Chronic virus shedding may stem from the acute, primary infection or may result following reactivation of latent virus. Chronic virus shedding may be important for reseeding latent virus reservoirs. In the immunocompetent host, the chronic infection is typically asymptomatic and is not associated with overt disease, although it has been associated with inflammatory and age-related disease including vascular disease (Pannuti et al., 1985; Zanghellini et al., 1999; Drew et al., 2003; Britt, 2008; Streblow et al., 2008). Endothelial and epithelial cells are key sites of chronic virus shedding. As an example, HCMV is commonly shed in breast milk in the postpartum period (Stagno et al., 1980). Further, virus may be shed for months to years from epithelial cells in the urinary tract of paediatric patients (Britt, 2008).

The latent infection is defined by a reversibly quiescent state in which viral genomes are maintained, but viral gene expression is highly restricted and no virus is produced. The reversibility of the latent infection, the ability of the virus to reactivate, is critical to the definition of latency as this feature distinguishes latency from an abortive infection. Importantly, loss of T-cell-mediated immune control or changes in the differentiation or activation state
of cells harbouring latent HCMV can result in reactivation of latent virus and production of viral progeny. While isolated reactivation events likely occur intermittently in the immunocompetent host, these events are controlled by existing T-cell-mediated immunity and do not result in clinical presentation. Severe HCMV disease is associated with reactivation of latent virus and chronic infection associated with states of insufficient T-cell control following stem cell or solid organ transplantation, HIV infection and intensive chemotherapy regimens for cancer (Britt, 2008; Boeckh and Geballe, 2011).

Despite decades of research, we have little more than a cursory understanding of the molecular basis of HCMV latency and how viral, cellular and organismal mechanisms are orchestrated to meet this objective. Efforts to understand HCMV latency are hampered by the restriction of HCMV to the human host. While HCMV infects a diverse number of cell types, latency is unique to specific cell types. Therefore, the current state of our knowledge is primarily borne from the use of primary human cell culture models, specifically those using primary haematopoietic progenitor (HPCs) or myeloid lineage cells and cell line models including the myeloid THP-1 and N-teratocarcinoma (T2) cell lines. Due to limitations in cell culture models, murine (MCMV) (reviewed in Redehease et al., 2002), rat (RCMV) (reviewed in Streblow et al., 2008), guinea-pig (reviewed in Schleiss, 2006) and the rhesus (RhCMV) viruses (reviewed in Powers and Fruh, 2008) are important models in understanding persistence in the context of the immunocompetent host (Kern, 2006). Despite the value of these animal models, differences between these viruses and HCMV with respect to the genome content, coding capacity and aspects of pathogenesis command studies using the human virus to understand unique mechanisms of persistence that arose through co-speciation.

**Virus-coded determinants**

Of the nearly 200 genes encoded by HCMV, less than one-fourth are essential for viral replication and conserved across herpesvirus subfamilies. Gene products for 37–60 open reading frames (ORFs) (depending on methods used) are detected following in vitro infection of CD34+ HPCs (Goodrum et al., 2004; Cheung et al., 2006). Gene products detected in CD34+ HPCs include the immediate early transcripts (Goodrum et al., 2002; 2004; Cheung et al., 2006; Petrucci et al., 2009) and proteins (IE1-72kDa and IE2-86kDa), which are transiently detected in CD34+ HPCs (Petrucci et al., 2009; Umashankar et al., 2011) as well as in CD14+ cells (Hargett and Shenk, 2010). Despite transient expression of IE genes, the full repertoire of genes required for replication is not detected. Indeed, the majority of ORFs expressed in CD34+ HPCs are non-essential for productive viral replication in fibroblasts and their function is unknown (Yu et al., 2003). Consistent with these findings, RCMV patterns of gene expression are defined by the cell type infected (Streblow et al., 2007). Due to limitations in current latency models, viral gene expression has not been globally analysed following the establishment of latency. However, several viral gene products reviewed in the following subsections have been detected in HPCs or myeloid cells from sero-positive individuals.

