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Cell Biology of Human Cytomegalovirus Latency: Implications for Pathogenesis and Treatment

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ABSTRACT

Human cytomegalovirus (HCMV), like all herpes viruses, can establish lifelong infections of the host. This is due to the capacity to establish latency—a defining characteristic of herpes virus infection. In healthy individuals, pathology associated with HCMV infection is rare due, in part, to a robust immune response that controls replication. Consequently, in patients with impaired immune responses substantial pathogenesis is observed due to a failure of immunological control. In this review, I discuss the biology of latency and reactivation with an emphasis on aspects important for our understanding of pathogenesis and treatment. In particular, I will represent how fundamental understanding of the cellular and molecular details of viral latency have, and will continue to be, pivotal for attempts to therapeutically target latent HCMV with a view to reducing the burden of disease. This will include pharmacological and immunological therapies that utilise the modulation of both host and viral functions important for latency and reactivation as well as strategies to harness the very well characterised and prodigious immune response directed against replicating HCMV to target latent infections as well.

1 | Introduction

The capacity of herpes viruses to establish latency, where viral genomes are maintained in the absence of infectious virion production, underpins the ability of these pathogens to maintain lifelong infections of the host. In healthy individuals, robust immune responses limit virus-induced pathology and, accordingly, severe herpes virus associated disease is mainly seen in populations with impaired immune function [1–4]. Pertinent for this review, it is often the reactivation of latent herpesviruses, and not latent carriage itself (perhaps with the exception of gamma herpesviruses [5, 6]), that is the major source of morbidity [7, 8]. Consequently, deciphering the underlying molecular and cellular principles that govern latency and reactivation has the potential to improve our basic understanding of this process with a long term view to therapeutic intervention. Here, I will discuss human cytomegalovirus (HCMV)—a beta herpes virus—with a focus on the contribution of latency to HCMV pathogenesis. Rather than provide an exhaustive review of the finer details of HCMV latency and reactivation (there are many excellent recent reviews covering aspects of this [9–12]) I will select fundamental molecular and cellular aspects considered crucial for understanding latency and, in turn, potentially represent novel targets for therapeutic or immunological intervention.

2 | The Cell Biology of HCMV Latency

HCMV primary infection occurs at mucosal membranes from where the virus disseminates—most likely via circulating monocytes [13] although in viraemic individuals it has also been

shown that circulating endothelial cells could also be a source of dissemination [14]. This can seed HCMV infections in multiple tissues but, important for this review, likely also leads to the seeding of virus in the bone marrow where infection of CD34+ haematopoietic progenitor cells establishes a major cellular reservoir of latent virus [15–17]. CD34+ haematopoietic progenitor cells give rise to the spectrum of differentiated immune cells that populate peripheral blood yet HCMV genomes are usually only detected in the cells of monocyte/myeloid lineage [18, 19]. Whether this reflects HCMV latency is established in a subset of CD34+ cells is not definitively known but it is an ongoing hypothesis being investigated [17, 20, 21]. Crucially, HCMV can replicate in differentiated myeloid cells, for example dendritic cells (DCs) [22, 23] and macrophages [24], and thus the differentiation of CD34+ progenitors (or monocyte derivatives) into these cells is concomitant with viral reactivation [25–27]. The frequency with which reactivation occurs in the host is hard to quantify: HCMV latency in the bone marrow has been estimated to be restricted to less than 0.01% of mononuclear cells [28] suggesting it could be an uncommon event. However, myelopoiesis itself is a frequent event [29] and represents the most common route out of the bone marrow for haematopoietic cells. Of note, not only is natural latency of HCMV routinely detected in bone marrow CD34+ progenitors [15] but also in CD14+ monocytes [19] and immature DCs [26] circulating in the blood of healthy seropositive individuals. Consequently, it is a possibility that the perceived 'restriction' of HCMV to myelo-monocytic lineage is purely a reflection of the normal cell biology of myelopoiesis—latent virus is maintained in bone marrow CD34+ progenitors and all their derivatives as they differentiate down the myeloid lineage as this is the most common exit route out of the bone marrow. However, in vivo evidence argues against this simple view as latent HCMV is not detectable in polymorphonuclear cells [30] yet these cells arise from the same granulocyte/macrophage progenitor cells carrying HCMV that also derive monocytes [17]. The actual scenario is likely to be somewhat more complex with specific subsets of CD34+ cells being better able to support HCMV latency [20, 21, 31], and this 'piggybacking' of viral genome maintenance onto myelopoiesis would clearly be evolutionarily advantageous as it provides a consistent route for viral genome to be directed to the periphery for onward transmission; it is also argued that HCMV latency-associated gene products promulgate a cellular environment which enhances certain pathways of myeloid cell commitment [32–36]. An intriguing aspect of these studies is the concept that the myeloid commitment is towards an immune-suppressive monocyte/macrophage phenotype potentially providing a micro-environment advantageous for HCMV reactivation [34, 37].

