Journal of General Virology (2006), **87**, 1763–1779 DOI 10.1099/vir.0.81891-0

Review

Latency and reactivation of human cytomegalovirus

John Sinclair and Patrick Sissons

Department of Medicine, University of Cambridge, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QQ, UK

Correspondence
John Sinclair
js@mole.bio.cam.ac.uk

Human cytomegalovirus (HCMV) persists as a subclinical, lifelong infection in the normal human host, maintained at least in part by its carriage in the absence of detectable infectious virus – the hallmark of latent infection. Reactivation from latency in immunocompromised individuals, in contrast, often results in serious disease. Latency and reactivation are defining characteristics of the herpesviruses and key to understanding their biology. However, the precise cellular sites in which HCMV is carried and the mechanisms regulating its latency and reactivation during natural infection remain poorly understood. This review will detail our current knowledge of where HCMV is carried in healthy individuals, which viral genes are expressed upon carriage of the virus and what effect this has on cellular gene expression. It will also address the accumulating evidence suggesting that reactivation of HCMV from latency appears to be linked intrinsically to the differentiation status of the myeloid cell, and how the cellular mechanisms that normally control host gene expression play a critical role in the differential regulation of viral gene expression during latency and reactivation.

Introduction

Human cytomegalovirus (HCMV) is a β-herpesvirus (*Human herpesvirus 5*, genus *Cytomegalovirus*, subfamily *Betaherpesvirinae*, family *Herpesviridae*) that causes widespread, persistent human infection. Its seroprevalence can vary from 50 to 90 %, mainly depending on the socio-economic status of the population. HCMV does not usually cause clinically obvious disease upon primary infection of an immunocompetent individual, but clinical disease is much more likely when infection occurs in the immunosuppressed, particularly transplant recipients and patients with AIDS (Drew, 1988; Rubin, 1990). Transplacental transmission during pregnancy or neonatal infection of premature newborns can lead to neurological damage, manifesting itself as deafness or learning disability in early life. Indeed, a study by the US Institute of Medicine that gave particular weighting to quality life years lost in consequence of an infection concluded that HCMV infection was one of the highest priorities for vaccine development (Arvin *et al.*, 2004). During active HCMV infection and during recovery from illness, virus can be detected in many cell types and is excreted in the urine, saliva and breast milk, indicating the involvement of a number of tissues as sites of productive infection and viral transmission during active infection (Stagno *et al.*, 1980; Sinzger *et al.*, 1995). However, exactly how HCMV infection in specific cell types translates into specific pathology during active HCMV infection and disease is far from clear.

During productive infection in the primary human fibroblast, a cell type used extensively *in vitro* for culture of HCMV, the viral genes are expressed in a temporal cascade, the first viral genes expressed being termed the immediate-early (IE) genes (Fig. 1). The most abundantly expressed viral genes at this IE time are transcribed from the major IE locus, which encodes the major IE72 and IE86 gene products (Stenberg, 1996). These viral gene products are generated by differential splicing and polyadenylation of a primary transcript from a single transcription start site (Fig. 1) and play a pivotal role in controlling viral and cellular gene expression to optimize the cellular environment for the production of viral DNA (Colberg-Poley, 1996; Stenberg, 1996; Fortunato *et al.*, 2000; Castillo & Kowalik, 2002; Goldmacher, 2004). After IE expression, transcription of viral early (E) genes and then late genes occurs. E genes generally encode functions associated with viral DNA replication and late genes encode virus structural proteins. Expression of both E and late genes is dependent on IE gene expression (Spector, 1996). Consequently, it has now become clear that expression of the viral major IE gene products is absolutely critical for this temporal cascade of viral gene expression and that the viral major IE proteins act to initiate the virus productive-infection programme.

However, an interesting biological property of HCMV, common to all herpesviruses and with obvious clinical importance, is the ability of the virus to establish lifelong persistence within the host following the initial, normally asymptomatic, infection. This strategy, used by all herpesviruses to persist in the infected individual, is the establishment of cellular sites of viral latency: virus persists in

Published online ahead of print on 15 March 2006 as DOI 10.1099/vir.0.81891-0.

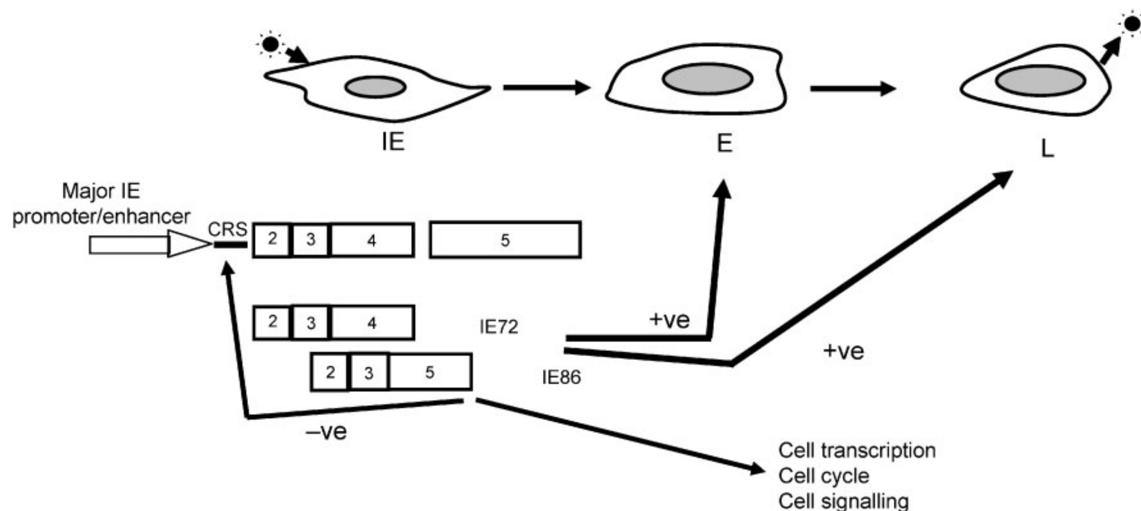

Fig. 1. Regulation of HCMV gene expression during productive infection. As with all herpesviruses, productive infection with HCMV results in a regulated cascade of viral gene expression designated IE, E and late (see text for details). Expression of the major IE gene products, IE72 and IE86 (the two predominant proteins at IE times of infection) is a result of differential splicing of the same primary transcript, IE72 comprising exons 2, 3 and 4 and IE86 comprising exons 2, 3 and 5. IE72 and IE86 act synergistically to activate viral E and late gene expression and IE86 can negatively autoregulate its own promoter by binding to the *cis*-repression signal (CRS). IE86, in particular, also has profound effects on cellular transcription, cell signalling and cell-cycle control.

specific sites in the host, but in the absence of any detectable production of infectious virus.

HCMV can remain latent throughout the lifetime of the host and sporadic reactivation events, if they occur, are generally well-controlled by cell-mediated immunosurveillance. However, as with primary infection, when reactivation occurs in immunocompromised AIDS patients or immunosuppressed transplant patients, HCMV replication can become uncontrolled and often leads to high levels of morbidity and mortality. Reactivation of latent HCMV in these settings presents a very serious clinical problem.

Analyses of virus strains during HCMV infection of, for instance, organ-transplant patients has shown that the predominant cause of infection is reactivation of the transplant recipient's own HCMV rather than virus transfer from the donor (Smyth *et al.*, 1991). Consequently, understanding where and how HCMV persists latently in the seropositive carrier, and particularly what regulates HCMV reactivation, is of major importance for the understanding of HCMV pathogenesis in the transplant scenario.

Consistent with the mechanism of maintenance of latency of other herpesviruses, such as Epstein–Barr virus (EBV) and herpes simplex virus type 1 (HSV1) (Speck *et al.*, 1997; Everett, 2000; Halford & Schaffer, 2001; Sinclair, 2003; Amon & Farrell, 2005), it now appears that what determines reactivation from latency of HCMV is expression of viral IE genes; it is whether expression of these crucial viral lytic gene products occurs that essentially determines commitment to the latent- or lytic-infection programme.

HCMV latency before the era of PCR

Viral latency is defined operationally as the persistence of the viral genome in the absence of production of infectious virions, but with the ability of the viral genome to reactivate under specific stimuli. Initial attempts to define cell types carrying HCMV in healthy, seropositive individuals and to what extent viral gene expression occurred in any such cell types were, for a long time, confounded by the difficulty in detecting HCMV at all in normal, healthy individuals. The ability of healthy, HCMV-seropositive blood donors to transmit HCMV infection to recipients and the fact that transmission by blood could be reduced by using leukocyte-depleted blood products suggested strongly that one site of carriage of this virus was in the peripheral-blood compartment (Yeager *et al.*, 1981; Adler, 1983; Tolpin *et al.*, 1985; de Graan-Hentzen *et al.*, 1989). However, HCMV is notoriously difficult to isolate directly from the blood of healthy donors (Jordan, 1983). Consequently, whilst the peripheral blood of healthy, seropositive individuals does not carry infectious virus, it must carry virus in a form that is reactivatable. Attempts to detect viral DNA, RNA or protein in such cell types by Southern, Northern and Western blot analyses, respectively, or from tissue biopsies of otherwise healthy individuals by *in situ* hybridization or immunohistochemistry produced data that were never reproduced when more sensitive and specific analyses became available (Schrier *et al.*, 1985; Gnann *et al.*, 1988; Hendrix *et al.*, 1997). One immunohistochemical analysis from post-mortem tissue did suggest that viral IE expression could be detected in most, if not all, tissues analysed (Toorkey & Carrigan, 1989), but the effects of post-mortem stress on viral gene

expression could never be ruled out and such levels of viral gene expression were never detected in live tissue biopsies (Gnann *et al.*, 1988). It was not until the technological breakthrough of PCR and its use as a highly sensitive detection tool for low copy numbers of DNA sequences that the question of sites of carriage of HCMV *in vivo* was really able to be addressed.

Cells of the myeloid lineage are an important site of carriage of HCMV

By using highly sensitive PCR strategies, a number of laboratories have demonstrated the presence of HCMV DNA in the peripheral blood leukocytes of healthy, HCMV-seropositive individuals (Taylor-Wiedeman *et al.*, 1991; Stanier *et al.*, 1992; Larsson *et al.*, 1998). These and other analyses, particularly using granulocyte–macrophage colony-stimulating factor (GM-CSF)-mobilized peripheral blood cells (Slobedman & Mocarski, 1999), suggest that the frequency of cells that carry the HCMV genome is extremely low (probably <1 in 10 000 peripheral blood mononuclear cells), requiring very sensitive PCR conditions. It is perhaps for this reason that the detection of HCMV DNA in the blood of healthy carriers by other detection techniques was so difficult and that some laboratories cannot detect HCMV DNA in donor blood by less-sensitive PCR protocols (Bitsch *et al.*, 1992; Meyer-Konig *et al.*, 1997). By using sorted peripheral blood-cell populations, however, it has now become clear that peripheral blood monocytes are a major site of carriage of HCMV DNA in healthy carriers (Taylor-Wiedeman *et al.*, 1991; Larsson *et al.*, 1998).

As with all lymphoid and myeloid cells, monocytes arise from pluripotent, $CD34^{+}$ stem cells present in bone marrow. These $CD34^{+}$ stem cells are believed to be the progenitors of all blood-cell lineages and capable of self-renewal (Metcalf, 1989). These $CD34^{+}$ stem cells differentiate along the myeloid lineage to monoblasts, then promonocytes, in the bone marrow and then enter the bloodstream, where they lose CD34 cell-surface antigen and develop into monocytes (Katz *et al.*, 1985). This lineage development depends upon responses to haematopoietic growth factors, particularly GM-CSF and interleukin 3 (IL-3), which stimulate cell division and differentiation (Adams & Hamilton, 1987).

Interestingly, HCMV DNA can also be detected in such $CD34^{+}$ bone-marrow progenitors (Mendelson *et al.*, 1996). Myeloid progenitors ($CD34^{+}$ cells) give rise not only to monocytes, but also to a number of other cell types including B cells, T cells and polymorphonuclear leukocytes (PMNLs). Consequently, it might be predicted that these cell types could also carry HCMV in the blood of normal, healthy carriers. However, viral genome does not appear to be carried in the PMNL, T-cell or B-cell fractions of peripheral blood (Taylor-Wiedeman *et al.*, 1991, 1993). How the HCMV genome is maintained selectively in only particular subsets of cells arising from common $CD34^{+}$ stem cells that carry viral DNA is not yet understood.

