Aspects of Human Cytomegalovirus Latency and Reactivation

M. Reeves, J. Sinclair(✉)

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Abstract Primary infection of healthy individuals with human cytomegalovirus (HCMV) is usually asymptomatic and results in the establishment of a lifelong latent infection of the host. Although no overt HCMV disease is observed in healthy carriers, due to effective immune control, severe clinical symptoms associated with HCMV reactivation are observed in immunocompromised transplant patients and HIV sufferers. Work from a number of laboratories has identified the myeloid lineage as one important site for HCMV latency and reactivation and thus has been the subject of extensive study. Attempts to elucidate the mechanisms controlling viral latency have shown that cellular transcription factors and histone proteins influence HCMV gene expression profoundly and that the type of cellular environment virus encounters upon infection may have a critical role in determining a lytic or latent infection and subsequent reactivation from latency. Furthermore, the identification of a number of viral gene products expressed during latent infection suggests a more active role for HCMV during latency. Defining the role of these viral proteins in latently infected cells will be important for our full understanding of HCMV latency and reactivation in vivo.

J. Sinclair
Department of Medicine, University of Cambridge, Addenbrooke's Hospital Cambridge, CB2 2QQ, UK
js@mole.bio.cam.ac.uk

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Introduction

The ability of human cytomegalovirus (HCMV), like all herpes viruses, to establish a lifelong persistent infection plays a crucial role in the long-term carriage of this opportunistic pathogen in the human host. It is likely that HCMV persistence, in vivo, involves sites in the host which continually produce low levels of virus. However, it is now clear that it also involves sites which carry the viral genome latently in the absence of any productive infection.

Although HCMV causes few overt symptoms following primary infection of healthy individuals, significant morbidity and mortality is observed in the immunonaïve, immunocompromised and immunosuppressed (Ho 1990; Zaia 1990). Primary infection is an important factor in HCMV-mediated disease (see the chapter by W. Britt, this volume), particularly following congenital infection (Griffiths and Walter 2005). However, reactivation from latency is a major cause of disease in certain transplant patients (both solid organ and bone marrow transplantation) and also in late-stage HIV patients suffering from AIDS (Adler 1983; Rubin 1990; Sissons and Carmichael 2002). Consequently, developing an understanding of the mechanisms that regulate latency and reactivation in vivo is of paramount importance for future clinical intervention.

In order to do this, a number of fundamental questions about the basic biology of HCMV need to be addressed: Firstly, in which cells does the latent virus reside? Secondly, in which cells does the virus reactivate. Thirdly, what regulates this latency and reactivation?

The ability to detect latent HCMV, particularly prior to the development of highly sensitive techniques such as the polymerase chain reaction (PCR), is in contrast to the ease of detecting productive HCMV infection during disease. Acute HCMV infection is manifest in numerous tissues (Rubin 1990; Sissons and Carmichael 2002). Epithelial, endothelial, smooth muscle, stromal, fibroblast and neuronal cells all support lytic HCMV replication in vivo (Sinzger et al. 1995; Plachter et al. 1996; see the chapter by C. Sinzger et al., this volume) and, consequently, HCMV pathology can be seen in a diverse range of organs throughout the body. In contrast, HCMV latency appears to be restricted to subpopulations of cell types.

Latency, Carriage and Reactivation of HCMV in the Cells of the Myeloid Lineage

Some of the first instructive observations regarding HCMV latency came from clinical studies. Although it was extremely difficult to detect infectious virus in the blood of normal healthy individuals, it was evident that blood transfusions from healthy seropositive donors often resulted in the transmission of HCMV to blood donor recipients (Adler 1983). However, the incidence of this transmission was significantly reduced if leukocyte-depleted blood products were used (Yeager et al. 1981; Tolpin et al.

1985; Gilbert et al. 1989), which strongly suggested that viral transmission was cell-based and not mediated by free virus. Consequently, one of the cellular sites of latency was believed to be in the peripheral blood compartment.