The important role of the haematopoietic cell system in the biology of latency and reactivation is not in doubt, yet this concept, the outcome of over 40 years of research, is periodically revisited. It is here, then, instructive to return to the early clinical observations that set investigators on this path. Specifically, it was identified that HCMV transmission and disease was substantially reduced if blood (which rarely contains infectious virus) destined for transfusion or use in transplantation was first depleted of white blood cells [38–41]. This is a simple but key observation: It tells us that HCMV is in peripheral blood, that virus is associated with white blood cells and likely latent but functional (i.e., it can reactivate). What is critical is that none of these studies preclude additional cellular sites of HCMV latency but, in turn, the potential existence of additional sites does not diminish the importance of the haematopoietic system in latency and pathogenesis of HCMV.

Arguably the haematopoietic system is an 'ideal home' for a latent infection to persist in as well as disseminate intra-host. It comprises multiple cell types whose identities depend on the complex regulation of differential gene expression (which, at the most simplistic level is what latency and reactivation is—differential viral gene expression in different phases of infection). Within this wide spectrum of cell identities in the haematopoietic lineage, there are also various activation statuses (e.g., resting vs. activated T cells in the context of HIV latency [42]) and this dynamic system provides a tractable vehicle for dissemination (including to sites of onward transmission). Indeed, many persistent infections are latent in cells of the haematopoietic system including multiple herpes viruses [18, 19, 43, 44] as well as HIV [45, 46] and other lentiviruses [47] which would be consistent with the premise that this biological system in the host is an 'ideal home'.

So what of other sites of HCMV latency? One of the key cell types postulated are endothelial cells (ECs) resident in tissue [48]. From studies of murine CMV (MCMV) it is clear that these cells are an important site of virus persistence in rodent animal models [49]. However, assessing this has proven trickier in human tissue—not least, in part, because early studies on post mortem tissue demonstrated the rapid and pervasive induction of viral replication post death making it hard to identify bona fide sites of latent infection and reactivation [50]. Using ECs derived from residual saphenous veins used in cardiac bypass surgery we could find no robust evidence of CMV genomes in these cells [51]. In contrast, Fish and colleagues reported strong evidence for the detection of non-lytic HCMV genomes in aortic ECs [52]. Interestingly, in vitro studies suggested infection of EC subsets could lead to 'smouldering non lytic' infections which could reflect an important site of tissue persistence of HCMV [52]. If this was the case, then this would represent an important source of HCMV in the context of pathogenesis and end organ disease in for example transplant patients—irrespective of the semantics of latent versus persistent infection—as it would provide another source of virus infection in the donated organ.

More recently, fibroblasts have also been suggested to be a possible cell type that could support latent infection [53]. Investigators reported that a subset of fibroblasts could be identified in mice following infection with murine CMV (MCMV) that were not lytically infected. It was then shown that these cells could retain genome and that same genome 'reactivated' to result in viral replication. By the simplest definition these observations are consistent with the definition of CMV latency. However, the direct infection of these same cells results in lytic infection suggesting that in vivo there is infection of a precursor that differentiates into these cells and so for reactivation to occur an additional stimulus is required. This latency versus. lytic phenotype in the same cell has some similarities with myeloid dendritic cells (DCs) in HCMV. Immature monocyte derived DCs are fully permissive for viral replication upon direct infection [23] but if they are generated from latently infected monocytes they will only

support reactivation if given additional inflammatory signals [25, 54, 55]. What perhaps remains to be understood is the biological significance of these observations for our understanding of HCMV? Is there a sub-population of fibroblasts within the human body that can truly serve as a long term virus reservoir which differentiates from a latently infected precursor? That said, even if fibroblasts represent a transient site of latency it could still be important in the context of transplantation and local reactivation in the donor organ as a source of viraemia. Ultimately, this is the crucial question—are they biologically important for pathogenesis in vivo or, alternatively, a potentially useful tool to study molecular mechanisms governing differential viral gene expression in latent/lytic switch in vitro?

Returning to the clinical perspective can we rationalise all these different ideas and observations to better understand the source of risk in vivo. A key underlying point is that, from a clinical perspective, in the solid organ transplant setting the risk is derived mainly from the donor and thus HCMV is being introduced via the transplanted tissue [2]. Thus this risk could be explained by multiple hypotheses that are not necessarily mutually exclusive (Figure 1).

