Differences between mouse and human cytomegalovirus interactions with their respective hosts at immediate early times of the replication cycle

Gerd G. Maul · Dmitri Negorev

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Abstract The promise of the mouse model of cytomegalovirus (CMV) research lies in a cost effective way to obtain significant data in in vivo settings. Keeping that promise requires a high degree of equivalency in the human and mouse virus. While genomic structure and many common proteins suggest that this system is appropriate to develop and test concepts in an organismal context, areas of difference have not been evaluated. Here we show that the major immediate early protein 1 (IE1) in MCMV binds the repressor Daxx suggesting that it serves a function performed by pp71 in HCMV. A Daxx binding pp71 equivalent at M82 could not be identified for MCMV. Differences in the mouse and human interferon upregulation of Daxx may have driven the need to have a Daxx-defeating function during reactivation, when pp71 is not present. The major immediate early protein 1 also differs in its chromatin binding properties between the two viruses. MCMV IE1 does not bind to chromatin, but HCMV IE1 does. It remains unclear whether this difference is functionally significant. The HCMV major immediate early protein 2 and its MCMV equivalent IE3 differ in their effect on the cell cycle; HCMV IE2 blocks the cell cycle, but MCMV IE3 does not, allowing MCMV to spread in infected mouse cells by cell division with continued expression of the major transactivating viral proteins. Actively transcribing genomes inducing immediate transcript environments are usually silenced and diminish during cell cycle progression. However, a recognizable desilencing and increase in immediate transcript environments takes place immediately after mitosis in MCMV infected cells. This raises the possibility

that desilencing happens during tissue transplantation, wound healing, or other injury where cells are induced to proliferate.

Keywords Human cytomegalovirus · Mouse cytomegalovirus · Immediate transcript environment · ND10 · IE1 · IE2 · IE3 · Daxx · pp71

Introduction

Part of the rationale for investigating mouse cytomegalovirus (MCMV) is the potential to use this virus as a stand-in for the human cytomegalovirus (HCMV) in the mouse model. In order to use MCMV for this purpose, the two species-specific viruses must behave similarly. The viruses' genetic make-up is very similar, and so is the gene structure of the major immediate early transcription unit, which is essential for both the lytic phase and for reactivation from latency. However, drawing robust analogies between the two systems requires a comparison of each established function at the molecular level. It might be presumed that the high species specificity of cytomegaloviruses is due to the long co-evolutionary development and that this has resulted in species-specific adaptations. These need to be known. Here we enumerate several functions of both viruses during immediate early times of infection, and infer functions for reactivation from latency. We will not review their detailed contributions to the success of the virus, as this has been done recently in various reviews [1, 2]. Instead, we will indicate where the two systems differ during the immediate early time of infection, and where additional information is needed to show whether proteins of the two viruses have comparable functions. Much of this discussion concerns the viral response to the transcriptional

G. G. Maul (✉) · D. Negorev
The Wistar Institute, 3601 Spruce Street,
Philadelphia, PA 19104, USA
e-mail: maul@wistar.org

repressor Daxx and the silencer histone deacetylase and differences of the major immediate early proteins.

Some time ago, we established for several nuclear replicating DNA virus families the importance of a nuclear domain, which appears as several protein aggregates throughout the nucleus. The aggregates contain various proteins such as PML, forming the matrix of ND10, and Daxx, a repressor or corepressor [3, 4]. We are also investigating an additional factor called Sp100 and find it to be suppressor of the HCMV major immediate early promoter (unpublished). Interferon up-regulates the expression of these proteins. We call this domain Nuclear Domain 10 (ND10), where the abbreviation fits the concepts of nuclear depot and an intrinsic nuclear defense. After HCMV infection, some viral genomes are found beside or juxtaposed to a few ND10 (Fig. 1a). During the first few hours the HCMV tegument protein pp71 is located at all ND10, and one finds a few ND10 juxtaposed to the HCMV major immediate early transactivator IE2 (Fig. 1b). These are the sites where viral transcripts originate, apparently penetrating through the IE2 aggregates (Fig. 1c) and spilling into the speckles or interchromosomal granule clusters, the accumulations of splicing factors. This linear arrangement has been defined as the “immediate transcript environment” since it also includes increased concentrations of the basic transcription factors TBP and TFIIB, which colocalize with HCMV IE2 [5]. The number of proteins believed to be involved in transcription in this environment has increased lately, including RNA polymerase II. The viral tegument protein UL69 and cellular proteins cyclin T1, as well as chromatin modifiers Brd4 and the chromatin silencers HDAC1 and 2, were also localized at concentrations above that in the rest of the nucleus [6]. For MCMV, the transcriptional repressor HDAC2, PML and mDaxx also accumulate in the equivalent environment [7]. The accumulation of pro-transcriptional and anti-transcriptional factors suggests a dynamic, mutually interfering site induced by the pres-