We now know that the latent load of HCMV in healthy carriers is around 1 genome-positive cell per 10,000 peripheral blood mononuclear cells (PBMCs) (Slobedman and Mocarski 1999), clearly below the detection limits of Northern, Southern and Western analyses. However, the use of a highly-sensitive PCR approach finally permitted the analysis of HCMV latency in vivo and defined the myeloid lineage as an important site of HCMV latency (Fig. 1). An experimental approach that isolated different fractions of cells from the blood of healthy seropositives showed that carriage of HCMV DNA occurred predominantly in the leukocyte fraction of peripheral blood - particularly in the $CD14^+$ monocyte population (Fig. 1a). HCMV was not found in the lymphocyte fraction or the polymorphonuclear cells (Fig. 1b) (Bevan et al. 1991; Taylor-Wiedeman et al. 1991, 1993; Stanier et al. 1992). Monocytes, however, represent a short-lived continually renewable population of cells that arise from haematopoietic cell precursors ($CD34^+$ cells) present in the bone marrow (Metcalf 1989). These cells were also shown to be HCMV genome-positive, suggesting that the bone marrow represents one latent reservoir of virus (Mendelson et al. 1996; Sindre et al. 1996). Although $CD34^+$ cells are sites of latency for HCMV and are a common precursor of both lymphoid and myeloid cells, the carriage of virus appears to be restricted to cells of the monocyte/myeloid lineage by, as yet, undefined mechanisms (for review see Sinclair and Sissons 2006).

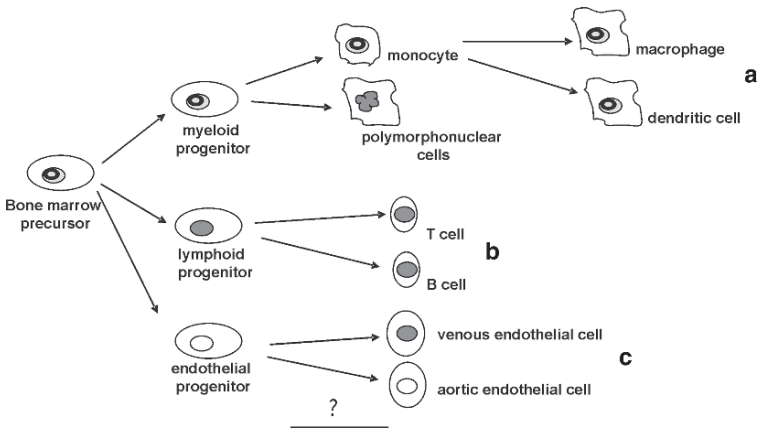

Fig. 1 HCMV latency is established in bone marrow progenitors and is carried in the myeloid lineage. During natural latency, HCMV DNA can be detected in bone marrow progenitor cells that give rise multiple lineages. However, the carriage of viral genomes is detected in the myeloid lineage (**a**) and not the lymphoid lineage (**b**). A third endothelial lineage (**c**) has been proposed, but not proven (*?*), which may also provide another site of HCMV latency in aortic, and not venous, endothelial cells

One major difference between viral latency and reactivation is characteristically defined by the absence of lytic gene transcription during latent carriage of virus. Although transcripts arising from the major IE region of HCMV have been detected during latency (Kondo and Mocarski 1995; Kondo et al. 1996) (see Sect. 4 below), the major IE transcripts IE72 or IE86 are not expressed in naturally latent $CD34^+$ cells or monocytes (Taylor-Wiedeman et al. 1994; Mendelson et al. 1996). Indeed, it is only upon terminal differentiation of these cells to mature macrophage or dendritic cell phenotypes that viral lytic gene expression is observed, which, under certain conditions, can result in complete reactivation and release of infectious virus (Taylor-Wiedeman et al. 1994; Soderberg-Naucler et al. 1997; Soderberg-Naucler et al. 2001; Reeves et al. 2005b). However, attempts to dissect the mechanisms of HCMV latency and reactivation have been hampered by the frequency of seropositive cells in vivo and a lack of a robust tissue culture model which allows a more thorough, large-scale analysis of natural latency.