Firstly, upon primary infection a long term latent (or persistent) infection could be established in tissue resident cells (e.g., endothelial cells or tissue resident myeloid cells). Indeed, a number of important pathogens can be transmitted in the transplantation setting due to their capacity to establish persistent infections in the transplanted organ (e.g., Hepatitis B (liver) and BK polyoma virus (renal)) [56, 57]. Notably, HCMV is a problem in most organ transplant settings suggesting widespread seeding of HCMV throughout the organ system under this model [2]. It is therefore interesting to highlight a recent study which suggested macrophages could be a potential reservoir for latent HCMV in tissue [58]. This would be consistent with the reactivation of HCMV seen in alveolar macrophages in patients with bronchial inflammation [59].

Secondly, low level persistent infection in tissues could likely be controlled by the immune response in healthy people but potentially is being re-seeded by circulating myeloid cells infiltrating into tissue. We know there is substantial anti-CMV immunity in the tissue [60–62]. For example, we recently observed that the anti-CMV quality of the incoming donor immunity in liver transplants correlates with clinical outcome post-transplant [63]. Indeed, similar studies have also suggested T cell quality could be important in determining protection from congenital infection [64]. Under this model HCMV in organs would be transient due to clearance from local immunity but could be re-seeded by infiltrating myeloid cells. It is here the D+R+ transplant population could be useful if there was a way to address whether the infection in the donated organ remains donor derived or is eventually replaced by the recipient strain.

Thirdly, it is a possibility that the transmitted virus in this setting is purely indicative of the presence of circulating

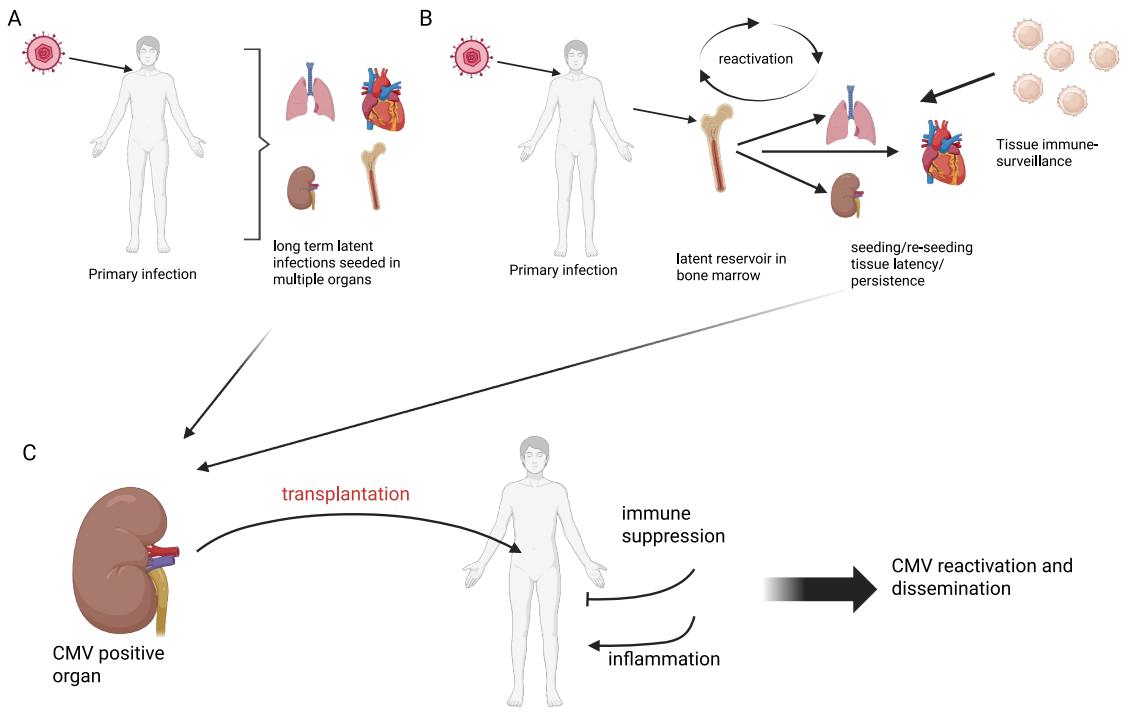

FIGURE 1 | Tissue resident HCMV is the source of HCMV in transplant patients. (A, B) Primary infection with HCMV results in the establishment of a lifelong latent infection of the host. Dissemination from mucosal infection either leads to direct infection and establishment of a long latent/persistent infection of multiple organs (A) including the bone marrow. Alternatively, tissue resident immunity (B), potentially, could target and eliminate HCMV in organs. However, cycles of reactivation from the bone marrow could contribute to re-seeding of latent infection in the organ—including circulating myeloid cells infiltrating tissue. (C) In both scenarios transplant of the latently infected organ leads to HCMV reactivation in the recipient due to inflammation associated reactivation and concomitant immune-suppression. Created in BioRender. M Reeves, M (2025) https://BioRender.com/uur9yg7.

monocytes in the organ carrying HCMV present at the time of transplant. Theoretically it is possible that the ischaemia in the organ (particularly in deceased donors' tissue) is reactivating HCMV in myeloid cells [65] and this is seeding lytic infection in the tissue which is the sources of viraemia in the patient.