Analysis of the conformation of HCMV DNA in peripheral blood mononuclear cells has also been assessed by using electrophoretic separation on native agarose gels in combination with PCR detection and has shown that the viral genome migrates as a circular plasmid (Bolovan-Fritts *et al.*, 1999) – consistent with carriage of virus genome in these cells as an episome. Whether these viral genomes are replicating actively has been difficult to address. What is known is that little or no viral lytic gene expression can be detected during carriage of HCMV in $CD34^{+}$ progenitors or monocytes, although this conclusion has been contentious. For instance, whilst some analyses using *in situ* hybridization have demonstrated the presence of RNA from the major IE region of HCMV in mononuclear cells of healthy carriers (Schrier *et al.*, 1985), other analyses have failed to detect HCMV IE RNA in seropositive donor tissue, even though biopsies taken after transplant of these tissues into seronegative recipients showed high levels of HCMV IE RNA (Gnann *et al.*, 1988). Such conflicting results may reflect limitations in the specificity or sensitivity of the detection method. However, more sensitive analyses using reverse transcription followed by PCR (RT-PCR) to detect HCMV lytic gene expression in monocytes or monocyte progenitors of healthy, seropositive carriers also failed routinely to detect viral IE gene expression, even though they clearly carried HCMV DNA (Taylor-Wiedeman *et al.*, 1994; Mendelson *et al.*, 1996), consistent with the inability to culture virus from cells. Consequently, at least in monocytic cells and their precursors, it would appear that HCMV is carried in a true latent state, with little or no accompanying viral IE gene expression.

Carriage of HCMV genomes in myeloid cells in healthy, seropositive individuals is, therefore, not associated with any substantial level of lytic infection, arguing against virus genome persistence in these cell types by, for instance, low-level productive infection. However, the fact that there is evidence suggesting that virus genomes are probably carried in such monocytic cells in an episomal form (Bolovan-Fritts *et al.*, 1999) begs the question of whether HCMV DNA is maintained by some type of latent viral DNA replication akin to that observed for EBV (Adams, 1987; Leight & Sugden, 2000). There is a notable lack of direct evidence for a latent origin of replication in HCMV or for any HCMV genes homologous to latent genome-maintenance factors such as the EBV-encoded Epstein–Barr nuclear antigen 1 (EBNA1) and, unlike EBV, there are no established cell lines that are able to carry HCMV stably, which could help to identify such functions. However, Mocarski *et al.* (2006) observed that deletion of DNA sequences close to the viral major IE coding region appears to affect the maintenance of viral genomes in experimentally infected undifferentiated granulocyte–macrophage precursors (GMPs) maintained in long-term culture, an experimental latency system that maintains viral genomes in the absence of productive infection (see below). Whether this indicates a latent origin of replication awaits further investigation.

Alternatively, it is possible that carriage of HCMV genomes in differentiating myeloid cells, at least in the peripheral blood, does not involve replication of viral DNA at all. The differentiation and development of haematopoietic cells is quite rapid *in vivo*; a $CD34^+$ bone-marrow progenitor may take only 2–3 days to exit the marrow and differentiate to terminally differentiated myeloid cell types, such as macrophages or dendritic cells (DCs) (Strobl, 2003). Therefore, it is conceivable that HCMV DNA is carried passively by these myeloid progenitors as they exit the bone marrow into the peripheral blood, where they differentiate, and the resulting macrophages and DCs then reactivate virus; new, latently infected myeloid cells are simply reseeded into the peripheral blood from the bone marrow by, perhaps, a small population of self-renewing, $CD34^+$, pluripotent stem cells. However, if this is the case, then whilst there may be no active maintenance of viral genomes in peripheral blood monocytic cells, it is likely that carriage of virus genomes in any self-renewing stem-cell population would require some form of active viral genome maintenance and segregation to daughter cells or, alternatively, it might result from a continual low-level productive infection of, for instance, bone-marrow stromal cells. What actually happens *in vivo* is still unclear.

Attempts to shed light on this aspect of HCMV biology by trying to identify viral transcripts associated with latent carriage of the virus in naturally infected myeloid cells have been confounded by the extremely low frequency of HCMV genome-positive cells in seropositive carriers, and it is not possible to isolate $CD34^+$ cells or monocytes enriched for the carriage of HCMV genomes from healthy, seropositive individuals to enable comprehensive analyses of viral transcription. This has led to extensive use of experimental infection of differentiating myeloid cell cultures, which takes advantage of the differentiation-dependent permissiveness of myeloid cells for productive infection *in vitro* with HCMV. Experimental infection of, for instance, pluripotent or totipotent bone-marrow progenitor cells results in the carriage of viral genomes for 2–3 weeks in the absence of any substantial production of infectious virus. Virus production can, however, be reactivated after differentiation of these long-term cultures (Kondo *et al.*, 1994; Minton *et al.*, 1994; Hahn *et al.*, 1998; Maciejewski & St Jeor, 1999; Goodrum *et al.*, 2002; Reeves *et al.*, 2005a). Cell systems supporting this type of experimental latency of HCMV have been used to try to identify putative latent viral transcripts; expression of such transcripts can then be confirmed, or otherwise, in naturally infected individuals.

Viral gene expression associated with experimental infection in latent model systems

Kondo and colleagues (Kondo *et al.*, 1994; Kondo & Mocarski, 1995), using experimentally infected GMPs derived from fetal liver cells, originally identified several HCMV transcripts that were expressed in the absence of virus production in these long-term cultures. These cytomegalovirus latency-specific transcripts (CLTs) included novel spliced and unspliced RNA transcripts mapping to both strands of the HCMV major IE region and included open reading frames (ORFs) that were, in some cases, recognized at the protein level by antisera from infected individuals (Kondo *et al.*, 1996). Whilst some of these transcripts were also identified in healthy, seropositive carriers (Kondo & Mocarski, 1995; Kondo *et al.*, 1996; Hahn *et al.*, 1998) and antibodies to some putative CLT ORFs were detected in healthy carriers (Landini *et al.*, 2000), many of the transcripts were also detected in infected cells in culture (Lunetta & Wiedeman, 2000). Hence, the true role of these CLTs, if any, in HCMV latency is yet to be established fully. Initial analysis of one major ORF (ORF94) using an ORF94-deletion virus failed to observe any effect on latency or reactivation after experimental infection of GMPs (White *et al.*, 2000). So far, no viral gene products from these CLTs have been shown to play a role in latency.

The same experimental model of latency has also identified a novel transcript from the unique long (UL) region of the viral genome (UL111.5A), which is predicted to encode a viral homologue of IL-10 (vIL-10). IL-10 is a cytokine that inhibits immune responses and the authors suggested that expression of a homologue may help to avoid host immunosurveillance during latency. However, the role of this transcript in latency is not clear and is somewhat confounded by the observation that detection of this putative viral transcript is not correlated with HCMV serostatus; the transcript was also detected in monocytes from HCMV-seronegative individuals (Jenkins *et al.*, 2004).

Other types of experimentally infected primary haematopoetic cells have also been used to try to identify viral transcripts associated with latent carriage of viral genomes. Long-term growth of $CD34^+$ progenitors from healthy, seropositive individuals on murine stromal cells has been used for a much more comprehensive analysis of viral gene expression using HCMV gene arrays, although the exact phenotype of these cells after their long-term growth was not established (Goodrum *et al.*, 2002). Such experiments originally identified multiple viral RNAs associated with carriage of the virus in the absence of production of infectious virions; many, if not all, of which are also detected during productive infection (Goodrum *et al.*, 2002). However, it was not determined whether any were also detectable in mononuclear cells of normal, healthy carriers. A more detailed analysis of subpopulations of these $CD34^+$ bone-marrow progenitors suggested that different subpopulations of $CD34^+$ progenitor cells are able to support different levels of HCMV infection, ranging from productive to abortive infection to latent carriage of the virus, depending on the phenotype, but, again, this study did not define any latent-specific transcripts (Goodrum *et al.*, 2004).

Based on transcripts identified by Goodrum *et al.* (2002), a more recent analysis of RNA isolated from monocytes of healthy, seropositive carriers has identified viral RNAs antisense to the UL81–82 region of the viral genome (UL81–82ast). Detectable in bone-marrow mononuclear

cells of seropositive but not seronegative individuals (Bego *et al.*, 2005), this transcript contains an ORF of 133 aa that can be identified by Western blot analysis during lytic infection of fibroblasts. As this transcript is antisense to the viral UL82 gene, which encodes the viral pp71 tegument protein, a known transcriptional activator of the viral major IE promoter/enhancer (MIEP), it has been suggested that the transcript or its protein product may be involved in restricting IE gene expression, perhaps helping to maintain latency (Bego *et al.*, 2005). However, confirmation of its role in HCMV latency awaits further study.

One other study using abortive infection of a myelomonocytic cell line (THP1) as a model of latency has also identified the viral US28 gene product as a putative latent transcript (Beisser *et al.*, 2001). However, virus was never reactivated from these cells and the authors did not attempt to detect US28 RNAs in naturally latently infected individuals.

Attempts to identify viral transcription associated with carriage of HCMV in myeloid cells from naturally latently infected individuals has clearly been frustrated by the low frequency of cells carrying latent HCMV *in vivo*. The model systems described above, based on experimental infection of undifferentiated myeloid cells, have succeeded in identifying a number of putative latent viral RNAs. However, a definitive role for any of these RNAs in HCMV latency has yet to be shown.

If latent viral RNAs do exist, it is likely that they are either associated with maintenance of the viral genome directly or that they confer on the cell a gene-expression profile more conducive to carriage of viral genomes. On this basis, experimental infection of undifferentiated myeloid cells, resulting in long-term carriage of virus genomes in the absence of overt productive infection, has also been used to try to identify changes in cellular gene expression that may be associated with HCMV latency.

Cellular gene expression associated with infection in latent model systems

Changes in cellular gene expression associated with productive infection of numerous cell types by HCMV have been studied extensively, either at the single-gene level or at a global level by microarray analyses (Zhu *et al.*, 1998; Challacombe *et al.*, 2004). However, studies to determine changes in the cellular transcriptome of latently infected cells have, once again, been frustrated by the low level of cells carrying viral genomes *in vivo*. Consequently, long-term carriage of experimentally infected, undifferentiated cells has also been used as a model to identify these types of changes in cellular gene expression that may be associated with 'latent' infection.

Initial observations have shown that, whilst a number of cell-surface markers appear not to be altered on experimentally infected GMP cultures, GMPs that carry viral genomes downregulate major histocompatibility class II (MHC II) expression at the protein level (Slobedman *et al.*, 2002). What mediates this MHC class II downregulation is unknown, but it appears to be independent of the previously characterized MHC I and MHC II immunomodulatory genes in the US2–11 region of HCMV, which are known to function during productive infection. Similarly, why MHC class II expression should be targeted in this way is also unknown, but it may help latently infected cells to avoid immunosurveillance (Slobedman *et al.*, 2002). The same latent model system has also recently been used to carry out a global survey of changes in cellular gene expression associated with carriage of experimentally infected virus in GMPs by microarray analysis (Slobedman *et al.*, 2004). This analysis failed to recapitulate the downregulation of MHC class II expression at the RNA level observed previously, but identified an assortment of other cellular transcripts that were up- or downregulated in experimentally infected GMPs carrying HCMV in the absence of productive infection. Many of these changes involved transcripts from cellular genes associated with the immune response, cell growth, signalling and transcriptional regulation, but the viral gene products that mediate these changes and the mechanisms by which they are altered await further analysis, as does the biological impact of such cellular changes on virus–host-cell interactions *in vivo*. Underlying these studies is the crucial question of whether experimental infection of undifferentiated myeloid progenitors and their long-term culture with concomitant carriage of viral genomes in the absence of virus production can really ever recapitulate natural latency *in vivo*. It is very difficult to rule out the possibility that changes in cellular gene expression observed in such cultures result from the initial insult of the experimental infection (normally at very high m.o.i.s) or that cells of a specific phenotype are the only ones that actually support the initial infection *in vitro*. The ability to directly address such cell-specific changes during natural latency awaits technological breakthroughs (perhaps as innovative as PCR was 15 years ago) that will allow the identification and enrichment of bone-marrow progenitor cells or other myeloid cells that specifically carry latent viral genomes from healthy, seropositive individuals.

Similarly, the question as to what changes in cellular gene expression accompany true reactivation of HCMV in the natural setting is of equal importance, yet will only be addressed comprehensively when techniques to identify naturally infected latent cells *in vivo* have been developed.