Models of HCMV Latency Using Experimental Infection

The low frequency of latently infected cells in vivo has resulted in a number of studies of HCMV latency which have been used in experimental infection of cord blood $CD34^+$ cells, fetal liver $CD34^+$ cells (GM-Ps), G-CSF mobilised $CD34^+$ cells and $CD34^+$ cells isolated from bone marrow aspirates (Kondo et al. 1994; Minton et al. 1994; Hahn et al. 1998; Maciejewski and St Jeor 1999; Goodrum et al. 2002; Slobedman et al. 2002; Reeves et al. 2005a). These experimentally infected latent model systems can result in 20%-90% of cells carrying latent virus depending on the cell type, virus strain and multiplicity of infection used. Consequently, such experimental latency model systems result in an increase in the level of latently infected cells in the experimental population and have made it possible to perform more comprehensive analyses which can then be tested in naturally latently infected cells.

Although it is not possible to fully review the wealth of data obtained from these studies here (for reviews see Streblow and Nelson 2003; Bego and St Jeor 2006; Sinclair and Sissons 2006), a number of instructive observations have been made. In general, they suggest that long-term carriage of viral genomes during latency occurs in the absence of any significant viral gene expression and the carriage of latent genomes appears to be specific to certain cell populations which include the precursors of monocytes (CD34/CD33/CD14) and dendritic cells (CD34/CD33/CD1a) (Hahn et al. 1998). Furthermore, reactivation of lytic gene expression requires terminal differentiation of such progenitors to macrophages or dendritic cells (Maciejewski and St Jeor 1999; Reeves et al. 2005a). Thus, there is good agreement between studies on experimental and natural latency and, consequently, these experimental models have been used extensively to address one of the more intriguing aspects of HCMV latency: latent viral gene expression.

Viral Gene Expression Associated with HCMV Latency

Using an experimental latent model system involving in vitro infection of GM-Ps, Kondo et al. first identified cytomegalovirus latency-expressed transcripts (CLTs) from both strands of the MIE region of the genome (Kondo and Mocarski 1995; Kondo et al. 1996). Subsequently, some of the CLTs were identified in the cells of healthy seropositives' bone marrow aspirates and antibodies to these six ORFs were detected in infected individuals (Kondo et al. 1996; Landini et al. 2000). However, their specific role, if any, in latency is unclear as they are expressed during productive infection and deletion of one CLT (pORF94) had no impact on the ability of the virus to establish latency or to reactivate in vitro (White et al. 2000).

Using the same experimentally latent GM-P model, another putative latency-associated transcript has been identified (Jenkins et al. 2004): the UL111a transcript, also expressed during lytic infection (Kotenko et al. 2000). UL111a encodes a viral homologue of interleukin-10 (vIL-10). Unlike its cellular counterpart, vIL-10 encodes only the immunosuppressive functions associated with cellular IL-10 (Spencer et al. 2002) and thus may play a role in avoiding immune surveillance (see the chapter by C. Powers et al., this volume). This provides an attractive mechanism for the increased survival of latently infected cells in vivo. However, detection of the transcript in vivo did not correlate with HCMV serostatus: monocytes from some seronegative individuals were also positive for the v-IL10 transcript. Whether such seronegative donors were DNA-positive or were, perhaps, sero-converting at the time of the analysis was never determined.

More comprehensive analyses of viral gene expression associated with experimental latency have also been carried out using microarrays (Goodrum et al. 2002; Cheung et al. 2006). These detected a large number of viral RNAs, including IE transcripts that were expressed transiently following infection of $CD34^+$ cells or GM-Ps. Consequently, whether all these viral RNAs represent truly latent transcripts requires more in depth analysis. The possibility that some of these viral RNAs may reflect detection of low-level persistent infection in some cells of the experimental latent cultures needs to be completely ruled out. Although the possibility that expression of these virals RNAs are required to establish latent infection, which are progressively switched off during long-term latency, needs consideration (Cheung et al. 2006).

One transcript, however, initially identified by Goodrum et al. with their microarray analysis (Goodrum et al. 2002), has also been shown to be expressed during natural latency in some seropositive monocytes and $CD34^+$ cells (Goodrum et al. 2007). This transcript, encoded by UL138 of the viral genome, may be required for HCMV latency as recombinant viruses lacking UL138 have an impaired ability to establish a latent infection in an experimental model system (Goodrum et al. 2007). However, the exact role of this transcript during latency will require further investigation.