**CMV latency-associated transcripts**

Transcripts and proteins encoded from a region encompassing the major immediate early region are detected in haematopoietic cells following infection in vitro as well as in latently infected individuals (Kondo et al., 1996; Landini et al., 2000). It should be noted that the structure of these transcripts differs from those produced during a productive infection in fibroblasts. While the role of these transcripts in infection is not known, the encoded ORF94 protein is dispensable for establishing latency in granulocyte-macrophage progenitor cells infected in vitro (White et al., 2000). However, ORF94 inhibits interferon-induced 2′,5′-oligoadenylate synthetase (OAS) during infection in fibroblasts (Tan et al., 2011), an activity also attributed to TRS1 and IRS1 (Child et al., 2004; Marshall et al., 2009). As discussed in later sections, intrinsic and innate defences to viral infection represent an important control point that impacts viral persistence.

**cmvIL-10**

The UL111A gene encodes a viral interleukin-10 homologue, cmvIL-10, with 27% identity to human IL-10 (Jenkins et al., 2004). While cmvIL-10 is not required for the establishment of the latent infection in vitro (Cheung et al., 2009), roles for cmvIL-10 in modulating cellular differentiation, cytokine production and the immune response were recently described and may underlie an important role for cmvIL-10 in persistence. cmvIL-10 suppresses pro-inflammatory cytokine production (Avdic et al., 2011) and inhibits the differentiation of infected progenitors into dendritic cells (Reeves et al., 2005). As dendritic cells provide a permissive environment for viral replication, this activity may contribute to maintenance of latency and a latent reservoir. cmvIL-10 may also compromise dendritic cell (DC) function to limit immune clearance of the virus. Further, cmvIL-10 decreases MHC class II expression in CD34+ HPCs and limits CD4+ T-cell recognition of infected cells (Cheung et al., 2009).

The RhCMV viral IL-10 orthologue dampens the innate immune response by decreasing the overall number of infiltrating immune cells thereby reducing the quality of the response.
ensuing adaptive response (Chang and Barry, 2010). Consistent with a role for an IL-10-like activity in latency, cellular IL-10 has been shown to restrict CMV-specific memory T-cell inflation and increases the latent load during infection with MCMV, which does not encode a viral IL-10 (Jones et al., 2010). Intriguingly, HCMV-infected CD34+ HPCs express increased cellular IL-10 due to decreased expression of the cellular miRNA targeting IL-10, hsa-miR-92a (Poole et al., 2011). These results suggest that in addition to cmvUL-10, HCMV has other mechanisms to modulate IL-10 activity. Taken together, these findings suggest an important role for IL-10, whether encoded by the virus or by the host cell, in shaping the immune response to cytomegalovirus infection and contributing to viral persistence.

**UL133–UL138 locus**

The UL133–UL138 locus is encoded within the ULb′ region of the genome that is unique to clinical isolates of HCMV. The UL133–UL138 locus is defined as a regulator of infection outcomes as the loss of this locus results in three context-dependent phenotypes; it is dispensable for replication in fibroblasts, suppresses replication in haematopoietic cells and is required for replication in primary endothelial cells (Umashankar et al., 2011). The protein encoded by UL138, pUL138, was originally identified as a viral determinant important for the establishment and/or maintenance of a latent infection in CD34+ HPCs infected in vitro (Goodrum et al., 2007; Petrucelli et al., 2009). pUL138 is expressed during both productive and latent infections in a variety of cell types (Petrucelli et al., 2009; Reeves and Sinclair, 2010; Umashankar et al., 2011) and UL138 transcripts are detected in CD34+ and CD14+ cells from latently infected individuals (Goodrum et al., 2007).

pUL138 is expressed from multiple polycistronic transcripts also encoding pUL133, pUL135 and pUL136 (Petrucelli et al., 2009; Grainger et al., 2010). While the roles of pUL135 and pUL136 are not yet known in infection, pUL133 functions similarly to pUL138 in that the disruption of the genes encoding either of these proteins results in increased replicative efficiency in CD34+ HPCs infected in vitro (Umashankar et al., 2011). As pUL133, pUL135, pUL136 and pUL138 are expressed together from polycistronic transcripts and localize together in the Golgi (Umashankar et al., 2011), these proteins likely function co-ordinately in viral infection. Given the localization of pUL133, pUL135, pUL136 and pUL138 to the secretory pathway, they may play unique roles in persistence that have not been described for other herpesviruses. The UL133–UL138 locus is conserved in chimpanzee CMV (ChCMV), but orthologues are absent in viruses infecting lower mammals and, therefore, may represent a novel primate host-specific viral adaptation acquired through co-speciation (Umashankar et al., 2011).