Are there other sites of latency or long-term carriage of HCMV besides myeloid cells?

Although there is now a real consensus that myeloid cells are an important site of true latency of HCMV *in vivo*, the possibility that other sites of latency occur in normal, healthy carriers requires serious consideration. A vast number of cell types quickly become infected productively upon clinical reactivation, begging the question of whether or not virus in these cells results from reactivation of HCMV

per se or reflects *de novo* infection after rapid dissemination from reactivating mononuclear cells.

Similarly, $CD34^+$ bone marrow-derived cells may also give rise to endothelial cells (ECs) (Quirici *et al.*, 2001) and, hence, the possibility that ECs may be one other reservoir of latent virus has been suggested (Jarvis & Nelson, 2002), perhaps lending support to the circumstantial evidence that has linked HCMV and atherosclerosis (Epstein *et al.*, 1996).

However, we have been unable to detect latent HCMV genomes in ECs or vascular smooth-muscle cells from saphenous vein of healthy, seropositive individuals, even though HCMV DNA can be detected readily in monocytes at the same time (Reeves *et al.*, 2004), suggesting that cells of the microvasculature are unlikely to be important sites of HCMV latency *in vivo*. Whether or not cells of the macrovasculature, such as aortic ECs, are sites of carriage of the virus *in vivo* is more difficult to address, not least because of the obvious difficulty in obtaining such cells *ex vivo* from healthy donors. For the same reason, it has not been possible to analyse many other cell types for latent carriage of HCMV *in vivo*.

The possibility that HCMV may persist in certain cells of healthy carriers as a low-level productive infection with little or no pathology or cell lysis has also been raised. Work using experimental infection of cultured ECs from different sources has suggested that HCMV might persist as a low-level productive infection in aortic ECs in the absence of cytopathic effects (Fish *et al.*, 1996; Streblow & Nelson, 2003) and would go some way to explaining the speed with which a wide variety of cell types become infected *in vivo* during reactivation resulting from, for instance, immunosuppression. However, other workers have observed that ECs, regardless of tissue of origin, show full cytopathic changes upon HCMV infection that always progress to complete lysis (Kahl *et al.*, 2000). Whether other sites *in vivo* (such as bone-marrow stromal cells) might, similarly, act as a virus reservoir maintained by low-level productive infection in the absence of cell lysis has not been addressed fully. An argument against this would be the observation that HCMV appears to undergo a fully lytic, productive infection in bone-marrow fibroblasts and myofibroblasts *in vitro* (Apperley *et al.*, 1989; Michelson *et al.*, 2001).

Nevertheless, these types of considerations do suggest that carriage of the virus *in vivo* is likely to be a complicated affair, with latency (no productive infection) and persistence (low-level productive infection) linked intimately and occurring simultaneously in different cell types. Clearly, myeloid cells represent one true site of latency in normal, healthy carriers, with little or no lytic gene expression. However, terminally differentiated macrophages and DCs in the same individual are likely sites of continual, subclinical reactivation. Consequently it is likely that, *in vivo*, HCMV reactivation occurs routinely in normal, healthy virus carriers, but that this is unlikely to present a problem in the immunocompetent, due to a robust $CD8^+$ cytotoxic T-lymphocyte (CTL) response to the virus. Consistent with this is the observation that the T-cell repertoire of healthy, seropositive individuals contains a strikingly high frequency of CTLs that recognize HCMV epitopes (Borysiewicz *et al.*, 1988; Riddell *et al.*, 1991; McLaughlin-Taylor *et al.*, 1994; Wills *et al.*, 1996; Sylwester *et al.*, 2005). This recognition is dominated by a relatively small number of major viral peptide epitopes, several of them derived from viral structural proteins, suggesting that the immune response to HCMV is subject to regular restimulation, with consequent focusing of the CTL response on a limited number of epitopes (McLaughlin-Taylor *et al.*, 1994; Wills *et al.*, 1996, 2002; Kern *et al.*, 2002; Sylwester *et al.*, 2005) – an event possibly mediated by sporadic levels of subclinical reactivation.

It is also still unclear whether any increased frequency of cells reactivating HCMV from latency results from immunosuppression *per se*; reactivation itself could be stimulated greatly by the numerous cytokines elicited by other infections, allogeneic stimulation, transplant rejection or graft-versus-host disease – all of which often result in, or are treated by, immunosuppression.

The question of what cell types *in vivo* carry HCMV DNA and whether these cells are infected lytically or truly latently is crucial to our understanding of HCMV reactivation and pathogenesis. However, addressing such questions is problematic, mainly because it is difficult to obtain tissue samples for analysis from healthy individuals. Consequently, our knowledge of the sites of carriage of HCMV in the healthy seropositive, other than in cells of the myeloid lineage, and the extent of virus gene expression in those sites is far from comprehensive.

Reactivation of HCMV from naturally latently infected myeloid cells requires differentiation

Nevertheless, the myeloid lineage has provided a wealth of information about the possible mechanisms of latency and reactivation of HCMV *in vivo*.

None of the myeloid cells identified in peripheral blood of healthy individuals that do carry viral DNA produce infectious virus or express viral lytic genes to any appreciable extent (Taylor-Wiedeman *et al.*, 1994; Mendelson *et al.*, 1996); they thus appear to be sites of classical viral latency. However, what has now become apparent is that, once these myeloid cells differentiate to macrophages and DCs, a fundamental change in their ability to support viral IE gene expression occurs. Differentiation of monocytes from healthy, seropositive donors *in vitro* to macrophages is known to result in reactivation of viral IE gene expression (Taylor-Wiedeman *et al.*, 1994), but there was no evidence of reactivation of infectious virus from these cells. However, complete reactivation of infectious virus from peripheral blood mononuclear cells has been reported after their long-term culture and differentiation in the presence of supernatant medium containing cytokines produced by the allogeneic stimulation of T cells (Söderberg-Nauclér *et al.*, 1997). Despite attempts to characterize the specific factors in

the culture media that might be responsible for causing this reactivation, their nature remained unresolved (Söderberg-Nauclér *et al.*, 2001). More detailed analyses have been complicated somewhat by the fact that this approach to reactivating HCMV from naturally latent peripheral blood mononuclear cells has not yet been reproduced independently by other investigators. Nevertheless, cells from which HCMV reactivated in this study were seen to carry macrophage and some DC markers. DCs are highly specialized cells that present antigen to cells of the immune system and thus play a key role in the induction of the immune response. There are at least two types of myeloid lineage-derived DCs: the Langerhans DCs that are located in the epithelium and the interstitial DCs, which reside in deeper-lying tissues (Banchereau *et al.*, 2000).

Consistent with the observation that the cells within naturally infected peripheral blood mononuclear cells from which HCMV reactivates after allogeneic T-cell stimulation carried some DC markers, our laboratory has observed full reactivation of infectious HCMV after *ex vivo* differentiation of $CD34^+$ bone-marrow progenitors to a mature DC phenotype consistent with that of mature Langerhans DCs (Reeves *et al.*, 2005b). These data were based entirely on direct analysis of material isolated from healthy virus carriers and show how the virus selectively utilizes the differentiation pathway of key antigen-presenting cells to persist in its human host. However, only three out of five HCMV-seropositive samples tested reactivated endogenous latent virus, suggesting that some, but not all, donors respond to differentiation-dependent reactivation for reasons that are not yet clear. It was, however, noticed that donors from whom infectious virus could be routinely reactivated tended to carry higher levels of latent viral genome, suggesting that higher HCMV 'latent loads' in a donor may make it easier either to reactivate virus *per se*, as has been suggested for HSV1 (Sawtell, 1998), or to detect reactivation (Reeves *et al.*, 2005b). Consequently, the robustness of this as a system for the routine reactivation of HCMV from naturally infected cells also awaits independent confirmation from other laboratories.

These caveats notwithstanding, there appears to be a fundamental correlation between cellular differentiation and productive infection and the implications of this with respect to the characteristics of HCMV persistence are discussed extensively below.

Permissiveness of myeloid cells for experimental HCMV infection requires cellular differentiation

The differentiation-dependent reactivation of viral lytic gene expression from myeloid cells infected naturally with HCMV, perhaps not surprisingly, also mimics well the relative permissiveness of these different myeloid cell types for experimental infection *in vitro* with virus.

It has been known for some time that peripheral blood monocytic cells are generally non-permissive for experimental infection with HCMV (Rice *et al.*, 1984; Einhorn *et al.*, 1985), whereas their differentiation to a macrophage-like phenotype results in cells that are fully permissive for infection (Ibanez *et al.*, 1991; Lathey & Spector, 1991). More recently, other work (Riegler *et al.*, 2000; Hertel *et al.*, 2003) has shown that, unlike their monocytic or $CD34^+$ progenitors, mature DCs are also fully permissive for experimental HCMV infection and this is consistent with the observations that cells that reactivated naturally acquired latent virus after allogeneic T-cell stimulation of peripheral blood monocytes expressed some DC markers (Söderberg-Nauclér *et al.*, 1997), as well as with the demonstration of HCMV reactivation after specific differentiation of $CD34^+$ cells to mature DCs (Reeves *et al.*, 2005b); clearly, cells that reactivate naturally acquired latent virus would be predicted to be fully permissive for HCMV after experimental infection.

A number of analyses of this differentiation-dependent permissiveness for HCMV infection *in vitro* have shown that this block in permissiveness for HCMV infection in undifferentiated cells is due to their inability to support viral IE gene expression (Riegler *et al.*, 2000; Hertel *et al.*, 2003) and is not due to the inability of cells to bind or internalize virus. Similarly, this block is also observed when using myelotropic and EC-tropic strains of HCMV, which carry about 20 kb of additional DNA sequence that is known to be lost upon long-term carriage of clinical isolates of HCMV in fibroblasts *in vitro* (Cha *et al.*, 1996); loss of specific genes in this region generally leads to the inability to passage fibroblast-adapted strains of HCMV in ECs or DCs (Hahn *et al.*, 2004; Gerna *et al.*, 2005; Wang & Shenk, 2005).

The difficulty in obtaining primary cells from blood for these types of analyses has led investigators to try to identify immortalized or transformed human cell lines that could recapitulate differentiation-dependent permissiveness for HCMV infection, and thus permit a more thorough analysis of what actually may be restricting viral IE gene expression in such a differentiation-dependent manner. Two such cell lines identified were the myelomonocytic THP1 cell line (Weinshenker *et al.*, 1988) and the teratocarcinoma cell line NT2D1 (Gonczol *et al.*, 1984). Both of these lines showed a clear restriction in their ability to support HCMV infection, but their differentiation resulted in a cell type that was fully permissive for full, productive infection.

Clearly, such blocks in virus productive infection could occur at many stages of the viral life cycle and, indeed, there is evidence that some cultured cells are able to express the virus major IE72 protein after infection, but are unable to replicate viral DNA (LaFemina & Hayward, 1988). However, many experiments have concluded that the lack of viral lytic gene expression in undifferentiated NT2D1 or THP1 cells is also not due to a lack of binding or internalization of virus, nor an inability to transport virus genome to the nucleus in these cells, but rather is due to a specific block in expression of viral IE genes, which then prevents later classes of viral gene expression. Differentiation of NT2D1

cells to a neuronal phenotype with retinoic acid (RA) or of THP1 to a macrophage phenotype with phorbol esters (PMA) lifts this block in IE expression, resulting in productive infection (Gonczol *et al.*, 1984; LaFemina & Hayward, 1986; Nelson & Groudine, 1986; Weinshenker *et al.*, 1988; Shelbourn *et al.*, 1989).

These cell types, therefore, represent tractable systems in which permissiveness for viral IE expression and productive infection is differentiation-dependent, similar to that observed for peripheral blood myeloid cells *in vitro*. A number of laboratories have used these cell systems to try to identify differentiation-specific regulators of IE gene expression on the basis that they may help to define factors that play a role in viral latency and reactivation.

Fortunately, the restriction in viral major IE gene expression upon virus infection of undifferentiated cell types *in vitro* also extends to the ability of the viral MIEP to express in transient-transfection analyses in these cell types (LaFemina & Hayward, 1986; Nelson & Groudine, 1986; Shelbourn *et al.*, 1989; Sinclair *et al.*, 1992). This has permitted analyses of the regions of the viral MIEP responsible for such differentiation-dependent repression.