Another recently identified putative latency-associated transcript encoded by HCMV is the LUNA (also known as latency-associated nuclear antigen) transcript (Bego et al. 2005). Identified in a screen of a monocyte cDNA library prepared

from RNA isolated from a healthy HCMV-seropositive individual, this transcript is partially anti-sense to the viral UL81 and UL82 genes. Although a protein product is expressed during productive infection, it has been suggested that LUNA could function as an anti-sense RNA during latency, perhaps mediating the inhibition of pp71 expression from the UL82 ORF. As pp71 is a potent transactivator of the MIEP (major immediate-early promoter) (Bresnahan and Shenk 2000), such suppression of the MIEP could help maintain latency.

Key Aspects of HCMV Latency and Reactivation

Although RNAs expressed in experimentally infected latent models do need to be carefully interpreted, these model systems have also been used extensively to attempt to address other key aspects of viral latency, namely latency establishment and maintenance as well as reactivation.

The Establishment of HCMV Latency

One of the critical steps for establishing latency is likely to include the silencing of the viral MIEP: such control of viral major IE gene expression is a credible mechanism by which all subsequent viral lytic gene expression will be regulated (Fig. 2). Thus, what regulates the MIEP? The MIEP appears to be regulated by multiple cellular transcription factors and higher-order chromatin structure during both lytic (Meier and Stinski 1996; Nevels et al. 2004; Ioudinkova et al. 2006; Reeves et al. 2006) and latent infection (Sinclair and Sissons 2006). Promoter transfection assays have identified a number of factors that repress the MIEP: including YY1 (ying yang 1) (Liu et al. 1994), ERF (ets-2 repressor factor) (Bain et al. 2003) and Gfi-1 (growth factor independent-1) (Zweidler-Mckay et al. 1996). These factors are expressed at high levels in nonpermissive cells and, interestingly, ERF and YY1 are known to interact with chromatin-modifying enzymes (Thomas and Seto 1999; Wright et al. 2005). Consistent with this, during both experimental and natural latency, the transcriptionally inactive MIEP is associated with markers of repressed chromatin, such as Heterochromatin protein 1 (HP1), and is responsive to the histone deacetylase inhibitor Trichostatin A (TSA) providing a model for silencing of the MIEP during experimental (Meier 2001; Murphy et al. 2002; Reeves et al. 2005a) and natural latency (Reeves et al. 2005b). Interestingly, during experimental latency in GM-Ps, cellular factors associated with the formation of repressive chromatin (i.e. AML-1b) are known to be upregulated (Slobedman et al. 2004). Therefore, such cellular responses to latent infection, coupled with a cellular environment already high in levels of repressors of the MIEP, may be critical determinants for the establishment and maintenance of latent carriage of viral genomes.

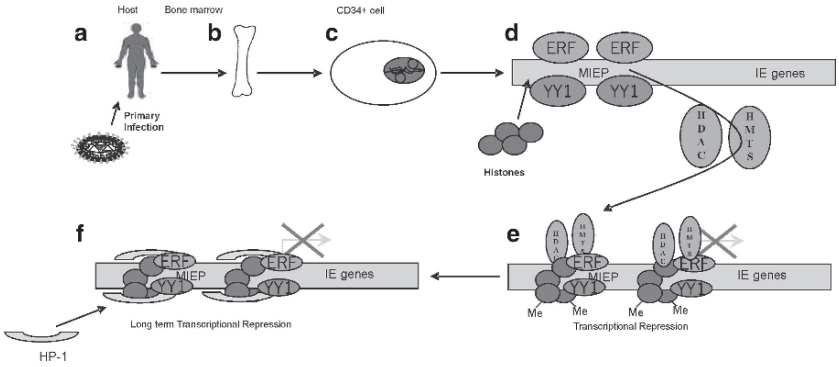

Fig. 2 The establishment of HCMV latency is promoted by chromatin structure. Following infection (**a**), HCMV infects the cells of the bone marrow (**b**) and establishes a latent infection of the $CD34^+$ cells resident therein (**c**). High levels of cellular transcriptional repressors such as ERF and YY1 (**d**) repress the MIEP. As well as transcription factor binding, histone proteins are recruited to the MIEP which become targets for histone deacetylases and histone methyltransferases that are recruited by YY1 and ERF (**e**). These methylated histones become targets for the recruitment of HP-1, which augments repression and the establishment of latency (**f**). Whether any viral products expressed during latency that are important for the repression of the MIEP in this model is, to date, unknown