Two groups have recently shown that pUL138 enhances cell surface levels of tumour necrosis factor receptor (TNFR) (Le et al., 2011; Montag et al., 2011). This action restores susceptibility of HCMV-infected cells to TNF-α-induced activation of NFκB, pUL138 expression during the context of infection or overexpression in reporter assays results in modest increases in both major immediate early promoter activation and immediate early protein (IE1-72kDa and IE2-86kDa) accumulation (Petrucelli et al., 2009), an activity that is enhanced by treatment with TNF-α (Montag et al., 2011). The role of pUL138 in sensitizing cells to TNF-α-mediated activation of NFκB and subsequent IE gene expression is consistent with a proposed role in reactivation of viral gene expression (Montag et al., 2011). However, this model is inconsistent with the demonstrated role for pUL138 in suppressing viral replication to promote latency in CD34+ HPCs (Goodrum et al., 2007; Petrucelli et al., 2009; Umashankar et al., 2011). While it is yet unclear what role NFκB plays in HCMV latency or reactivation, the activation of NFκB is critically important for stabilizing γ-herpesvirus latency, including that of Epstein Barr virus (EBV), Kaposi’s sarcoma-associated herpesvirus (KSHV) and murine γ-68 virus (Speck and Ganem, 2010).

**US28**

US28 is one of four G protein-coupled receptors expressed by HCMV and has homology to CC-chemokine receptors (Gao and Murphy, 1994). US28 binds multiple CC-chemokines, including RANTES, MCP-1, MIP1α and MIP-1β and the CX3C-chemokine Fractalkine (Kuhn et al., 1995). In addition to productive infections, US28 expression is detected in latently infected individuals as well as in the THP-1 monocytic cell line infected in vitro (Beisser et al., 2001). US28 expression in monocytes increases IL-8 secretion and alters the adhesion and migration of these cells suggesting that it may contribute to the dissemination of latently infected cells (Randolph-Habecker et al., 2002). Further, fractalkine stimulation of cells expressing US28 induces the migration of macrophages, but not smooth muscle cells, indicating cell type-specific functions of US28 (Vomaske et al., 2009). Taken together, these findings suggest an important role for US28-mediated signalling in virus dissemination. US28 activates signalling and cell proliferation through IL-6-JAK1-STAT3 signalling axis (Singer et al., 2010). Consistent with this activity, transgenic mice expressing US28 develop neoplasia and have increased susceptibility to inflammatory-induced tumours (Bongers et al., 2010).
LUNA

Transcripts antisense to UL81–UL82 encode the 16 kDa latent undefined nuclear antigen, LUNA. LUNA transcripts and antibodies are detected in latently infected individuals (Bego et al., 2005; 2011). While the function of this protein in infection is undetermined, transcript levels of LUNA diminish as immediate early transcripts increase during differentiation of CD34+ cells into dendritic cells and reactivation (Reeves and Sinclair, 2010). As is true of the major immediate early genes, LUNA expression depends on IE1-72kDa to relieve Daxx/ATRX-mediated repression of the LUNA promoter (Reeves et al., 2010).

CMV-miRNAs

Eleven microRNAs (miRNAs) are encoded throughout the HCMV genome (Grey et al., 2005). While many CMV-miRNA targets are unknown, miR-UL112 targets the major immediate early transcript encoding the IE1-72kDa regulator protein (Grey et al., 2007; Murphy et al., 2008). miR-UL112 also targets UL114, reducing its activity as a uracil DNA glycosylase (Stern-Ginossar et al., 2009). Consistent with these activities, miR-UL112 inhibits viral replication in fibroblasts (Grey et al., 2007; Murphy et al., 2008) and could favour the establishment of latency. Intriguingly, miR-UL112 also averts natural killer cell recognition by targeting the cellular stress-inducible MICB ligand for the NKG2D activating receptor (Stern-Ginossar et al., 2009). Taken together, this work begins to define an elegant mechanism by which miR-UL112 co-ordinately downmodulates viral replication and the immune response for viral persistence. Two additional HCMV-coded miRNAs, miR-US25-1 and miR-US25-2, inhibit viral DNA synthesis and viral replication of HCMV (Stern-Ginossar et al., 2009). Similar to these findings for HCMV, HSV-1 expresses at least two miRNAs in latently infected neurons that target the ICP0 and ICP4, and therefore, may contribute to the establishment and maintenance of latency by inhibiting immediate early and early gene expression (Umbach et al., 2008). Herpesvirus-coded miRNAs offer an intriguing potential for regulating viral infection for latency and provide an attractive mechanism that does not require expression of a protein antigen. Studies in rats using RCMV demonstrate that viral miRNA expression is tissue specific and that some are uniquely expressed during states of viral persistence (Meyer et al., 2011).