Cellular transcription factors that may be involved in mediating latency and reactivation of HCMV

HCMV major IE gene expression is under the control of the MIEP (Fig. 2). This region is extremely complex and contains one of the strongest-known transcriptional enhancers (made up of an array of 17, 18, 19 and 21 bp-repeat elements) and a far-upstream element, which has been termed the modulator and which includes an imperfect dyad symmetry (Boshart *et al.*, 1985; Lubon *et al.*, 1989; Meier & Stinski, 1996). The modulator region has been shown to be highly sensitive to DNase I and the extent of hypersensitivity changes upon differentiation of cells to a phenotype permissive for HCMV infection (Nelson *et al.*, 1987; Lubon *et al.*, 1989). It has, therefore, long been suggested that changes in binding of cellular transcription factors to the MIEP may play an important role in determining the permissiveness of cells for HCMV IE expression (Lubon *et al.*, 1989; Ghazal *et al.*, 1990; Nelson *et al.*, 1990) and, hence, may help to identify cellular factors controlling virus latency and reactivation *in vivo*.

Transfection and electrophoretic mobility-shift assays have identified a number of cellular transcription factors that can positively regulate expression of the major IE transcription unit (reviewed by Meier & Stinski, 1996). For example, nuclear factor κB (NF-κB) (Sambucetti *et al.*, 1989), cAMP response element-binding protein (CREB) (Hunninghake *et al.*, 1989) and Sp1 (Lang *et al.*, 1992) are known to activate the MIEP by binding to the 18 (NF-κB) and 19 (CREB and SP1) bp-repeat sequences.

However, sequence analysis of the MIEP regions has also identified putative binding sites for a number of other cellular transcription factors that are known to act as transcriptional repressors. These include proteins such as modulator-binding factors (Shelbourn *et al.*, 1989; Kothari *et al.*, 1991), Yin Yang 1 (YY1) (Liu *et al.*, 1994), methylated

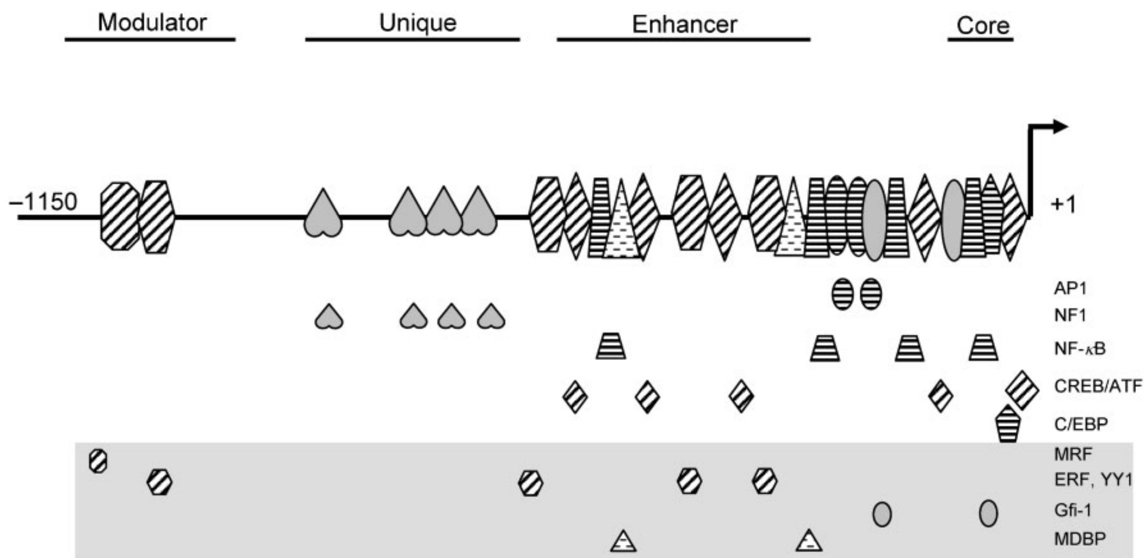

Fig. 2. The HCMV major IE promoter/enhancer (MIEP). The MIEP drives expression of the viral major IE gene products IE72 and IE86 and comprises a core promoter, an enhancer and unique and modulator regions. Within the enhancer, binding sites for known cellular transcription factors have been identified (see text for details). NF-κB, CREB/ATF and YY1/ERF bind to the 18, 19 and 21 bp repeats, respectively. The transcription start site is designated by the forward arrow at +1. Negative-regulatory factors are highlighted by the shaded background.

DNA-binding protein (Zhang *et al.*, 1991), modulator-recognition factor (Huang *et al.*, 1996), growth factor independence-1 (Zweidler-McKay *et al.*, 1996) and Ets-2 repressor factor (ERF) (Bain *et al.*, 2003). Consequently, it was suggested that a balance of these positively and negatively regulating factors determines whether a specific cell type is permissive or non-permissive for viral major IE gene expression and that a change in this balance accompanies differentiation of cells to a permissive phenotype (Sinclair & Sissons, 1996).

Consistent with this, we have found that differentiation-specific repression of the MIEP in non-permissive NT2D1 and THP1 cells is due, at least in part, to DNA sequences within the modulator region and the 21 bp-repeat elements of the enhancer (Shelbourn *et al.*, 1989; Kothari *et al.*, 1991; Liu *et al.*, 1994). These DNA sequences within the MIEP bind YY1 and ERF specifically *in vitro* (Fig. 2) and both YY1 and ERF can mediate repression of the MIEP in transient co-transfection experiments (Liu *et al.*, 1994; Bain *et al.*, 2003). Whilst many groups have shown consistently that the modulator, 21 bp repeats and other upstream elements of the MIEP play a clear role in inhibiting IE gene expression in transfection assays (Shelbourn *et al.*, 1989; Kothari *et al.*, 1991; Zhang *et al.*, 1991; Liu *et al.*, 1994; Huang *et al.*, 1996; Zweidler-McKay *et al.*, 1996; Bain *et al.*, 2003), the question of whether these repressor sites play any role in the context of the virus genome is a valid one.

Recent studies using short interfering RNAs to specifically inhibit expression of some of these putative repressors in normally non-permissive cells have asked whether knock-down of their expression permits expression from the viral MIEP. Unfortunately YY1, as well as negatively regulating the viral MIEP, is a cellular housekeeping gene whose knock-down leads to profoundly detrimental effects on the cell (Bain & Sinclair, 2005).

Other studies using deletion mutants of HCMV lacking the binding sites for these factors in the MIEP have argued against these sites playing a role in the differentiation-dependent repression of the MIEP (Meier & Stinski, 1997). However, the large deletions in the viruses used in such studies have also been shown by the same workers to have perturbed IE gene expression even in permissive cells (Meier & Pruessner, 2000) and this may cause difficulties in such comparisons of IE gene expression in non-permissive and permissive cell types. Consequently, confirmation or otherwise of the role of the modulator and 21 bp repeats in repression of the viral MIEP in the context of the virus awaits more defined, specific virus deletions or mutations.

This notwithstanding, transfection analyses of MIEP reporter constructs in such differentiation-dependent permissive cell lines do suggest that the control of the MIEP by differentiation-specific cellular transcription factors could be a mechanism by which viral lytic gene expression is restricted *in vitro* and that these factors may also be involved in the regulation of viral latency and reactivation *in vivo*.

Until recently, the mechanism by which cellular transcription factors such as YY1 or ERF might act to repress the MIEP was unclear. YY1 is expressed differentially in NT2D1 and THP1 cells and appears to be controlled post-translationally, in that steady-state levels of YY1 RNA are constant throughout differentiation, whereas YY1 protein levels are reduced substantially in differentiated NT2D1 (Pizzorno, 2001) and THP1 cells. In contrast, differentiation of both NT2D1 and THP1 cells is not associated with decreases in steady-state levels of ERF protein (Bain *et al.*, 2003). Consequently, it cannot simply be that differentiation results in a global decrease in protein expression of these MIEP-binding factors. It has now become clear that the mechanism by which YY1 and ERF repress the viral MIEP involves the ability of these proteins to interact physically with and recruit chromatin-modification enzymes to the viral MIEP (Wright *et al.*, 2005); specifically, histone deacetylases (HDACs) that remove acetyl groups from core histone tails. It is the changes in post-translational modification of histones around the viral MIEP resulting from such HDAC recruitment that appear to play a pivotal role in the differentiation-dependent regulation of the MIEP during virus infection of undifferentiated and differentiated cell types.

Chromatin structure of the MIEP upon infection determines the permissiveness of cell lines for productive infection *in vitro*

How do such changes in the post-translational modification of histones regulate the transcriptional activity of specific promoters? It is now very clear that the chromatinization of eukaryotic DNA, mediated by its association with histone proteins, has the dual role of both tightly packaging the DNA into the nucleus and regulating the transcription of cellular genes (Weintraub & Groudine, 1976; Fig. 3). This regulation is imparted by post-translational modification of specific histones (Strahl & Allis, 2000). Generally, histone acetylation resulting from activity of specific histone acetylases is a marker of transcriptionally active chromatin (Lusser, 2002; Kuo & Allis, 1998; Eberharter & Becker, 2002). In contrast, deacetylated histones, resulting from the activity of HDACs, become targets for methylation and the subsequent recruitment of transcription-silencing proteins such as heterochromatin protein 1 (HP1) – methylated histones and HP1 binding both being markers of transcriptionally silenced chromatin (Bannister *et al.*, 2001; Khochbin *et al.*, 2001; Schotta *et al.*, 2002; Rice *et al.*, 2003; Khorasanizadeh, 2004). Recent work has suggested that expression of key genes from a number of viruses is also regulated by their chromatin structure. Indeed, changes in chromatinization of both EBV and HSV1 have been implicated strongly in the control of reactivation of these herpesviruses (Jenkins *et al.*, 2000; Arthur *et al.*, 2001; Kubat *et al.*, 2004; Amon & Farrell, 2005).

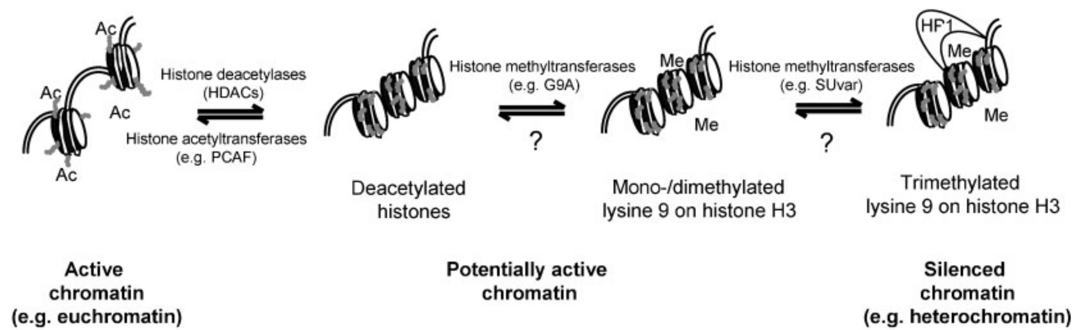

Fig. 3. Regulation of promoter activity by histone acetylation/deacetylation. Expression from certain regions of DNA is turned on and off by modifying histone proteins in the nucleosome. Specific patterns of histone modifications on nucleosomes attract or repel regulatory proteins to chromatinized gene promoters. The levels of histone modification and patterns of modification dictate promoter activity – this is the so-called histone-code hypothesis. Note that whilst there is now evidence of specific mechanisms for histone demethylation of arginine residues on H3 and H4 and lysine 27 on H3, no such specific function has been identified for demethylation of lysine 9 on H3.

A working model for differentiation-dependent, chromatin-mediated regulation of HCMV IE gene expression, which may also be involved in regulation of latency and reactivation during natural infection, is that cellular factors such and YY1 and ERF recruit HDACs specifically to the MIEP in non-permissive cells, resulting in changes in histone modification that impose a transcriptionally repressive environment. Differentiation of normally non-permissive cells to a differentiated, permissive phenotype appears not to be concomitant with a reduction in absolute levels of repressors such as YY1 and ERF themselves (Bain *et al.*, 2003; Wright *et al.*, 2005), but to be due, at least in part, to a reduction in co-factor repressors, such as HDACs, that YY1 and ERF recruit to the MIEP (Murphy *et al.*, 2002; Wright *et al.*, 2005).

A prediction of this would be that, in undifferentiated NT2D1 and monocytic cells, which are refractory to viral IE gene expression, HCMV infection results in association of the viral MIEP with methylated histones and silencing proteins such as HP1. In contrast, infection of differentiated cells, in which IE gene expression is not repressed, should result in association of the viral MIEP with markers of transcriptional activation.