To date, there is a good consensus that HCMV infects $CD34^+$ haematopoietic stem cells and establishes a latent infection in them. Whilst this is demonstrably true, it has also been suggested that subsets of $CD34^+$ cells may show susceptibility for HCMV productive infection. A study by Goodrum et al. (2004) that analysed differential outcomes of HCMV infection in sorted populations of haematopoietic $CD34^+$ stem cells concluded that infection of one subset of $CD34^+$ cells ($CD34^+$ but $CD38^-$) established the hallmarks of a latent infection (Goodrum et al. 2004), i.e. no detectable virus production but the ability to reactivate upon cellular differentiation. In contrast, other $CD34^+$ cell subpopulations were fully productive for HCMV infection, whilst more mature $CD34^+$ stem cell subpopulations appeared to undergo abortive infection and failed to maintain latent viral genomes. This suggests that the outcome of infection of different $CD34^+$ stem cell subpopulations could depend on the exact phenotype of each stem cell subpopulation. Indeed, there is increasing evidence of early lineage commitment in the haematopoietic stem cell compartment such that a dendritic cell fate, although not irreversible (O'Garra and Trinchieri 2004), is thought to be determined at earlier stages of progenitor cell development (Olweus et al. 1997; Monji et al. 2002). Taken together, these observations could support the hypothesis that HCMV infection of $CD34^+$ stem cells, resulting in latent viral carriage, is restricted to certain subpopulations of $CD34^+$ stem cells. These cells are restricted to specific myeloid cell fates and this mechanism may explain why the carriage of HCMV genomes occurs in some but not all cell types of the myeloid lineage.

Alternatively, infection of $CD34^+$ stem cells could, in itself, promote lineage commitment of the latently infected cell to specific myeloid cell types. Although there is no direct evidence for this, differences in the cellular transcriptome of experimentally latently infected GM-Ps compared with uninfected GM-Ps suggest that such changes in cellular gene expression upon latent infection could, hypothetically, promote lineage commitment of these myeloid progenitor cells (Slobedman et al. 2004). Thus, whether viral genome carriage in only certain myeloid cells is a consequence of HCMV initially infecting specific $CD34^+$ subpopulations which are already committed to different lineages or is due to the latent infection itself, promoting lineage commitment to specific myeloid cell types, is unclear, but both are plausible.

It is clear that a critical determinant of whether the outcome of an infection is productive or latent is dependent on the regulation of IE gene expression: if repression of the MIEP prevails, latency will probably be established (Sinclair and Sissons 1996). However, whether this also involves expression of other specific viral genes is not clear. As stated previously, experimental latency models have identified a wide range of viral transcripts which appear to be expressed during latency. However, many of these are not exclusive to latent infection (Lunetta and Wiedeman 2000; Bego et al. 2005; Goodrum et al. 2007) and their expression has not been confirmed in natural latency (Beisser et al. 2001; Goodrum et al. 2002; Cheung et al. 2006). Consequently, they may simply represent noise from abortive or productive infection in certain subpopulations of progenitor cell types or they may represent a class of viral gene products required to establish latent infection (see Sect. 4, above).

The Maintenance of HCMV Latency

Latency is established within myeloid cells, a cell type with substantial proliferative capacity (Metcalf 1989). Between two and ten copies (Slobedman and Mocarski 1999) of the HCMV genome are carried in an episomal form in mononuclear cells in the peripheral blood of healthy seropositive individuals (Bolovan-Fritts et al. 1999). How the viral genome is maintained in these dividing progenitor cells is unclear. Contrast this with the gamma herpesvirus Epstein-Barr Virus (EBV), which has a defined latent origin of replication (Yates et al. 1984; Adams 1987) and encodes a number of latently expressed genes. Some of these viral genes have defined roles in viral latent genome maintenance in B cells (Leight and Sugden 2000; Young et al. 2000). HCMV does not have similar genes and does not have a known latent origin of replication. Genome replication does not appear to be due to any low-level persistence in the cells in which HCMV is carried in vivo. Recently, Mocarski et al. reported the identification of a segment of genome with proximity to the IE region of HCMV that appears to be important for carriage of the viral genome in an experimentally infected GM-Ps (Mocarski et al. 2006). Since the carriage of viral genomes in these cells occurs without lytic gene expression (Hahn

et al. 1998), and presumably without lytic DNA replication, this region of the viral genome may act as a latent origin of replication or encode a viral gene product essential for maintenance of the viral genome. Characterisation of this region awaits further study.