The balance of cellular responses to infection and viral countermeasures

HCMV masterfully evades all levels of the host response to infection, including intrinsic, innate and adaptive responses. Further, HCMV skilfully manipulates cellular controls including regulation of the cell cycle and gene silencing. Overcoming cellular defences and control of proliferation and gene expression is essential for successful viral replication and persistence. Therefore, the suppressive forces provided by cellular defences and controls may aid the establishment of latency. The balance between the virus–host interactions centred around these cellular responses depend on the context of infection and the repertoire of viral genes expressed. The following subsections will discuss aspects of cell biology that necessarily or potentially impact viral persistence.

Intrinsic defences

As intrinsic cellular defences stand ready to respond at the point of virus binding to the cell surface, they represent an exceptionally important control point for viral infection. These defences include cellular restriction factors that are constitutively expressed and active independent of pathogen encounter. Viruses that are unable to adequately disarm these defences ultimately fail to replicatively (reviewed in Bieniasz, 2004). By extension, these defences could provide a pressure for the virus to establish a latent infection in cell types where the full complement of viral factors required for disarmament are either not expressed or not functional.

Nuclear domain 10 (ND10s), also referred to as promyelocytic leukaemia (PML) oncogenic domains (PODs) or PML nuclear bodies, are dynamic proteinaceous structures comprised of PML, Sp100, hDaxx and ATRX, which play key roles in the intrinsic defence to viral infection. ND10s are juxtaposed with viral genomes, which become viral centres of genome synthesis and replication (Maul, 1998; 2008). Recent studies demonstrate a dynamic relationship between ND10s and viral infection, suggesting that ND10s are recruited to viral genomes (Tavalai et al., 2006; Dimitropoulou et al., 2010). The proteins associated with ND10s negatively impact HCMV replication (Saffert and Kalejta, 2006; Tavalai et al., 2006; 2011; Woodhall et al., 2006; Lukashchuk et al., 2008; Tavalai and Stamminger, 2011). The importance of ND10 to antiviral defence is exemplified in the fact that multiple families of viruses encode functions to disperse or degrade ND10 components including adenovirus, papillomavirus, polyomavirus and arenavirus, in addition to herpesvirus family members (Korioth et al., 1996; Ahn and Hayward, 1997; Everett and Chelbi-Alix, 2007; Lukashchuk et al., 2008; Tavalai and Stamminger, 2008). In HCMV, both the tegument protein pp71 and IE1-72kDa function to disrupt ND10s (reviewed in Maul, 2008; Tavalai and Stamminger, 2011).

The viral tegument protein, pp71, is important during the initial stages of infection (prior to viral gene expression) in establishing a permissive cellular environment for
viral replication. pp71 facilitates transactivation of the major immediate early promoter (MIEP) by degrading the cellular repressor hDaxx and evicting ATRX from ND10 (Ishov et al., 2002; Cantrell and Bresnahan, 2005; 2006; Preston and Nicholl, 2006; Saffert and Kalejta, 2006; 2007; Hwang and Kalejta, 2007), thereby preventing chromatinization and repression of the MIEP (Woodhall et al., 2006). While this strategy facilitates activation of the MIEP for replication in fibroblasts, pp71 is retained in the cytoplasm and cannot inactivate hDaxx in the Ntera2 and THP-1 cell lines or CD34+ HPCs (Saffert and Kalejta, 2007; Saffert et al., 2010). Artificial knock-down of hDaxx permits immediate early gene expression in these cells. This provides a possible strategy by which the viral chromosome is repressed epigenetically in haematopoietic cells for latency. These findings suggest that ND10 disruption is a pivotal control point in controlling the outcome of infection. Consistent with a possible role in creating a repressive environment important for viral latency, cells latently infected with EBV have intact ND10s and their dispersal is coincident with lytic replication (Bell et al., 2000).