Consistent with this prediction is the observation that treatment of undifferentiated NT2D1 cells with HDAC inhibitors, such as trichostatin A, leads to an increase in IE gene expression in these normally non-permissive cells after experimental infection *in vitro* (Meier, 2001; Murphy *et al.*, 2002). Analysis of the viral MIEP by chromatin immunoprecipitation assays also shows very clearly that, in undifferentiated NT2D1 or monocytic cells, experimental infection results in the association of the viral MIEP with methylated histones and HP1 protein. In contrast, experimental infection of differentiated NT2D1 cells or monocytic cells results in the association of the MIEP with acetylated histones (Murphy *et al.*, 2002), all entirely consistent with the inability of undifferentiated, but not differentiated, cells to support viral IE expression.

Similar experiments using an *in vitro* latency system based on experimental infection of $CD34^+$ cells, their subsequent long-term culture in the absence of lytic gene expression and differentiation to mature DCs resulting in virus reactivation (Reeves *et al.*, 2005a) have shown identical changes in MIEP chromatin acetylation/deacetylation and recruitment of HP1; again, this was correlated specifically to reactivation of viral IE expression and production of infectious virus (Reeves *et al.*, 2005a).

Chromatin structure of the MIEP regulates latency and reactivation of HCMV in natural infection *in vivo*

Clearly, the use of cell lines or myeloid cell-culture systems, which are conditionally permissive for experimental HCMV infection due to their inability to support viral IE gene expression whilst undifferentiated, but not when differentiated, has given enormous insight into the mechanisms by which cell differentiation regulates HCMV lytic gene expression and productive infection. However, whether such mechanisms play a role in the control of latency and reactivation *in vivo*, even though there is a clear link between differentiation of myeloid cells and reactivation of both viral IE expression (Taylor-Wiedeman *et al.*, 1994) and infectious virus (Söderberg-Nauclér *et al.*, 1997; Reeves *et al.*, 2005b) in naturally latently infected cells as well as in experimental latent systems, is a key question.

Recent work from our laboratory has now shown that such differentiation-dependent chromatin remodelling of the viral MIEP by post-translational modification of histones is also involved in the control of latency and reactivation of HCMV *in vivo* (Reeves *et al.*, 2005b). Analysis of the chromatin state of the HCMV MIEP carried out directly on cells isolated from naturally infected, healthy, seropositive donors has shown that, in latent HCMV genomes in

CD34$^+$ cells and monocytes of naturally infected, healthy carriers, the MIEP is also associated with HP1 and not with acetylated histones; this is consistent with transcriptional repression. However, the differentiation of both CD34$^+$ cells and monocytes to mature DCs results in chromatin remodelling of the viral MIEP. Specifically, HP1 is lost from the MIEP and the histones bound to the MIEP become acetylated, consistent with transcriptional activation of viral gene expression. These changes in chromatinization of the viral MIEP correlate precisely with reactivation of infectious virus from the terminally differentiated, mature DCs (Reeves *et al.*, 2005b). Consequently, reactivation of HCMV from true latency appears to be linked intrinsically to the differentiation status of the myeloid cell and, hence, to the cellular mechanisms that normally control host gene expression (Fig. 4).

Interestingly, this chromatin remodelling of the MIEP upon *ex vivo* differentiation of DC progenitors to mature DCs was also linked with changes in expression of specific cellular proteins identified as playing a putative role in the differentiation-dependent regulation of the viral MIEP *in vitro*. Whilst YY1 and ERF showed no differential expression, differentiation of DC progenitors to mature DCs resulted in the downregulation of HDAC-1 protein, a known co-repressor of a number of transcriptional repressors and involved with transcriptional repression mediated by both YY1 (Thomas & Seto, 1999) and ERF (Wright *et al.*, 2005).

Clearly, the differentiation of CD34$^+$ cells or monocytes to DCs does not result in global acetylation of histones bound to all cellular promoters and, hence, their universal transcriptional activation. So what determines this differentiation-specific acetylation of histones associated with the viral MIEP? It is known that a number of cellular promoters are activated specifically upon myeloid differentiation; this includes promoters driving expression of a number of inflammatory cytokines and chemokines required for the function of DCs (Saccani & Natoli, 2002). The activation of such cellular genes is promoted by the activation of the p38–mitogen-activated protein kinase (MAPK) pathway by extracellular signals, such as mitogens and stress signals. The overall result of the activation of the p38–MAPK pathway is to promote the phosphorylation of a number of cellular proteins, including histone H3. This phosphorylation leads to the recruitment of histone acetyltransferases, subsequent acetylation of the histones and ultimately the activation of the target promoter. This response is restricted to a subset of cellular genes that are NF-κB-responsive (Saccani *et al.*, 2002) and it is possible that the activation of the MIEP, an NF-κB-responsive promoter itself, may be due to the activation of the p38–MAPK pathway. Indeed, the stimuli for the

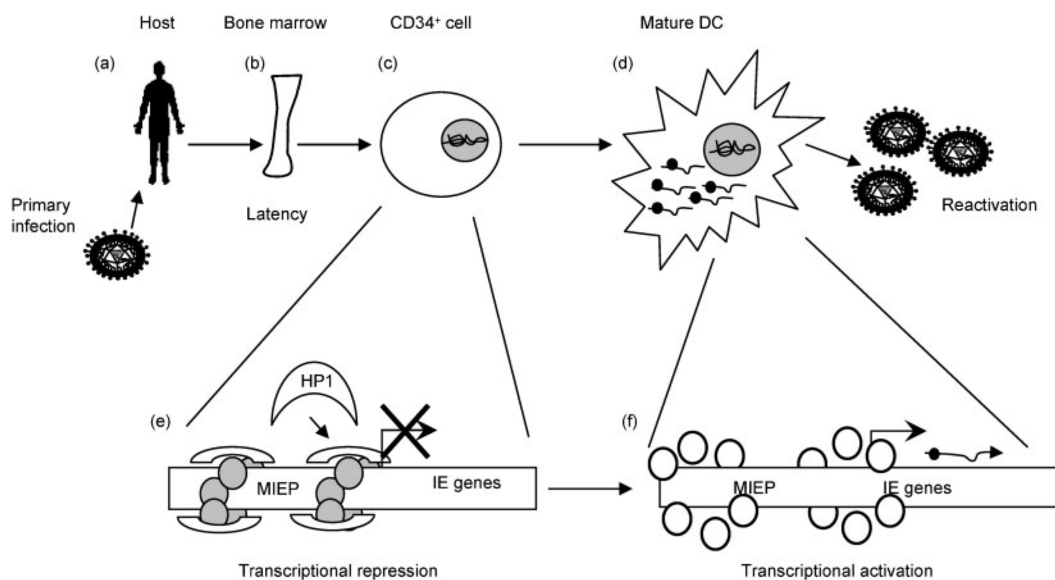

Fig. 4. Regulation of HCMV latency by chromatin remodelling of the MIEP. Following primary infection (a), HCMV establishes a latent infection of the CD34$^+$ bone-marrow mononuclear cells (b). The viral genome persists in the CD34$^+$ cells in the absence of viral lytic gene expression (c) due to the action of cellular transcriptional repressors that bind to the MIEP. These repressors recruit enzymes that modify histones bound to the MIEP (○) such that the MIEP remains in a transcriptionally repressed state (e). However, if the CD34$^+$ cells are differentiated to a mature DC phenotype, the reactivation of lytic gene expression occurs from the previously quiescent genome, which ultimately results in the release of infectious virus progeny (d). The reactivation of lytic gene expression is concomitant with chromatin remodelling of the MIEP into a transcriptionally active state (f). Such chromatin remodelling of the MIEP allows the reactivation of viral lytic gene expression to occur, following specific differentiation of the CD34$^+$ cells to mature DCs. Reproduced with permission from Reeves *et al.* (2005c).

activation of the p38–MAPK pathway in mature DCs can be delivered either by signals resulting from engagement of a cell-surface molecule on DCs, called CD40, or by lipopolysaccharide-induced maturation of DCs – an event that also appears to be crucial for reactivation of HCMV in DCs (Reeves *et al.*, 2005b). Thus, it is likely that differentiation-dependent activation of the viral MIEP requires the coordinated expression of positive regulators of the MIEP that are not found in undifferentiated cells (Meier & Stinski, 2006), as well as changes in expression of specific negative regulators of IE gene expression, and may well be mimicking the positive regulation of inflammatory genes normally associated with DC maturation.

Conclusion

Our understanding of HCMV latency and reactivation has clearly been hampered by the lack of a good model system. However, sensitive detection techniques that have allowed the analysis of sites of carriage of virus, as well as studies of specific cell types that can be infected with HCMV *in vitro*, have begun to give insights into the mechanisms by which HCMV is carried in the healthy host. The difficulty in obtaining samples for analysis from healthy carriers has generally restricted these analyses to the peripheral blood. It cannot be overstated that a comprehensive analysis of other sites, besides the peripheral blood, that may act as true sites of latency/persistence of HCMV in the healthy host is an important goal in HCMV research. For instance, a number of observations hint at the possibility of other, non-haematopoietic sites of latency: the speed with which HCMV reactivation occurs in ductal epithelial cells, the much higher incidence of HCMV transmission in solid-organ compared with bone-marrow transplants and the known ability to detect locally restricted virus reactivation in breast milk during lactation suggest that epithelial cells, as well as other cell types, may harbour latent or persistent virus *in vivo*.

Indeed, analyses of latency of murine cytomegalovirus (MCMV), which we are unable to address in any detail in this review due to space constraints, suggest that latent infection in mice is associated with high loads of latent viral genomes in many tissues (Simon, 2006). At first glance, this seems incompatible with what we know, to date, about HCMV latency. However, it will be crucial to analyse other tissues systematically for the carriage of latent HCMV and to determine whether there really are such fundamental differences between HCMV and MCMV latency *in vivo*.

Still, a substantial body of work to date points clearly to the important role that myeloid cells play in the maintenance of HCMV in the normal host and to the role of cellular differentiation in the control of viral gene expression.

A speculative model of HCMV latency/persistence in the healthy, seropositive carrier would be that virus is maintained in monocytes and their progenitors and probably particularly in the DC precursors within this population, in a truly latent state with the absence of viral lytic gene expression – which in itself may help to evade the host immune response (Fig. 5). This results from the transcriptional milieu of the monocytes/DCs preventing viral IE expression and would correlate well with the known inability to productively infect monocytes *in vitro* or to culture virus from peripheral blood monocytes of healthy, seropositive carriers. However, as monocytes/DCs become terminally differentiated to macrophages/mature DCs, the block in viral IE expression is lifted and the viral genome becomes fully reactivated such that these sites in the host

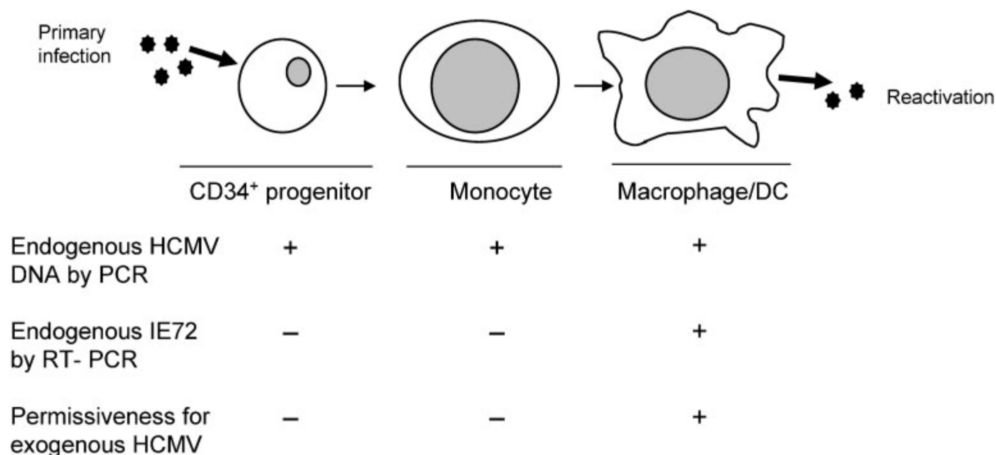

Fig. 5. Speculative model of HCMV latency and reactivation in the normal carrier. Although viral DNA can be detected in all cells of the myeloid lineage by sensitive PCR during natural latent infection, viral IE expression only occurs upon differentiation of cells to macrophages or DCs. This results in a transcriptional milieu conducive to IE expression, resulting in reactivation of infectious virus. This fits well with the ability of these cell types to support IE expression and full productive infection after experimental infection *in vitro*.

may be looked upon as being persistently infected. In this situation, however, the host's cytotoxic T-cell response acts efficiently to clear these productively infected cells unless they are immunocompromised, at which time virus disseminates, resulting in disease.