If latent replication of HCMV genomes does occur, how is segregation of viral genomes to the daughter cells accomplished? This is achieved in EBV and KSHV by using chromatin tethering proteins such as EBNA-1 (Leight and Sugden 2000) and LANA (Cotter and Robertson 1999), respectively. In the absence of an identified homologue in HCMV, how the viral genomes are replicated and segregated during latent carriage is not known.

One alternative, which needs to be considered, is that there is actually no latent replication of HCMV DNA. Instead, $CD34^+$ haematopoietic cells exiting the bone marrow are continually infected from a low level subclinical persistent infection in, for instance, surrounding stromal cells. Once these $CD34^+$ cells enter the blood-stream, they are relatively short-lived and quite quickly differentiate, which results in virus reactivation and virus production from these terminally differentiated myeloid cells. Whether or not any of these models are true will require further study, in perhaps more tractable model systems than we have at present.

The maintenance of HCMV latency is likely to require the continued repression of viral gene expression - particularly IE gene expression - to prevent the virus from re-entering the lytic cycle. In experimentally latent cells, the MIEP is associated with repressed chromatin upon infection (Murphy et al. 2002) and throughout long-term culture as $CD34^+$ cells (Reeves et al. 2005a). Similarly, in naturally latent cells from healthy individuals the MIEP is associated with repressed chromatin (Reeves et al. 2005b). This suggests that virus is kept latent, at least in part, by the recruitment of repressive chromatin factors to the MIEP. However, chromatin is a highly dynamic structure and thus it may be a possibility that yet undefined viral functions have a role in the maintenance of the MIEP in a repressed form, which may also impact on the carriage of the viral genome in a latent state.

Reactivation of HCMV from Latency

Latency is operationally defined as the persistence of the viral genome in the absence of viral lytic gene expression, but importantly, with the capacity of the virus to re-enter its lytic life cycle. The ease and rapidity with which HCMV reactivates in vivo, causing severe disease, is in stark contrast to the ability to reactivate in vitro.

Observations from both experimentally and naturally latent cells suggest that the cellular environment is a key factor in HCMV reactivation: changes in the cellular environment result in the induction of viral lytic gene expression and, hence, virus reactivation (Fig. 3). A number of functions associated with virus infection are known to augment viral IE gene expression. Virus binding on the surface of the cell results in significant changes to the cellular environment by targeting a number of