Many viral activities associated with viral entry or replication of progeny virions trigger programmed cell death or apoptosis. Certainly, the activation of intrinsic defences will ultimately result in apoptosis without viral-mediated intervention. HCMV actively subverts apoptosis during the productive infection through the action of several virus-coded inhibitors of apoptosis, including IE1 and IE2, UL36/vICA, UL37 exon 1/vMIA and UL38 (Brune, 2011). It is not known if these anti-apoptotic factors play a role in inhibiting cell death during latency. Two groups have recently demonstrated that HCMV infection upregulates myeloid cell leukaemia protein-1 (Mcl-1) (Chan et al., 2010; Reeves et al., 2012), a member of the Bcl-2 family which is important for myeloid cell survival. Mcl-1 activation was dependent on PI3K (Chan et al., 2010) or MAPK/ERK (Reeves et al., 2012) signalling initiated during early events in infection. These differing results suggest that multiple signalling pathways may lead to the same result, perhaps depending on the cell type infected. Subverting cell death pathways to ensure successful productive and latent infections is critical to all herpesviruses (Kaminskyy and Zhivotovsky, 2010).

Epigenetics

Once latency is established, the HCMV genome is presumably maintained as a chromatinized episome. Epigenetic regulation is a key mechanism in regulating viral genome expression during herpesvirus replication and latency (Knipe and Cliffe, 2008; Bloom et al., 2010; Paulus et al., 2010; Takacs et al., 2010; Nevels et al., 2011). During productive infection, all four core histones associate with the HCMV genome, and while nucleosome occupancy remains low, it is dynamic (Nitsche et al., 2008), as has also been shown for KSHV (Toth et al., 2010) and HSV-1 (Cliffe and Knipe, 2008). Low nucleosome occupancy during productive viral replication is likely mediated by viral gene products that prevent depositions or promote eviction of nucleosomes, as has been shown for a number of herpesviruses (reviewed in Paulus et al., 2010) (Reeves et al., 2006). Virus-coded latency determinants may also play an active role in chromatinizing the genome during latency (Wang et al., 2005; Giordani et al., 2008).

Studies in cell lines supporting a latent-like HCMV infection or CD34+ HPCs, demonstrate that the MIEP is associated with repressive heterochromatin protein 1 (HP1) and deacetylated histones (Murphy et al., 2002; Reeves et al., 2005), while the LUNA promoter is associated with activated acetylated histones (Reeves and Sinclair, 2010). While HCMV awaits a global characterization of the epigenetic signature of latency, epigenetic regulation of HSV-1 and KSHV has been more extensively studied (Cliffe et al., 2009; Kwiatkowski et al., 2009; Toth et al., 2010). From these studies, it is clear that latent genomes are not devoid of activating histone modifications (H3K9/K14-ac and H3K4-me3), but that polycomb group proteins concomitantly modify viral genomes with H3K27-me3, which represses transcription in the presence of activating marks (Gunther and Grundhoff, 2010; Toth et al., 2010). Bivalently modified viral genomes allow the genome to persist in a reversibly heterochromatin state poised for reactivation and reveal the difficulty in ascertaining the importance of any individual histone modification without considering the composite of the epigenetic landscape.

Signalling pathways

Pathways of cell signalling are critical to modulating the state of the cell and ultimately the host organism. HCMV initiates and mediates cellular signalling both prior to and following viral gene expression (Yurochko, 2008). In the first tier, signalling is initiated by viral glycoprotein interaction with cellular receptors and by constituents of the viral tegument following infection. In a second tier, cellular signalling may be initiated and modulated by viral gene products during the course of infection. Just as viral-mediated signalling is critical for successful viral replication, cellular signalling events will also contribute importantly to creating a cellular environment permissive for latency. Within the first hour of infection in monocytes, HCMV stimulates a downstream signalling event involving increased pEGFR, PI3K activity and MAPK activity (Bentz and Yurochko, 2008). The PI3K signalling is crucial for upregulating active actin nucleator, N-WASP, to induce...
monocyte motility, an activity favouring haematogenous dissemination and persistence (Chan et al., 2009). HCMV’s upregulation of PI3K signalling also increases Mcl-1, a Bcl-2 member, which inhibits apoptosis post infection (Chan et al., 2010). The signalling pathways initiated during HCMV infection are unique to cell type. For example, HCMV-induced phosphorylation of EGFR is transient in fibroblasts and trophoblasts but chronic in endothelial cells (Wang et al., 2003; LaMarca et al., 2006; Bentz and Yurochko, 2008).