At present, we know little about where or how latent viral genomes are maintained in the nucleus of the cell. It is likely to be in an episomal form, but whether it is associated with cellular chromosomes, as for EBV (Leight & Sugden, 2000), is not known. It is clear that during experimental infection *in vitro*, HCMV genomes, as with other herpesviruses, are deposited at or close to specific nuclear structures termed nuclear domain 10 (ND10), which have been suggested to be sites of suppression of viral IE gene expression (Tang & Maul, 2006). Whether such sites play any role in repression of IE gene expression during latency will need to be investigated actively.

Similarly, our understanding of the carriage of HCMV in bone marrow is also incomplete. Virus can clearly enter bone-marrow progenitors after experimental infection *in vitro* and persist without lytic gene expression during long-term culture of these cells; HCMV DNA can be detected by PCR in the absence of IE RNA in bone-marrow progenitors of naturally infected carriers. Taken together, these observations would be consistent with the bone marrow acting as a reservoir of latent HCMV after primary infection, which then seeds latent virus into the peripheral blood via the monocytes/DCs. Differentiation of these cells to tissue macrophages and DCs then results in local reactivation. This model of HCMV carriage and latency/persistence of HCMV in the healthy carrier, although still speculative, is consistent with many of the observations that have been made to date (Fig. 5).

The recent *in vivo* studies that we have outlined above now suggest a particularly strong relationship between HCMV and DCs and show that persistence of HCMV is associated intimately with the normal programme of myeloid-cell differentiation; it is the changes in the internal cellular environment that accompany differentiation that promote virus reactivation. HCMV DNA remains latent in myeloid DC progenitors of naturally infected carriers and latent carriage of HCMV is maintained until specific differentiation of these cells to a mature DC phenotype. This results in reactivation of infectious virus from the DCs of some healthy, seropositive individuals, consistent with DC being a biologically significant site of viral reactivation *in vivo*.

As with other herpesviruses, the induction of IE lytic gene expression from latent virus represents the critical event required for the switch from latency to reactivation. Analyses *in vitro* have shown that transcriptional activation of viral IE gene expression from the viral MIEP requires the action of cellular transcription factors and changes in chromatinization of the MIEP. Consistent with this, chromatin remodelling of the viral MIEP also plays a crucial role in reactivation of HCMV from latency upon DC differentiation in naturally infected individuals. Latent HCMV in DC progenitors *in vivo* is in a closed, transcriptionally silent chromatin conformation. In contrast, differentiation of these progenitors to mature DCs results in specific chromatin remodelling of the MIEP to an open, transcriptionally active chromatin structure, resulting in reactivation of viral lytic IE gene expression and production of infectious virus.

Understanding the relationships between HCMV and such professional antigen-presenting cells in detail at the cellular level is important for our understanding of how this virus persists in the host and the complexities of its interaction with the immune system. For example, it is well-recognized that HCMV possesses an unusually large number of genes encoding functions designed to help it evade the cellular immune response; it now seems plausible that these may reflect the particular problems facing the virus in residing in, and in particular reactivating from, cells whose specific function is to initiate immune responses. This new knowledge may also aid the development of more effective treatments for the prevention of HCMV-mediated disease due to reactivation. Clearly, future studies will be needed to define precisely the biochemical triggers responsible for myeloid DC differentiation, as these also appear to promote the switch from viral latency to reactivation. These may help to generate specific strategies to more effectively control virus reactivation, an important aspect of HCMV biology that also leads to a substantial amount of HCMV-mediated disease – particularly that occurring in the context of solid-organ and bone-marrow transplantation.

Acknowledgements

We would like to thank many members of our laboratory, past and present, whose work has contributed to the studies described here. We also apologize to colleagues in the field whose work could not be cited due to space limitations. This work was supported by the Medical Research Council and the Wellcome Trust. We particularly thank Mark Bain, Matthew Reeves and Mark Wills for critical reading of the manuscript.

References

Adams, A. (1987). Replication of latent Epstein-Barr virus genomes in Raji cells. *J Virol* **61**, 1743–1746.

Adams, D. O. & Hamilton, T. A. (1987). Molecular transductional mechanisms by which IFN gamma and other signals regulate macrophage development. *Immunol Rev* **97**, 5–27.

Adler, S. P. (1983). Transfusion-associated cytomegalovirus infections. *Rev Infect Dis* **5**, 977–993.

Amon, W. & Farrell, P. J. (2005). Reactivation of Epstein-Barr virus from latency. *Rev Med Virol* **15**, 149–156.

Apperley, J. F., Dowding, C., Hibbin, J., Buiter, J., Matutes, E., Sissons, P. J., Gordon, M. & Goldman, J. M. (1989). The effect of cytomegalovirus on hemopoiesis: in vitro evidence for selective infection of marrow stromal cells. *Exp Hematol* **17**, 38–45.

Arthur, J. L., Scarpini, C. G., Connor, V., Lachmann, R. H., Tolkovsky, A. M. & Efstathiou, S. (2001). Herpes simplex virus type 1 promoter

activity during latency establishment, maintenance, and reactivation in primary dorsal root neurons in vitro. *J Virol* **75**, 3885–3895.

Arvin, A. M., Fast, P., Myers, M., Plotkin, S. & Rabinovich, R. (2004). Vaccine development to prevent cytomegalovirus disease: report from the National Vaccine Advisory Committee. *Clin Infect Dis* **39**, 233–239.

Bain, M. & Sinclair, J. (2005). Targeted inhibition of the transcription factor YY1 in an embryonal carcinoma cell line results in retarded cell growth, elevated levels of p53 but no increase in apoptotic cell death. *Eur J Cell Biol* **84**, 543–553.

Bain, M., Mendelson, M. & Sinclair, J. (2003). Ets-2 Repressor Factor (ERF) mediates repression of the human cytomegalovirus major immediate-early promoter in undifferentiated non-permissive cells. *J Gen Virol* **84**, 41–49.

Banchereau, J., Briere, F., Caux, C., Davoust, J., Lebecque, S., Liu, Y.-J., Pulendran, B. & Palucka, K. (2000). Immunobiology of dendritic cells. *Annu Rev Immunol* **18**, 767–811.

Bannister, A. J., Zegerman, P., Partridge, J. F., Miska, E. A., Thomas, J. O., Allshire, R. C. & Kouzarides, T. (2001). Selective recognition of methylated lysine 9 on histone H3 by the HP1 chromo domain. *Nature* **410**, 120–124.

Bego, M., Maciejewski, J., Khaiboullina, S., Pari, G. & St Jeor, S. (2005). Characterization of an antisense transcript spanning the UL81-82 locus of human cytomegalovirus. *J Virol* **79**, 11022–11034.

Beisser, P. S., Laurent, L., Virelizier, J.-L. & Michelson, S. (2001). Human cytomegalovirus chemokine receptor gene US28 is transcribed in latently infected THP-1 monocytes. *J Virol* **75**, 5949–5957.

Bitsch, A., Kirchner, H., Dupke, R. & Bein, G. (1992). Failure to detect human cytomegalovirus DNA in peripheral blood leukocytes of healthy blood donors by the polymerase chain reaction. *Transfusion* **32**, 612–617.

Bolovan-Fritts, C. A., Mocarski, E. S. & Wiedeman, J. A. (1999). Peripheral blood $CD14^{+}$ cells from healthy subjects carry a circular conformation of latent cytomegalovirus genome. *Blood* **93**, 394–398.

Borysiewicz, L. K., Hickling, J. K., Graham, S., Sinclair, J., Cranage, M. P., Smith, G. L. & Sissons, J. G. P. (1988). Human cytomegalovirus-specific cytotoxic T cells. Relative frequency of stage-specific CTL recognizing the 72-kD immediate early protein and glycoprotein B expressed by recombinant vaccinia viruses. *J Exp Med* **168**, 919–931.

Boshart, M., Weber, F., Jahn, G., Dorsch-Hasler, K., Fleckenstein, B. & Schaffner, W. (1985). A very strong enhancer is located upstream of an immediate early gene of human cytomegalovirus. *Cell* **41**, 521–530.

Castillo, J. P. & Kowalik, T. F. (2002). Human cytomegalovirus immediate early proteins and cell growth control. *Gene* **290**, 19–34.

Cha, T.-A., Tom, E., Kemble, G. W., Duke, G. M., Mocarski, E. S. & Spaete, R. R. (1996). Human cytomegalovirus clinical isolates carry at least 19 genes not found in laboratory strains. *J Virol* **70**, 78–83.

Challacombe, J. F., Rechtsteiner, A., Gottardo, R., Rocha, L. M., Browne, E. P., Shenk, T., Altherr, M. R. & Brettin, T. S. (2004). Evaluation of the host transcriptional response to human cytomegalovirus infection. *Physiol Genomics* **18**, 51–62.

Colberg-Poley, A. M. (1996). Functional roles of immediate early proteins encoded by the human cytomegalovirus UL36-38, UL115-119, TRS1/IRS1 and US3 loci. *Intervirology* **39**, 350–360.

de Graan-Hentzen, Y. C., Gratama, J. W., Mudde, G. C. & 7 other authors (1989). Prevention of primary cytomegalovirus infection in patients with hematologic malignancies by intensive white cell depletion of blood products. *Transfusion* **29**, 757–760.

Drew, W. L. (1988). Diagnosis of cytomegalovirus infection. *Rev Infect Dis* **10** (Suppl. 3), S468–S476.

Eberharter, A. & Becker, P. B. (2002). Histone acetylation: a switch between repressive and permissive chromatin. *EMBO Rep* **3**, 224–229.

Einhorn, L., Einhorn, S. & Wahren, B. (1985). Interferon induction in human leukocytes after in vitro exposure to cytomegalovirus or Epstein-Barr virus. *Intervirology* **23**, 140–149.

Epstein, S. E., Speir, E., Zhou, Y. F., Guetta, E., Leon, M. & Finkel, T. (1996). The role of infection in restenosis and atherosclerosis: focus on cytomegalovirus. *Lancet* **348** (Suppl. 1), S13–S17.

Everett, R. D. (2000). ICP0, a regulator of herpes simplex virus during lytic and latent infection. *Bioessays* **22**, 761–770.

Fish, K. N., Britt, W. & Nelson, J. A. (1996). A novel mechanism for persistence of human cytomegalovirus in macrophages. *J Virol* **70**, 1855–1862.

Fortunato, E. A., McElroy, A. K., Sanchez, I. & Spector, D. H. (2000). Exploitation of cellular signaling and regulatory pathways by human cytomegalovirus. *Trends Microbiol* **8**, 111–119.

Gerna, G., Percivalle, E., Lilleri, D., Lozza, L., Fornara, C., Hahn, G., Baldanti, F. & Revello, M. G. (2005). Dendritic-cell infection by human cytomegalovirus is restricted to strains carrying functional UL131–128 genes and mediates efficient viral antigen presentation to $CD8^{+}$ T cells. *J Gen Virol* **86**, 275–284.

Ghazal, P., Lubon, H., Reynolds-Kohler, C., Hennighausen, L. & Nelson, J. A. (1990). Interactions between cellular regulatory proteins and a unique sequence region in the human cytomegalovirus major immediate-early promoter. *Virology* **174**, 18–25.

Gnann, J. W., Jr, Ahlmen, J., Svalander, C., Olding, L., Oldstone, M. B. & Nelson, J. A. (1988). Inflammatory cells in transplanted kidneys are infected by human cytomegalovirus. *Am J Pathol* **132**, 239–248.

Goldmacher, V. S. (2004). Cell death suppressors encoded by cytomegalovirus. *Prog Mol Subcell Biol* **36**, 1–18.

Gonczol, E., Andrews, P. W. & Plotkin, S. A. (1984). Cytomegalovirus replicates in differentiated but not in undifferentiated human embryonal carcinoma cells. *Science* **224**, 159–161.

Goodrum, F. D., Jordan, C. T., High, K. & Shenk, T. (2002). Human cytomegalovirus gene expression during infection of primary hematopoietic progenitor cells: a model for latency. *Proc Natl Acad Sci U S A* **99**, 16255–16260.