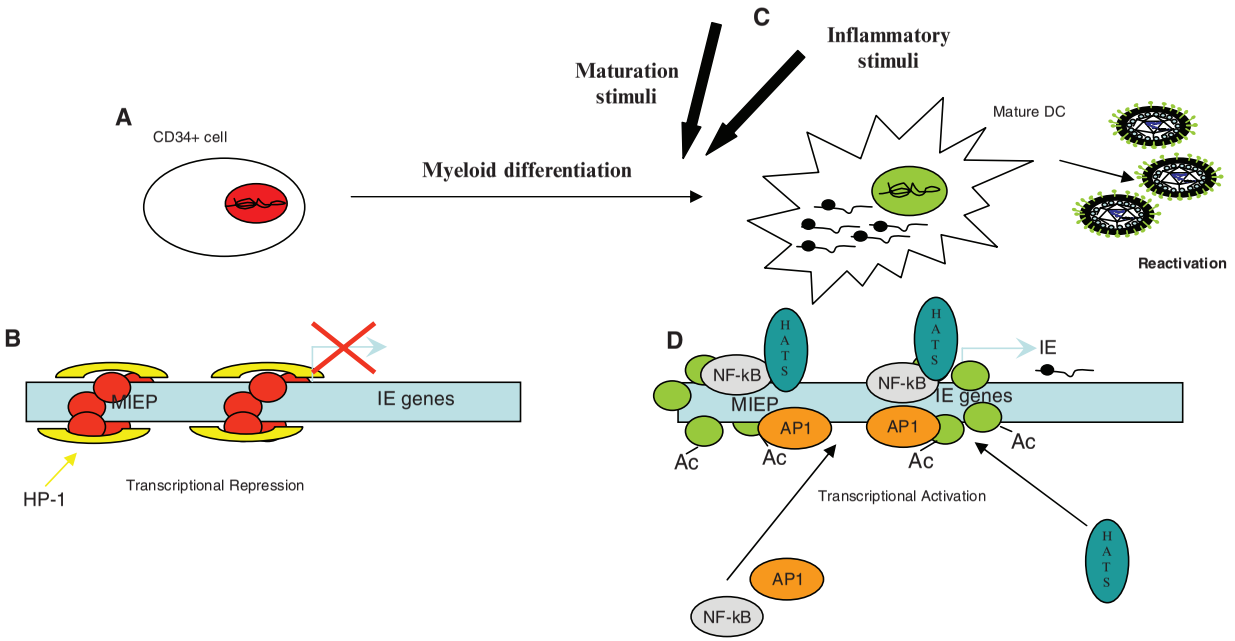

Fig. 3 Reactivation may be promoted by pro-inflammatory signals and is differentiation dependent. CD34⁺ cells carry the HCMV genome in a latent form (**A**), maintained by a repressive chromatin structure around the MIEP (**B**). Exposure of CD34⁺ cells to growth factors and/or inflammatory cytokines promotes myeloid differentiation to mature macrophages and dendritic cells (**C**). Differentiation is concomitant with changes in the levels of specific cellular transcription factors and co-factors. These changes promote the reactivation of HCMV immediate-early gene expression, which is consistent with changes in the chromatin structure of the MIEP from a repressed to an active conformation (**D**). Whether this change is totally regulated by cellular factors or requires a viral product remains unknown

signaling pathways (Fortunato et al. 2000; Simmen et al. 2001; Johnson and Hegde 2002; see the chapters by A. Yurochko and M.K. Isaacson et al., this volume). Viral tegument proteins are also delivered to the cell which can target cellular functions (Everett 2006; see the chapter by G. Maul, this volume). Finally, some of these viral tegument proteins delivered by the virion particle are known to transactivate gene expression to promote high levels of viral IE transcription (Liu and Stinski 1992; Bresnahan and Shenk 2000; Schierling et al. 2004; see the chapter by R; Kaletja, this volume). However, none of these are likely to be involved in reactivation from latency as no virions will be present in the apparent absence of these events during latency; the switch from a latent to a reactivating phenotype requires a latency breaking step. Whether this is a virally encoded latent function or is a consequence of changes to the cellular environment is presently under intense investigation in a number of laboratories.

In our laboratory, we have shown that reactivation of viral gene expression and productive infection in natural (Reeves et al. 2005b) or experimental latency (Murphy et al. 2002; Reeves et al. 2005a) is associated with differentiation of $CD34^+$ cells to a DC phenotype. Histone acetylation at the MIEP facilitates an open chromatin conformation which is permissive for MIEP transcription (Reeves et al. 2005b). Consequently, the implication is that normal changes in cellular transcriptional regulators which occur upon terminal differentiation of myeloid cells could be enough to trigger reactivation of virus IE gene expression.

The likelihood that reactivation from latency occurs in the absence of virally encoded transactivators of IE gene expression implies that the viral genome senses reactivation signals from cellular mediators. The first report of reactivation in vitro from myeloid cells involved the stimulation of monocytes with cytokines derived from allogeneically stimulated T cells (Soderberg-Naucler et al. 1997), including TNF-alpha, interferon-gamma, interleukins and GM-CSF (Soderberg-Naucler et al. 2001). Pro-inflammatory factors or induction of myeloid cell differentiation have been responsible for promoting reactivation of viral major IE gene expression. This scenario may have strong clinical relevance, considering the known association of virus reactivation and CMV disease with transplantation (Sissons et al. 2002). Attempts to further characterise the role of the cytokines have, so far, proved inconclusive and await further study.