While HCMV clearly alters cellular signalling pathways through interaction with cellular receptors and early events following viral entry, HCMV also encodes trans-activating proteins and viral receptors with the ability to modulate cellular signalling through the course of infection. As discussed previously, US28 is an HCMV-coded G-protein coupled receptor. US28-mediated chemokine signalling events enhance macrophage migration, which likely contributes to viral dissemination and persistence (Yomaske et al., 2009). The role of viral receptors, signalling decoys or homologues in latency represents an important frontier for future work. It will be critical to understand how cellular signalling pathways and the viral modulation of those pathways are integrated to influence outcomes of infection and viral persistence.

Inflammatory, stress and differentiation signals are tightly associated with viral reactivation due to a high density of NFκB, AP1 and CRE transcription factor binding sites in the MIEP (Meier and Stinski, 2006). HCMV dramatically alters the transcriptome of infected monocytes favouring a pro-inflammatory state and differentiation into pro-inflammatory M1 macrophages (Chan et al., 2008a; 2008b). Further, reactivation of the MIEP in NT2 cells can occur through PKCδ signalling and depends on CREB and NFκB binding sites in the MIEP (Liu et al., 2010) or through the PKA-CREB-TORC2 signalling axis (Yuan et al., 2009). HCMV reactivation in DC is associated with IL-6 activation of the ERK/MAPK pathway (Reeves and Compton, 2011).

As CMV initiates signalling cascades in a variety of cells, it is likely that viral-induced or -mediated signalling may also be important for creating an environment permissive for latency in ways that are not yet understood. CMV may differentially mediate signalling pathways depending on the cell type infected and the repertoire of viral genes expressed. Consistent with a key role for signalling in the establishment and maintenance of HCMV latency, latency membrane protein 1 (LMP1) of EBV activates EGFR, ERK and STAT3 (Kung et al., 2011) and NFκB activation is critical for γ-herpesvirus latency (reviewed in Speck and Ganem, 2010). A comprehensive understanding of the signalling events that support HCMV latency awaits further investigation.

Cell cycle regulation

Cell cycle and checkpoint control are intimately connected to the outcome of herpesvirus infection. The complexity inherent to this virus–host interaction is becoming ever-more apparent. G1/G0 cells, but not S/G2 cells, are permissive to HCMV IE expression and viral replication (Sanchez and Spector, 2008). It has been recently shown that high CDK activity, but not PML or Daxx, is the basis for the block to replication in S/G2 (Zydek et al., 2010; 2011). The block is overcome by inhibiting CDK activity either through inducing p21waf1/cip1 or by treating cells with the CDK inhibitor, roscovitine. Treatment with roscovitine also relieves repression of the MIEP in NT2 cells (Zydek et al., 2010). Consistent with these findings, productive EBV replication requires the accumulation of p53 and p21waf1/cip1. The EBV BXLF1 protein both positively and negatively affects p53 levels, a function that may constitute a mechanism by which BXLF1 modulates the switch between latent and productive infection (Sato et al., 2010). Similarly, Tip60, a component of an acetyltransferase complex and upstream regulator of the DNA damage response, is activated by the BGLF 4 kinase in EBV infection and is required for efficient EBV replication (Li et al., 2011). For HCMV, pUL27 has recently been shown to induce p21waf1/cip1 by degrading Tip60 (Reitsma et al., 2011), positively implicating the DNA damage repair pathway and cell cycle arrest in viral replication. These studies suggest a pivotal role for cell cycle checkpoints in modulating permissivity to IE gene expression and possibly regulating the balance between latent and productive states of infection.