Goodrum, F., Jordan, C. T., Terhune, S. S., High, K. & Shenk, T. (2004). Differential outcomes of human cytomegalovirus infection in primitive hematopoietic cell subpopulations. *Blood* **104**, 687–695.

Hahn, G., Jores, R. & Mocarski, E. S. (1998). Cytomegalovirus remains latent in a common precursor of dendritic and myeloid cells. *Proc Natl Acad Sci U S A* **95**, 3937–3942.

Hahn, G., Revello, M. G., Patrone, M. & 9 other authors (2004). Human cytomegalovirus UL131-128 genes are indispensable for virus growth in endothelial cells and virus transfer to leukocytes. *J Virol* **78**, 10023–10033.

Halford, W. P. & Schaffer, P. A. (2001). ICP0 is required for efficient reactivation of herpes simplex virus type 1 from neuronal latency. *J Virol* **75**, 3240–3249.

Hendrix, R. M. G., Wagenaar, M., Slobbe, R. L. & Bruggeman, C. A. (1997). Widespread presence of cytomegalovirus DNA in tissues of healthy trauma victims. *J Clin Pathol* **50**, 59–63.

Hertel, L., Lacaille, V. G., Strobl, H., Mellins, E. D. & Mocarski, E. S. (2003). Susceptibility of immature and mature Langerhans cell-type dendritic cells to infection and immunomodulation by human cytomegalovirus. *J Virol* **77**, 7563–7574.

Huang, T. H., Oka, T., Asai, T., Okada, T., Merrills, B. W., Gertson, P. N., Whitson, R. H. & Itakura, K. (1996). Repression by a differentiation-specific factor of the human cytomegalovirus enhancer. *Nucleic Acids Res* **24**, 1695–1701.

Hunninghake, G. W., Monick, M. M., Liu, B. & Stinski, M. F. (1989). The promoter-regulatory region of the major immediate-early gene of human cytomegalovirus responds to T-lymphocyte stimulation and contains functional cyclic AMP-response elements. *J Virol* **63**, 3026–3033.

Ibanez, C. E., Schrier, R., Ghazal, P., Wiley, C. & Nelson, J. A. (1991). Human cytomegalovirus productively infects primary differentiated macrophages. *J Virol* **65**, 6581–6588.

Jarvis, M. A. & Nelson, J. A. (2002). Mechanisms of human cytomegalovirus persistence and latency. *Front Biosci* **7**, d1575–d1582.

Jenkins, P. J., Binné, U. K. & Farrell, P. J. (2000). Histone acetylation and reactivation of Epstein-Barr virus from latency. *J Virol* **74**, 710–720.

Jenkins, C., Abendroth, A. & Slobedman, B. (2004). A novel viral transcript with homology to human interleukin-10 is expressed during latent human cytomegalovirus infection. *J Virol* **78**, 1440–1447.

Jordan, M. C. (1983). Latent infection and the elusive cytomegalovirus. *Rev Infect Dis* **5**, 205–215.

Kahl, M., Siegel-Axel, D., Stenglein, S., Jahn, G. & Sinzger, C. (2000). Efficient lytic infection of human arterial endothelial cells by human cytomegalovirus strains. *J Virol* **74**, 7628–7635.

Katz, F. E., Tindle, R., Sutherland, D. R. & Greaves, M. F. (1985). Identification of a membrane glycoprotein associated with haemopoietic progenitor cells. *Leuk Res* **9**, 191–198.

Kern, F., Bunde, T., Faulhaber, N. & 10 other authors (2002). Cytomegalovirus (CMV) phosphoprotein 65 makes a large contribution to shaping the T cell repertoire in CMV-exposed individuals. *J Infect Dis* **185**, 1709–1716.

Khochbin, S., Verdel, A., Lemercier, C. & Seigneurin-Berny, D. (2001). Functional significance of histone deacetylase diversity. *Curr Opin Genet Dev* **11**, 162–166.

Khorasanizadeh, S. (2004). The nucleosome: from genomic organization to genomic regulation. *Cell* **116**, 259–272.

Kondo, K. & Mocarski, E. S. (1995). Cytomegalovirus latency and latency-specific transcription in hematopoietic progenitors. *Scand J Infect Dis Suppl* **99**, 63–67.

Kondo, K., Kaneshima, H. & Mocarski, E. S. (1994). Human cytomegalovirus latent infection of granulocyte–macrophage progenitors. *Proc Natl Acad Sci U S A* **91**, 11879–11883.

Kondo, K., Xu, J. & Mocarski, E. S. (1996). Human cytomegalovirus latent gene expression in granulocyte–macrophage progenitors in culture and in seropositive individuals. *Proc Natl Acad Sci U S A* **93**, 11137–11142.

Kothari, S., Baillie, J., Sissons, J. G. P. & Sinclair, J. H. (1991). The 21bp repeat element of the human cytomegalovirus major immediate early enhancer is a negative regulator of gene expression in undifferentiated cells. *Nucleic Acids Res* **19**, 1767–1771.

Kubat, N. J., Tran, R. K., McAnany, P. & Bloom, D. C. (2004). Specific histone tail modification and not DNA methylation is a determinant of herpes simplex virus type 1 latent gene expression. *J Virol* **78**, 1139–1149.

Kuo, M.-H. & Allis, C. D. (1998). Roles of histone acetyltransferases and deacetylases in gene regulation. *Bioessays* **20**, 615–626.

LaFemina, R. & Hayward, G. S. (1986). Constitutive and retinoic acid-inducible expression of cytomegalovirus immediate-early genes in human teratocarcinoma cells. *J Virol* **58**, 434–440.

LaFemina, R. L. & Hayward, G. S. (1988). Differences in cell type-specific blocks to immediate early gene expression and DNA replication of human, simian and murine cytomegalovirus. *J Gen Virol* **69**, 355–374.

Landini, M. P., Lazzarotto, T., Xu, J., Geballe, A. P. & Mocarski, E. S. (2000). Humoral immune response to proteins of human cytomegalovirus latency-associated transcripts. *Biol Blood Marrow Transplant* **6**, 100–108.

Lang, D., Fickenscher, H. & Stamminger, T. (1992). Analysis of proteins binding to the proximal promoter region of the human cytomegalovirus IE-1/2 enhancer/promoter reveals both consensus and aberrant recognition sequences for transcription factors Sp1 and CREB. *Nucleic Acids Res* **20**, 3287–3295.

Larsson, S., Söderberg-Nauclér, C., Wang, F. Z. & Moller, E. (1998). Cytomegalovirus DNA can be detected in peripheral blood mononuclear cells from all seropositive and most seronegative healthy blood donors over time. *Transfusion* **38**, 271–278.

Lathey, J. L. & Spector, S. A. (1991). Unrestricted replication of human cytomegalovirus in hydrocortisone-treated macrophages. *J Virol* **65**, 6371–6375.

Leight, E. R. & Sugden, B. (2000). EBNA-1: a protein pivotal to latent infection by Epstein-Barr virus. *Rev Med Virol* **10**, 83–100.

Liu, R., Baillie, J., Sissons, J. G. P. & Sinclair, J. H. (1994). The transcription factor YY1 binds to negative regulatory elements in the human cytomegalovirus major immediate early enhancer/promoter and mediates repression in non-permissive cells. *Nucleic Acids Res* **22**, 2453–2459.

Lubon, H., Ghazal, P., Hennighausen, L., Reynolds-Kohler, C., Lockshin, C. & Nelson, J. (1989). Cell-specific activity of the modulator region in the human cytomegalovirus major immediate-early gene. *Mol Cell Biol* **9**, 1342–1345.

Lunetta, J. M. & Wiedeman, J. A. (2000). Latency-associated sense transcripts are expressed during *in vitro* human cytomegalovirus productive infection. *Virology* **278**, 467–476.

Lusser, A. (2002). Acetylated, methylated, remodeled: chromatin states for gene regulation. *Curr Opin Plant Biol* **5**, 437–443.

Maciejewski, J. P. & St Jeor, S. C. (1999). Human cytomegalovirus infection of human hematopoietic progenitor cells. *Leuk Lymphoma* **33**, 1–13.

McLaughlin-Taylor, E., Pande, H., Forman, S. J., Tanamachi, B., Li, C. R., Zaia, J. A., Greenberg, P. D. & Riddell, S. R. (1994). Identification of the major late human cytomegalovirus matrix protein pp65 as a target antigen for CD8+ virus-specific cytotoxic T lymphocytes. *J Med Virol* **43**, 103–110.

Meier, J. L. (2001). Reactivation of the human cytomegalovirus major immediate-early regulatory region and viral replication in embryonal NTera2 cells: role of trichostatin A, retinoic acid, and deletion of the 21-base-pair repeats and modulator. *J Virol* **75**, 1581–1593.

Meier, J. L. & Pruessner, J. A. (2000). The human cytomegalovirus major immediate-early distal enhancer region is required for efficient viral replication and immediate-early gene expression. *J Virol* **74**, 1602–1613.

Meier, J. L. & Stinski, M. F. (1996). Regulation of human cytomegalovirus immediate-early gene expression. *Intervirology* **39**, 331–342.

Meier, J. L. & Stinski, M. F. (1997). Effect of a modulator deletion on transcription of the human cytomegalovirus major immediate-early genes in infected undifferentiated and differentiated cells. *J Virol* **71**, 1246–1255.

Meier, J. L. & Stinski, M. F. (2006). Major immediate-early enhancer and its gene products. In *Cytomegaloviruses: Molecular Biology and Immunology*, pp. 150–166. Edited by M. J. Reddehase. Wymondham, UK: Caister.

Mendelson, M., Monard, S., Sissons, P. & Sinclair, J. (1996). Detection of endogenous human cytomegalovirus in $CD34^+$ bone marrow progenitors. *J Gen Virol* **77**, 3099–3102.

Metcalf, D. (1989). The molecular control of cell division, differentiation commitment and maturation in haemopoietic cells. *Nature* **339**, 27–30.

Meyer-Konig, U., Hufert, F. T. & von Laer, D. M. (1997). Infection of blood and bone marrow cells with the human cytomegalovirus in vivo. *Leuk Lymphoma* **25**, 445–454.

Michelson, S., Rohrlich, P., Beisser, P. & 7 other authors (2001). Human cytomegalovirus infection of bone marrow myofibroblasts enhances myeloid progenitor adhesion and elicits viral transmission. *Microbes Infect* **3**, 1005–1013.

Minton, E. J., Tysoe, C., Sinclair, J. H. & Sissons, J. G. P. (1994). Human cytomegalovirus infection of the monocyte/macrophage lineage in bone marrow. *J Virol* **68**, 4017–4021.

Mocarski, E. S., Jr, Hahn, G., White, K. L., Xu, J., Slobedman, B., Hertel, L., Aguirre, S. A. & Noda, S. (2006). Myeloid cell recruitment and function in pathogenesis and latency. In *Cytomegaloviruses: Molecular Biology and Immunology*, pp. 465–481. Edited by M. J. Reddehase. Wymondham, UK: Caister.

Murphy, J. C., Fischle, W., Verdin, E. & Sinclair, J. H. (2002). Control of cytomegalovirus lytic gene expression by histone acetylation. *EMBO J* **21**, 1112–1120.

Nelson, J. A. & Groudine, M. (1986). Transcriptional regulation of the human cytomegalovirus major immediate-early gene is associated with induction of DNase I-hypersensitive sites. *Mol Cell Biol* **6**, 452–461.

Nelson, J. A., Reynolds-Kohler, C. & Smith, B. A. (1987). Negative and positive regulation by a short segment in the 5′-flanking region of the human cytomegalovirus major immediate-early gene. *Mol Cell Biol* **7**, 4125–4129.

Nelson, J. A., Gnann, J. W., Jr & Ghazal, P. (1990). Regulation and tissue-specific expression of human cytomegalovirus. *Curr Top Microbiol Immunol* **154**, 75–100.

Pizzorno, M. C. (2001). Nuclear cathepsin B-like protease cleaves transcription factor YY1 in differentiated cells. *Biochim Biophys Acta* **1536**, 31–42.

Quirici, N., Soligo, D., Caneva, L., Servida, F., Bossolasco, P. & Deliliers, G. L. (2001). Differentiation and expansion of endothelial cells from human bone marrow CD133$^+$ cells. *Br J Haematol* **115**, 186–194.

Reeves, M. B., Coleman, H., Chadderton, J., Goddard, M., Sissons, J. G. P. & Sinclair, J. H. (2004). Vascular endothelial and smooth muscle cells are unlikely to be major sites of latency of human cytomegalovirus *in vivo*. *J Gen Virol* **85**, 3337–3341.