Apropos this, there is the curiosity of the common clinical manifestation of HCMV pathogenesis in HIV/AIDS patients of CMV retinitis [66]. This could be attributed to the capacity of latently infected cells to infiltrate the retina due to changes in the structure of the eye in these unfortunate patients with circulating myeloid cells the most likely source [67].

It is very clear that these different hypotheses are not mutually exclusive and likely all scenarios could contribute to the pathogenesis we see in vivo in very scenario-specific settings.

3 | The Molecular Biology of Latency

3.1 | Transcriptional and Epigenetic Control of Latency

Our understanding of the molecular details of latency comes predominantly from studies of HCMV infection in the haematopoietic system [68]. The first seminal observations regarding HCMV latency and reactivation demonstrated that myeloid cell differentiation and activation of latently infected progenitor cells was sufficient to promote HCMV reactivation in both experimental [69–74] and, crucially, natural latency [17, 25, 27, 54, 75, 76]. Changes in the cellular proteome induced by inflammatory signalling and differentiation are likely key to this [77, 78] and again, in the simplest terms, leads to the induction of major immediate early gene (MIE) expression through differential regulation of the MIE promoter (MIEP) or, intriguingly, an alternative internal set of promoters of MIE gene expression located downstream of the MIEP [79].

The pivotal importance of MIE gene expression for HCMV reactivation resulted in a concerted effort aimed at understanding how differential regulation of the MIEP is achieved—which is now clear to be via the same host mechanisms that govern differential regulation of eukaryotic gene expression. The MIEP is highly complex with binding sites for both transcriptional repressors (expressed at high levels in cells such as CD34+ cells which support latent infection) and transcriptional activators (that are elevated in more differentiated myeloid cells after response to inflammation and activation) [80]. Furthermore, accessibility of the MIEP is controlled by higher order chromatin structure—in itself a dynamic structure—whereby the silenced MIEP in CD34+ cells is associated with methylated histones and transcriptional repressor heterochromatin protein-1 (HP-1) and the activated MIEP in reactivating cells is associated with acetylated histones [25]. This 'chromatin switch' phenotype of latency and reactivation faithfully reflects the eukaryotic histone code hypothesis that states post-translational modifications of histones dictate gene expression [81].

Subsequently, virally encoded functions expressed during latency have been shown to help maintain that transcriptional phenotype of differential expression of the MIE genes. The specific details of the functions of these different gene products has been reviewed extensively elsewhere [9, 11, 82] but, in brief, the deletion of the GPCR US28 or UL138 genes from HCMV is deleterious to the establishment of latency in experimental models [83, 84]. The explanations for this are far ranging but include a failure to establish epigenetic silencing of the MIEP [85], disruption of EGFR signalling pathways [86] and silencing of crucial inflammatory pathways [87] suggested important for HCMV reactivation (e.g. NF-kB and ERK/MSK) [54, 74, 88]. It is important to also note that other transcripts [89–93] have also been reported to be expressed during, and contribute to, latency but (in the experimental models used) their deletion has not been observed to have as dramatic impact on the ability of HCMV to establish latency to the extent that UL138 and US28 do.