Subversion of T-cell recognition

T-cell surveillance plays a critical role in maintenance of viral latency, as reactivation from latency and HCMV disease is associated with a loss of T-cell immunity. HCMV is a master of evading recognition and elimination by CD8+ T-cells and NK cells. This is achieved through the action of a number of genes that prevent activation of NK cells and downregulate viral antigen presentation by the major histocompatibility complex I (MHC-I). The importance of this defence is exemplified in that HCMV encodes six gene products to impede antigen presentation, including US2, US3, US6, US8, U10 and US11, and a number of diverse proteins and miRNAs to evade NK cell recognition (Powers et al., 2008). While it is easy to envision an essential role for viral evasion of MHC-I antigen presentation in the primary infection, recent work reveals that the concerted action of RhCMV orthologues of the HCMV MHC-I evasion proteins is dispensable for the primary infection in rhesus macaques (Hansen et al., 2010). Nevertheless, these genes are required for HCMV
superinfection, suggesting an important role in viral persistence by permitting continual reinfection or by averting immune recognition of reactivating virus.

Despite possessing an exquisite ability to escape antigen presentation and T-cell-mediated clearance, infected individuals maintain exceptionally large populations of CD4+ and CD8+ T-cells specific to HCMV. If these inflated T-cell populations are dysfunctional and contribute to an ‘immune risk’ phenotype (Pawelec and Derhovanessian, 2011), then these findings suggest that HCMV persistence ultimately has a deleterious impact on the host. Recent studies to define immune senescence and the role of CMV in immune senescence in CMV-infected humans, rhesus macaques and mice illustrate the complexity of these questions (reviewed in Wills et al., 2011). Understanding the complicated relationship between HCMV and the host immune response will contribute importantly to our understanding of viral persistence.

Future directions

Herpesvirus latency, and particularly that of HCMV, is the sum total of intricate and multi-layered interactions between the virus and host (Fig. 1). While it is clear that both viral and host mechanisms contribute to persistence, we do not yet have comprehensive understanding of the mechanisms and molecular components involved. Further, how the individual contributions of viral and cellular mechanisms are integrated for viral persistence is not well understood and is the standing challenge for going forward. In the last decade, a number of viral factors and cellular processes have been associated with the latent infection. Understanding how these factors function...
and how the balance of replication promoting and replication suppressing factors is regulated in infection represents a critical next step in understanding mechanisms of viral persistence.

Most fundamental to the understanding of HCMV persistence is the cellular reservoirs for chronic virus shedding and latent genome maintenance in the infected host. While HCMV infects a wide variety of cells in the human host, not all cells are permissive for latency and reservoirs of latency remain to be definitively defined in the human host. The majority of latency studies have focused on haematopoietic cells; however, other cell types, including endothelial and epithelial cells remain important reservoirs that contribute to persistence in ways that are not well understood. Work to define reservoirs of latent and chronic infection is important for understanding cell type-specific interactions culminating in viral persistence and to identify targets for antiviral strategies aimed at latently infected cells.

Confinement of HCMV latency studies to cultured cells is the greatest impediment to understanding the mechanisms fundamental to HCMV persistence in the host. New models, including humanized mice, will permit studies in an intact organism where components of the human host and immune system can be explored (Smith et al., 2010). In addition to appropriate animal models, it is also important to advance relevant primary cell or cell line models to understand the molecular mechanisms underlying latency. Many latency models have used induction of IE gene expression as a marker of reactivation. While this certainly indicates reactivation of IE gene expression, it is problematic as a measure of full reactivation from latency. As elegantly shown in MCMV, resuming IE gene expression is only the first step in a cascade of events that are required to productively reactivate viral replication (Kurz et al., 1999). As the detection of IE transcripts may reflect non-productive reactivation, recovery of infectious virus should remain the gold standard for measuring reactivation.

Taking lessons from the α- and γ-herpesviruses, it will be critical to understand how epigenetic, cellular stress and signalling pathways contribute to an intracellular state required for latency and how the virus may tweak these pathways for the purpose of latency or reactivation. While taken as true, the existence of an episomal genome is largely inferred and the mechanisms by which it is maintained and replicated in latently infected cells are not known. Future studies aimed at understanding regulation of the viral chromosome offer the exciting promise of further advancing our understanding of how cellular intrinsic defence, DNA damage repair pathways and nuclear architecture converge at an epigenetic control point for infection. Emerging technologies and discovery-based approaches, including next-generation sequencing and quantitative proteomics, combined with refined cellular models will be critical to understanding how complex host–virus interactions converge and contribute to viral persistence. Through advanced technologies and refined models, we will define the key virus–host interactions underlying states of infection important to viral persistence.

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