Reeves, M. B., Lehner, P. J., Sissons, J. G. P. & Sinclair, J. H. (2005a). An *in vitro* model for the regulation of human cytomegalovirus latency and reactivation in dendritic cells by chromatin remodelling. *J Gen Virol* **86**, 2949–2954.

Reeves, M. B., MacAry, P. A., Lehner, P. J., Sissons, J. G. P. & Sinclair, J. H. (2005b). Latency, chromatin remodeling, and reactivation of human cytomegalovirus in the dendritic cells of healthy carriers. *Proc Natl Acad Sci U S A* **102**, 4140–4145.

Reeves, M., Sissons, P. & Sinclair, J. (2005c). Reactivation of human cytomegalovirus in dendritic cells. *Discov Med* **5**, 170–174.

Rice, G. P. A., Schrier, R. D. & Oldstone, M. B. A. (1984). Cytomegalovirus infects human lymphocytes and monocytes: virus expression is restricted to immediate-early gene products. *Proc Natl Acad Sci U S A* **81**, 6134–6138.

Rice, J. C., Briggs, S. D., Ueberheide, B., Barber, C. M., Shabanowitz, J., Hunt, D. F., Shinkai, Y. & Allis, C. D. (2003). Histone methyltransferases direct different degrees of methylation to define distinct chromatin domains. *Mol Cell* **12**, 1591–1598.

Riddell, S. R., Rabin, M., Geballe, A. P., Britt, W. J. & Greenberg, P. D. (1991). Class I MHC-restricted cytotoxic T lymphocyte recognition of cells infected with human cytomegalovirus does not require endogenous viral gene expression. *J Immunol* **146**, 2795–2804.

Riegler, S., Hebart, H., Einsele, H., Brossart, P., Jahn, G. & Sinzger, C. (2000). Monocyte-derived dendritic cells are permissive to the complete replicative cycle of human cytomegalovirus. *J Gen Virol* **81**, 393–399.

Rubin, R. H. (1990). Impact of cytomegalovirus infection on organ transplant recipients. *Rev Infect Dis* **12** (Suppl. 7), S754–S766.

Saccani, S. & Natoli, G. (2002). Dynamic changes in histone H3 Lys 9 methylation occurring at tightly regulated inducible inflammatory genes. *Genes Dev* **16**, 2219–2224.

Saccani, S., Pantano, S. & Natoli, G. (2002). p38-dependent marking of inflammatory genes for increased NF-κB recruitment. *Nat Immunol* **3**, 69–75.

Sambucetti, L. C., Cherrington, J. M., Wilkinson, G. W. G. & Mocarski, E. S. (1989). NF-κB activation of the cytomegalovirus enhancer is mediated by a viral transactivator and by T cell stimulation. *EMBO J* **8**, 4251–4258.

Sawtell, N. M. (1998). The probability of in vivo reactivation of herpes simplex virus type 1 increases with the number of latently infected neurons in the ganglia. *J Virol* **72**, 6888–6892.

Schotta, G., Ebert, A., Krauss, V., Fischer, A., Hoffmann, J., Rea, S., Jenuwein, T., Dorn, R. & Reuter, G. (2002). Central role of *Drosophila* SU(VAR)3–9 in histone H3-K9 methylation and heterochromatic gene silencing. *EMBO J* **21**, 1121–1131.

Schrier, R. D., Nelson, J. A. & Oldstone, M. B. (1985). Detection of human cytomegalovirus in peripheral blood lymphocytes in a natural infection. *Science* **230**, 1048–1051.

Shelbourn, S. L., Kothari, S. K., Sissons, J. G. & Sinclair, J. H. (1989). Repression of human cytomegalovirus gene expression associated with a novel immediate early regulatory region binding factor. *Nucleic Acids Res* **17**, 9165–9171.

Simon, C., Seckert, C., Grzimek, N. & Reddehase, M. (2006). Murine models of cytomegalovirus latency and reactivation. In *Cytomegaloviruses: Molecular Biology and Immunology*, pp. 483–500. Edited by M. J. Reddehase. Wymondham, UK: Caister.

Sinclair, A. J. (2003). bZIP proteins of human gammaherpesviruses. *J Gen Virol* **84**, 1941–1949.

Sinclair, J. & Sissons, P. (1996). Latent and persistent infections of monocytes and macrophages. *Intervirology* **39**, 293–301.

Sinclair, J. H., Baillie, J., Bryant, L. A., Taylor-Wiedeman, J. A. & Sissons, J. G. P. (1992). Repression of human cytomegalovirus major immediate early gene expression in a monocytic cell line. *J Gen Virol* **73**, 433–435.

Sinzger, C., Grefte, A., Plachter, B., Gouw, A. S. H., The, T. H. & Jahn, G. (1995). Fibroblasts, epithelial cells, endothelial cells and smooth muscle cells are major targets of human cytomegalovirus infection in lung and gastrointestinal tissues. *J Gen Virol* **76**, 741–750.

Slobedman, B. & Mocarski, E. S. (1999). Quantitative analysis of latent human cytomegalovirus. *J Virol* **73**, 4806–4812.

Slobedman, B., Mocarski, E. S., Arvin, A. M., Mellins, E. D. & Abendroth, A. (2002). Latent cytomegalovirus down-regulates major histocompatibility complex class II expression on myeloid progenitors. *Blood* **100**, 2867–2873.

Slobedman, B., Stern, J. L., Cunningham, A. L., Abendroth, A., Abate, D. A. & Mocarski, E. S. (2004). Impact of human cytomegalovirus latent infection on myeloid progenitor cell gene expression. *J Virol* **78**, 4054–4062.

Smyth, R. L., Sinclair, J., Scott, J. P., Gray, J. J., Higenbottam, T. W., Wreghitt, T. G., Wallwork, J. & Borysiewicz, L. K. (1991). Infection and reactivation with cytomegalovirus strains in lung transplant recipients. *Transplantation* **52**, 480–482.

Söderberg-Nauclér, C., Fish, K. N. & Nelson, J. A. (1997). Reactivation of latent human cytomegalovirus by allogeneic stimulation of blood cells from healthy donors. *Cell* **91**, 119–126.

Söderberg-Nauclér, C., Streblow, D. N., Fish, K. N., Allan-Yorke, J., Smith, P. P. & Nelson, J. A. (2001). Reactivation of latent human cytomegalovirus in CD14$^+$ monocytes is differentiation dependent. *J Virol* **75**, 7543–7554.

Speck, S. H., Chatila, T. & Flemington, E. (1997). Reactivation of Epstein-Barr virus: regulation and function of the *BZLF1* gene. *Trends Microbiol* **5**, 399–405.

Spector, D. H. (1996). Activation and regulation of human cytomegalovirus early genes. *Intervirology* **39**, 361–377.

Stagno, S., Reynolds, D. W., Pass, R. F. & Alford, C. A. (1980). Breast milk and the risk of cytomegalovirus infection. *N Engl J Med* **302**, 1073–1076.

Stanier, P., Kitchen, A. D., Taylor, D. L. & Tyms, A. S. (1992). Detection of human cytomegalovirus in peripheral mononuclear cells and urine samples using PCR. *Mol Cell Probes* **6**, 51–58.

Stenberg, R. M. (1996). The human cytomegalovirus major immediate-early gene. *Intervirology* **39**, 343–349.

Strahl, B. D. & Allis, C. D. (2000). The language of covalent histone modifications. *Nature* **403**, 41–45.

Streblow, D. N. & Nelson, J. A. (2003). Models of HCMV latency and reactivation. *Trends Microbiol* **11**, 293–295.

Strobl, H. (2003). Molecular mechanisms of dendritic cell sublineage development from human hematopoietic progenitor/stem cells. *Int Arch Allergy Immunol* **131**, 73–79.

Sylwester, A. W., Mitchell, B. L., Edgar, J. B. & 9 other authors (2005). Broadly targeted human cytomegalovirus-specific CD4$^+$ and CD8$^+$ T cells dominate the memory compartments of exposed subjects. *J Exp Med* **202**, 673–685.

Tang, Q. & Maul, G. G. (2006). Immediate early interactions and epigenetic defense mechanisms. In *Cytomegaloviruses: Molecular Biology and Immunology*, pp. 131–149. Edited by M. J. Reddehase. Wymondham, UK: Caister.

Taylor-Wiedeman, J., Sissons, J. G. P., Borysiewicz, L. K. & Sinclair, J. H. (1991). Monocytes are a major site of persistence of human cytomegalovirus in peripheral blood mononuclear cells. *J Gen Virol* **72**, 2059–2064.

Taylor-Wiedeman, J., Hayhurst, G. P., Sissons, J. G. P. & Sinclair, J. H. (1993). Polymorphonuclear cells are not sites of persistence of human cytomegalovirus in healthy individuals. *J Gen Virol* **74**, 265–268.

Taylor-Wiedeman, J., Sissons, P. & Sinclair, J. (1994). Induction of endogenous human cytomegalovirus gene expression after differentiation of monocytes from healthy carriers. *J Virol* **68**, 1597–1604.

Thomas, M. J. & Seto, E. (1999). Unlocking the mechanisms of transcription factor YY1: are chromatin modifying enzymes the key? *Gene* **236**, 197–208.

Tolpin, M. D., Stewart, J. A., Warren, D. & 7 other authors (1985). Transfusion transmission of cytomegalovirus confirmed by restriction endonuclease analysis. *J Pediatr* **107**, 953–956.

Toorkey, C. B. & Carrigan, D. R. (1989). Immunohistochemical detection of an immediate early antigen of human cytomegalovirus in normal tissues. *J Infect Dis* **160**, 741–751.

Wang, D. & Shenk, T. (2005). Human cytomegalovirus UL131 open reading frame is required for epithelial cell tropism. *J Virol* **79**, 10330–10338.

Weinshenker, B. G., Wilton, S. & Rice, G. P. (1988). Phorbol ester-induced differentiation permits productive human cytomegalovirus infection in a monocytic cell line. *J Immunol* **140**, 1625–1631.

Weintraub, H. & Groudine, M. (1976). Chromosomal subunits in active genes have an altered conformation. *Science* **193**, 848–856.

White, K. L., Slobedman, B. & Mocarski, E. S. (2000). Human cytomegalovirus latency-associated protein pORF94 is dispensable for productive and latent infection. *J Virol* **74**, 9333–9337.

Wills, M. R., Carmichael, A. J., Mynard, K., Jin, X., Weekes, M. P., Plachter, B. & Sissons, J. G. P. (1996). The human cytotoxic T-lymphocyte (CTL) response to cytomegalovirus is dominated by structural protein pp65: frequency, specificity, and T-cell receptor usage of pp65-specific CTL. *J Virol* **70**, 7569–7579.

Wills, M. R., Okecha, G., Weekes, M. P., Gandhi, M. K., Sissons, P. J. G. & Carmichael, A. J. (2002). Identification of naive or antigen-experienced human CD8$^+$ T cells by expression of costimulation and chemokine receptors: analysis of the human cytomegalovirus-specific CD8$^+$ T cell response. *J Immunol* **168**, 5455–5464.

Wright, E., Bain, M., Teague, L., Murphy, J. & Sinclair, J. (2005). Ets-2 repressor factor recruits histone deacetylase to silence human cytomegalovirus immediate-early gene expression in non-permissive cells. *J Gen Virol* **86**, 535–544.

Yeager, A. S., Grumet, F. C., Hafleigh, E. B., Arvin, A. M., Bradley, J. E. & Prober, C. G. (1981). Prevention of transfusion-acquired cytomegalovirus infections in newborn infants. *J Pediatr* **98**, 281–287.

Zhang, X.-Y., Inamdar, N. M., Supakar, P. C., Wu, K., Ehrlich, K. C. & Ehrlich, M. (1991). Three MDBP sites in the immediate-early enhancer-promoter region of human cytomegalovirus. *Virology* **182**, 865–869.

Zhu, H., Cong, J.-P., Mamtora, G., Gingeras, T. & Shenk, T. (1998). Cellular gene expression altered by human cytomegalovirus: global monitoring with oligonucleotide arrays. *Proc Natl Acad Sci U S A* **95**, 14470–14475.

Zweidler-McKay, P. A., Grimes, H. L., Flubacher, M. M. & Tsichlis, P. N. (1996). *Gfi*-1 encodes a nuclear zinc finger protein that binds DNA and functions as a transcriptional repressor. *Mol Cell Biol* **16**, 4024–4034.