3.2 | Post-Transcriptional Regulation of Latency

Chromatin modification is an effective cellular mechanism for regulating the MIEP to control HCMV latency but like any biological system it has inherent 'noise'. A clear example of this is from work performed in ES cells that demonstrated transcription can arise from operationally 'silenced' cellular promoters [94]. The MIEP is arguably the most potent promoter in eukaryotic cell biology and thus, even in a repressed state, there is the potential for low level 'trickle through' transcription. This phenotype is revealed in CD34+ cells infected with HCMV encoding a deletion of a virally encoded miRNA, mirUL112.1. miRNAs are small regulatory RNAs that can bind to mRNA targets to promote destruction or, alternatively, prevent their translation. Either way, the potential for protein synthesis is reduced. In the case of UL112.1, it binds to viral MIE UL123 transcripts and prevents translation of the IE72 gene product [95]; thus providing a 'belt and braces' approach to silencing the MIEP during latency [96, 97]. Furthermore, it is interesting to note that at least one cellular miRNA (hsa-mir-200), which binds to MIE transcript UL122 (which gives rise to IE86 protein), is highly expressed in haematopoietic cells and will similarly reduce steady state levels of any IE86 RNA [98]. This is a concept that also pervades HSV whereby an elegant study demonstrated that HSV infection of neuronal cells and the silencing of ICP0 was mediated by hsa-mir-138 [99]. Similar mechanisms have been proposed for the gamma herpes viruses [100] and also the more distant HIV [101]. In the case of HCMV, whether these miRNAs are important for preventing reactivation is not clear—a more attractive hypothesis is that they are acting as an insulator to remove any spurious transcription where chromatin temporarily fails to fully repress the MIEP. This is likely important from an immunological point of view: HCMV infected individuals have high numbers of T cells that recognise HCMV IE72 protein and thus transient low level expression of MIE genes in latency may expose them to T cell killing [96].

In addition to this, HCMV has also been demonstrated to transiently upregulate a subset of cellular miRNAs upon initial infection of CD34+ cells [102]. The precise role for these miRNAs is unclear but contributing to silencing of the MIEP is one

likely outcome. Indeed, bioinformatic interrogation of these miRNAs led to the identification of a role for calcium channel signalling in HCMV reactivation [102]. Finally, long term latently infected cells also differentially regulate a subset of host miRNAs which are distinct in identity to those identified during the initial stages of infection [103]. This is most easily explained by the involvement of different processes and proteins during the establishment versus the long term maintenance of viral latency. For example, in long term latent infection HCMV targets host miRNAs to up-regulate expression of the key pioneer transcription factor, GATA-2 [103], which may contribute to the myeloid cell commitment associated with HCMV infection of progenitor cells [104].

3.3 | Immune Evasion During Latency

Anthropomorphically, the logical way to avoid immune detection during latency is to remain transcriptionally silent and express viral proteins only when the conditions are conducive for replication and onward transmission. From an evolutionary perspective, one could argue that, if it were possible, the 'optimal' HCMV latency phenotype would be no transcription—so no viral proteins, which could be targeted by host immune responses, would be expressed. However, it is very clear that HCMV expresses a subset of gene products during latency which implies there exists a clear trade-off between potentially deleterious immune detection and pro-viral functions associated with viral persistence. Put another way, the need for latently expressed functions must compensate for any threat of immune detection of these viral functions—or, alternatively (or additionally), encode for strategies that limit that immunological threat. T cells that recognise latency-associated antigens have been reported in a number of studies [105, 106, 111] although, interestingly, the T cells that recognise latent antigens are more likely to display an immune-suppressive T cell phenotype [111]. Furthermore, HCMV propagates an immune-suppressive phenotype during latency through induction of immune-modulatory cytokines such as TGF-b and IL-10 [108] as well as the expression of a viral homologue of IL-10 (LavIL-10) [91]—one of the first latent transcripts to be identified during natural latency—which has multiple functions including down-regulation of HLA-DR expression and protection from cell death [91, 108]. From a clinical perspective this becomes of major importance—it reveals that latently infected cells could be potentially immunogenic but the virus has evolved strategies to dampen the capacity of the immune system to target latent cells. Thus, tipping the balance to change the relationship between the latent cell and the immune system could have important implications for the control of HCMV and the limiting of pathogenesis in vivo [109].

It is worth noting that studies that suggest latent HCMV propagates a microenvironment to promote immune-suppressive T cells against latent antigens is focused on studies of infected CD34+ cells and thus implicitly suggest these events are occurring in the bone marrow. However, if there are sites of tissue latency beyond bone marrow then it is a sensible hypothesis that this could also occur in other tissues. Indeed, these immune-suppressive T cells directed against latent antigens are circulating in blood [110] and thus could derive from multiple potential tissue sites of latency. Related to this idea of tissue specific immune responses is work in the murine models of CMV persistence/latency suggesting immunological control of CMV differs depending on tissue [111]. For example, it has been demonstrated regulatory T cells antagonise CD8+ effector function promoting persistence in the spleen yet also limit IL-10 secretion by Fox3p CD4+ cells in salivary glands to prevent reactivation [112]. More generally, CD8+ T cells appear important for control of MCMV reactivation in the lung [113] whereas CD4+ T cells appear crucial in the salivary gland [114].