Besides the basic regulation of viral IE expression, it is also clear that the interplay between the host immune system and reactivating virus has a profound role in HCMV reactivation in vivo (Sissons et al. 2002; Peggs and Mackinnon 2004). Possibly, HCMV reactivation is a sporadic event, occurring infrequently when certain inflammatory conditions are encountered locally in the host. Alternatively, it could be a more common event, occurring whenever latently infected myeloid cells naturally differentiate. In both cases, any reactivation and virus dissemination, which could result in severe disease, is efficiently controlled by a robust immune response. This may be the reason for the unprecedented number of memory T cells that recognise lytic HCMV antigens from healthy carriers in vivo (Riddell et al. 1991; McLaughlin-Taylor et al. 1994; Wills et al. 1996; Sylwester et al. 2005). Both scenarios are possible and it is likely that that the exact mechanism could lay some-

where between these two extremes; either could account for the success of this virus as an opportunistic pathogen.

In contrast, during transplantation and likely accompanying immune suppression, the cytokine storm induced by transplantation could drive virus reactivation which is uncontrollable in the absence of a good cytotoxic T cell (CTL) response. However, studies to address these types of questions are extremely difficult, requiring analyses with naturally, latently infected cells and homologous or nonhomologous CTLs. Given the frequency of HCMV DNA-positive cells, this would be a challenging exercise. It is becoming increasingly clear that conducting these and other analyses pertinent to HCMV latency will require a system for enriching for HCMV-positive cells in cell populations from naturally infected individuals.

Other Sites of HCMV Latency

Whilst most analyses of HCMV latency have focused on the myeloid lineage, it must be acknowledged that there may be other cellular sites of HCMV latency in vivo. Upon virus reactivation, a diverse number of tissues become infected very rapidly. Whilst this could be attributed to mononuclear cell-mediated dissemination of the virus, it could also equally be attributed to other sites of reactivation (Fig. 1c).

$CD34^+$ stem cells are also believed to be the precursors of some endothelial cells (Quirici et al. 2001), and latent carriage and/or reactivation in these cells has been suggested to be involved in atherosclerotic disease (Epstein et al. 1996; see the chapter by D.N. Streblow et al., this volume). Although the data linking atherosclerosis and HCMV is circumstantial and contentious (Danesh et al. 1997), HCMV reactivation in endothelial cells (ECs) could result from changes in cellular gene expression (Maussang et al. 2006; Reinhardt et al. 2006). Countering this interpretation is the observation that latent HCMV genomes have not been detected in ECs isolated from saphenous vein tissue of seropositive donors (Reeves et al. 2004). Although all ECs are likely to arise from the same progenitor, such an analysis does not preclude the possibility that aortic ECs are sites of latent carriage of HCMV (Jarvis and Nelson 2007). Alternatively, the possibility that a selective segregation of viral genomes also occurs between venous and aortic ECs, as appears to be the case in the myeloid linage, cannot be ruled out (Fig. 1c).

Another possibility is that ECs are sites of low-level persistent HCMV infection, in vivo, rather than sites of true viral latency. Some studies have shown that infected ECs are sites of virus production which show little sign of cell lysis (Fish et al. 1995, 1998). However, other studies have suggested that the infection of ECs is fully cytopathic, resulting in complete cell lysis (Kahl et al. 2000). Whether these differences result from different types of ECs used in the studies or virus strain variation is unclear. What is clear, though, is that the difficulty in obtaining aortic tissue from healthy individuals means it is almost impossible to analyse directly whether these cells are latently or persistently infected.

Conclusion

Fully understanding the establishment and maintenance of HCMV latent infection as well as reactivation from latency remains a weighty problem. As molecular techniques continue to progress, it is likely that global analyses of the effects of HCMV infection on the cellular transcriptome and proteome will become even more possible. As our understanding of the functional heterogeneity of populations of CD34+ haematopoietic precursors increases, it may become possible to further clarify the mechanisms which promote viral latency in only specific cell types. Why the maintenance of HCMV genomes occurs only in particular subsets of cells derived from a common ancestor is unknown. As a consequence, what conditions in these cells facilitate virus reactivation is also unknown.

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