4 | Targeting Functions Important for Latency and Reactivation for Therapeutic Interventions

The aim of this review has not been to provide an exhaustive list of host and virally encoded gene products and their potential roles in viral latency but, rather, to focus on aspects of their latency-associated functions that may lead to better a understanding of ways to target HCMV therapeutically. Thus, for the remainder of this review I will discuss these in more detail. It is important to highlight that all current anti-virals directed against HCMV target functions associated with replicating virus—such as viral DNA replication (e.g., targeted by valganciclovir) and genome processing from concatamers (e.g., targeted by letermovir)—and, for this reason, resistance has been documented for all current anti-virals [115]. That said, current anti-virals have proven powerful tools to control replicating HCMV. In principle, strategies that target latent HCMV are aiming to act prior to replication which will likely limit the risk of resistance arising against them.

4.1 | Shock and Kill Approaches

Shock and kill approaches have long been considered an attractive way to purge latent infections. First proposed for HIV [116] it is becoming increasingly evident that it could be applicable to herpes viruses [117]. The approach is predicated on the understanding that epigenetics regulates latency and reactivation and thus, through the use of pharmacological inhibitors of epigenetic modifiers, you can 'force' the latent virus to express lytic genes and expose itself to immune elimination. It is becoming increasingly evident, at least in the HIV field, that the elimination of the virus through this approach is going to be incredibly difficult [118] and, if any HIV persists, this will rapidly re-seed latency. For the herpes viruses, the replication of which is much less than HIV in a persistently infected host, reducing the viral latent load may be sufficient to achieve clinical impact. We know from studies of the natural history of HCMV in transplantation that viral load is the key parameter of disease [119]. Thus, reducing the latent load (either in recipient, or the donor organ) has the potential to limit the impact of HCMV reactivation in this setting without the need (or bar) of achieving eradication.

The key next step to establish any major clinical impact is to understand what the best epigenetic modifiers to use. The first experiments have relied on histone deacetylase inhibitors (HDACi) [120] but it is possible that the pervasive effects these

have on viral (and host) gene expression may be a problem—HCMV encodes many immune evasion genes and, thus, any concomitant activation of these genes by HDACi along with IE gene expression (which would be the target for T cell-mediated anti-viral responses) may limit the clearance. Thus, key to this approach is the selective activation of the MIE genes—which has been shown using BET inhibitors (BETi) which target Bromodomain proteins that only activate the MIE promoter [121]. The next stage for clinical impact is to consider how these would be deployed. Epigenetic modifiers can be well tolerated in vivo (e.g., HDACi, such as valproic acid, is routinely used to treat epilepsy [122]). However, an emerging concept is rather than reduce the size of the latent reservoir in live donors by their pre-treatment with HDACi, it may prove more attractive to purge latent virus from donor organs prior to transplant. Of course, this approach of purging virus from the organs prior to transplant by shock and kill with epigenetic modifiers would require the tissue resident immune system in the donor organ to be active against HCMV under pre-transplant conditions ex vivo or, alternatively, to be rapidly deployed in the recipient once the organ was transplanted under physiological conditions. Essentially, the premise is to treat the HCMV that causes major pathogenesis (i.e., the HCMV in the donor organ) rather than achieve complete elimination.

4.2 | US28

GPCRs are a class of transmembrane proteins that are almost ubiquitously expressed in eukaryotic cells and manipulate the function of diverse cellular processes and, consequently, their dysfunction has been associated with many disease states. They are considered highly attractive and 'druggable' targets in a number of disease scenarios with around 30%–50% of all pharmacological targets being directed against this class of proteins [123].

HCMV encodes the GPCR, US28, which is expressed during both lytic [124] and latent infection [82]. Expression during latent infection was first reported in 2001 in a study of HCMV infection of the THP1 myelomonocytic cell line [125] and has later been reported in other model systems of HCMV latency [84, 126, 127]. The phenotype associated with US28 deletion from HCMV is quite marked in experimental latency the virus fails to establish latency due to an inability to silence MIE gene expression [82]. This is linked to the capacity of US28 to have a complex differential signalling activity whereby, depending on cell, type, it has almost opposing effects on the same signalling pathway within the cell [87]. How US28 achieves this is still not entirely clear but, suffice to say, it restricts the activity of multiple components of pathways associated with activation of the MIEP during latency but, in permissive cells, US28 signalling activity strongly up-regulates the same pathways. It is also important to highlight here that this reported latency phenotype of US28 is not a consensus view - other investigators have argued the US28 deletion has no impact on establishment of latency but fails to reactivate efficiently [127].

It is also noteworthy that US28 has been implicated in the control of myeloid cell commitment with evidence from experimental latency suggesting US28 manipulates STAT3/NO signalling to repress the MIEP, whilst simultaneously driving a suppressive monocyte phenotype [33]. How US28 hypothetically achieves this differential signalling activity is likely due to cell type specific differences in the interactions between US28 and host cell proteins which modulates the complex signalling landscape associated with that specific cell protein. Given the strong evidence for a role of US28 in regulating viral latency, it is considered that modulating its function, specifically inhibiting its repressive activity against the MIEP in latency, may derepress the MIEP resulting in expression of MIE gene products which can be surveilled and targeted by the host to the immune system [109]. Indeed, recent proof of concept of this principle has been demonstrated in two separate studies: In one study, nanobodies were used to modulate US28 activity and promote IE gene expression in latently infected myeloid cells [128]. More recently, investigators conjugated US28 antibodies with endogenous degradation domains which remove US28 from latent cells. Both these strategies release the repression of the MIEP leading to IE protein production and T cell killing [128, 129].

However, an intriguing alternative approach is to not to necessarily inhibit US28 function directly but, instead, use it as a targeting mechanism akin to a 'magic bullet' therapy first proposed as a general concept to treat disease by Paul Ehrlich in 1907 [130] and adopted by oncologists as a strategy for treating cancer many years ago [131]. US28 is expressed on the surface of latently (and lytic) infected cells meaning it can potentially be targeted using antibody mediated therapeutic targeting to deliver a toxin [132]. Subsequently, it has been demonstrated this approach can be used to lower the latent load in populations of latently infected mononuclear cells [133]. The key to success in any approach like this depends on the specificity of the antibody to ensure only the intended targets are killed-if any level of off target killing were observed, it is not likely such an approach would be approved for treatment of healthy individuals. However, an alternative approach could be to target and kill latent cells from a graft prior to transplantation. Again, under these conditions it is critical that the treatment does not have toxicity for the organ but this type of approach returns to the premise that HCMV latency could be targeted in situations where any latent load could be a source of pathogenesis rather than a general strategy to purge HCMV completely from infected, but otherwise low risk, individuals.

4.3 | Targeting Other Latent Gene Products

HCMV latency is concomitant with the expression of a subset of viral transcripts that, unlike reported for US28 and UL138, do not have as large phenotypes when analysed using in vitro experimental latent systems. However, that does not necessarily preclude them as potential targets. Indeed, when US28 and UL138 are compared it is US28 that has proven more attractive therapeutically which, in part, can be linked to expression at the plasma membrane which makes it easier to target immunologically. We also have to consider that UL138 has not proven possible to target therapeutically as it does not possess intrinsic enzymatic function—with enzymes, historically, being another important class of druggable protein along with GPCRs.

One latent gene product, LUNA, originally identified in the St Jeor laboratory [90] has since been demonstrated to encode for deSUMOylase activity [134]—an enzymatic activity that remove the SUMO post-translational modification from proteins to change their function and interactions. Inhibition of deSUMOylases is possible although a number of host encoded deSUMOylases exist [135, 136] which raises the next issue with targeting enzymatic functions likely encoded also by the host—a high potential for cytotoxicity. Thus understanding how these latent gene products manipulate the latent cell could prove important. Rather than targeting the viral protein directly you, again, target a host function targeted by the viral protein that is important for maintaining latency. The consequence being that disabling that may promote exposure of the latent virus to the immune system via aberrant IE gene expression, for example. These approaches, theoretically, would be transient reducing the potential for toxicity associated with targeting host-associated functions.

In addition, although latent products may not be able to be targeted pharmacologically it is possible that they could be immune targets. T cells against latent antigens are detectable in humans including effector CD8 T cells [111, 137]. Adoptive T cell transfer against lytic antigens to control HCMV replication in at risk patients is an ongoing approach being developed and tested [138]. Thus it remains possible that T cells against latent antigens may prove effective at reducing the latent load in individuals which could have downstream consequences of reduced reactivation and viraemia.

4.4 | Inhibition of Initiation of Viral Reactivation

Any pathogenesis associated with HCMV latency is, of course, actually due to viral reactivation and that reactivation is overtly pathogenic only in specific clinical situations—usually where immunological control of virus reactivation is impaired [2]. Routinely, active virus replication resulting from reactivation can be blocked by existing anti-virals. However, developing strategies that block the initiation of reactivation in myeloid cells could potentially be important. A key event in HCMV reactivation is the re-initiation of MIE gene expression [80]. Inflammation and cellular differentiation, within the myeloid cell compartment, is central for the activation of MIE gene expression. Thus, understanding how the latent MIEP is activated has long been considered crucial in order to fully understand reactivation. What we do know is that, although chromatin is pivotal, it is difficult to pinpoint a single pathway responsible for reactivation of the MIEP. Indeed, the viral MIEP has binding sites for transcription factors activated by a wide range of inflammatory molecules (e.g., tumour necrosis factor alpha, interleukin-6, prostaglandins, Glucocorticosteroids [54, 71, 74, 139, 140]) which could explain the rapidity with which HCMV reactivates in vivo in clinical settings—the ability of virus to respond to multiple reactivation signals likely results in multiple escape routes for latent virus via reactivation. Furthermore, we have no real appreciation of the role the alternative MIE promoters play during in vivo reactivation events [79, 141], which appear to be regulated by stress-responsive regulatory elements [142], but what is clear that these alternative promoters add even more potential exit strategies from latency [55]. What is going to be crucial, perhaps, is whether we can identify whether any specific pathway is the predominant driver of clinically important HCMV reactivation in specific disease settings.

4.5 | Vaccination

The final aspect of this review concerns the prospect of a vaccination strategy to target latency. It raises the question of whether generating a T cell or humoural response against latent antigens is a viable solution to the latency elimination conundrum? This is probably unlikely given we know the host makes substantial T cell responses and that the ones directed against latent antigens clearly are not sterilising. That said, vaccination against the viral IL10 as a strategy has been suggested from studies of IL-10 in rhesus models. Here it is postulated that IL-10 drives more efficient horizontal transmission events and thus removing that activity reduces this [143]. What was interesting is that vaccination reduced horizontal CMV acquisition in the animal studies - this is clearly not targeting latency per se but likely reduces the frequency of establishment in the population [143]. I highlight that studies of human LaCMVIL-10 using in vitro models suggest it could have a role in latency and enhanced immune evasion [91, 107, 144]. Thus, removing this viral Il-10 effect may be enough to skew the immune response to better target and eliminate latent cells which, over time, may be sufficient for elimination of the reservoir as well as provide the protective effects from initial infection reported in the primate studies.

One of the unanswered questions concerning HCMV latency is whether there is truly a cell type in the bone marrow that is a site of long term latency or, alternatively, there is regular re-seeding of the latent reservoir. Arguably, any long term persistence of HCMV in the dividing bone marrow cells without re-seeding would require a mechanism for latent genome replication and maintenance. Whether this is the case remains to be definitively answered; although chromatin tethering functions have been linked to a sub-domain of IE72 protein (IE1exon4) [93], evidence for a bona fide latent origin of replication—as has been clearly demonstrated for EBV [145]—remains elusive. Thus, if we return to the latter hypothesis that there is ongoing infection/re-seeding of CD34+ cells, that is latent virus in undifferentiated myeloid cells in the bone marrow reactivates in differentiated myeloid cells in the periphery then reinfects the bone marrow, then a vaccination strategy that limits viral replication may also tangentially result in a reduction in the latent load if it prevents re-seeding of the latent reservoir. This could be tested using longitudinal studies of vaccinated individuals and simple measurement of latent loads in mononuclear cells. Although not a primary outcome of any HCMV vaccination strategy, it could well be a very fortuitous secondary outcome in seropositive individuals—particularly if it induces both cellular and antibody responses that limit haematogenic spread of HCMV [128].

5 | Concluding Remarks

HCMV latency and reactivation makes a substantial contribution to the disease we see in vivo. In this review, I have focused

on the direct consequences on pathology associated with HCMV latency and reactivation. Thus, it is clear, even when considering these aspects, interventions directed against latent virus could have an enormous impact on health. Additionally, it is worth remembering that the impact of living with long term persistent HCMV infection has never been unequivocally quantified [146]. HCMV is a virus that is constantly challenging the immune system (best exemplified by the immunological resources devoted to the control of HCMV over the host's life time) with the potential for driving important immunological changes in the functionality of the immune response against HCMV and other infections and diseases (i.e., immune-senescence). These so-called 'indirect effects' of HCMV remain hard to quantify but the recent data that demonstrates infection with EBV (another herpes virus capable of establishing latency that is highly immunogenic) is a risk factor for Multiple Sclerosis [147] is a prescient example of the threat latent herpes viruses could pose to healthy individuals as well as the very well established threat to those with dysfunctional immune systems. Thus, understanding the basic tenets that govern latency and reactivation of HCMV will remain important as we seek to understand the spectrum of HCMV pathologies in vivo with the long term aim of interdicting and controlling HCMV, and its' impact, in the host.

Author Contributions

M.B.R. conceived and wrote the review.

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Conflicts of Interest

M.B.R. is a co-inventor of WO Patent WO 2022/129937A1 assigned to University College London (UCL), entitled 'hCMV antibody and vaccine target', which deals with a novel antigenic domain on HCMV glycoprotein B (gB). The author declares no other competing interests.

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