ence of the activated viral genome. These factors are not present at other, presumably silenced, viral genomes in the same nucleus.

Differences in the viral suppression of the host repressor Daxx

The transcription repressor Daxx has been identified as a strong intranuclear repressor of HCMV [8]. It does so independently of another repressor, PML [9], with which it interacts through the PML small ubiquitin modifier (SUMO)-Daxx SUMO interaction motive (SIM) [10]. HCMV has evolved countermeasures to the host's repressive activity. The UL 82 gene product, the tegument protein pp71, has been reported to destroy much of the cellular Daxx by a proteasome-dependent but ubiquitin-independent pathway. The strict interpretation from that observation that Daxx seems to disappear before the major immediate early transcription unit is activated and can only be activated after the removal of all hDaxx [8], does not hold on the single cell level. As shown in Fig. 1b, IE2 is present while pp71 is still mostly segregated at ND10 through the binding of Daxx to PML. The argument that hDaxx has not been shown to hold pp71 at ND10 can be refuted. In addition, the protein levels of hDaxx are also not totally eliminated and come back rather fast (Fig. 2c). Daxx appears to be continuously produced in human cells. However, it is apparent that pp71 substantially reduces the repressive effect of hDaxx [11].

For MCMV no pp71 equivalent has been identified, although some sequence homology exists in the M82–84 region [12]. Transfection of 3T3 cells with a plasmid that expresses this MCMV region had no discernable effect on the amount of mDaxx in the cell, and did not cause aggregation of a protein product at ND10 (unpublished observation). Because an absence of mDaxx correlates with higher

Fig. 1 Identification of the immediate transcript environment. **a** HCMV infected human fibroblast 3 h p.i. In situ hybridization of viral DNA after RNase treatment. ND10 are labeled with anti-PML antibodies. Arrows point to viral genomes juxtaposed to ND10. **b** Same as **a**, but probed with anti-pp71 and anti IE2. pp71 occupies ND10 by

Daxx-PML binding. **c** Same as **A**, but in situ hybridization of viral RNA (IE1/2 transcripts). The IE1/2 transcripts are present only in association with ND10 (PML) and with IE2, which sits like a collar around the emerging transcripts

Fig. 2 Effect of IE1 on Daxx and PML in MCMV and HCMV infected fibroblasts. **a** Wild type MCMV-GFP_{IE1/3} infected 3T3 cells. IE1 and IE3 move with near identical mobilities. **b** MCMV-GFP_{IE3} infected 3T3 cells (IE1 was removed by fusing exon3 and 5). **c** BJ human fibroblasts infected with HCMV AD169. Parallel cultures were infected and treated with the histone deacetylase inhibitor TSA and the proteosomal inhibitor MG132. The titer used resulted in approximately equal numbers of cells infected (~75%) as judged by GFP expression at 6 h p.i. Western blots were successively striped and reprobed with antibodies to IE1/IE3, mDaxx, mPML and tubulin or antibodies to HCMV IE1/2 and hDaxx

apparent infectivity [13], we must assume that some MCMV protein fulfills the role of the HCMV pp71. If there is no Daxx-defeating pp71 equivalent in MCMV, which protein would be able to suppress mDaxx repressive activity? mDaxx produces a very early effect, and presently only MCMV IE1 is known to interact with mDaxx [7].

Comparison of the wild type MCMV and an IE1 deletion mutant showed that there is no change in the mDaxx amount (Fig. 2a). However, the mDaxx from cells infected with the IE1 deletion mutant showed an extra slower-migrating band. This seems, but has not been proven, to be a more highly phosphorylated form. For hDaxx the phosphorylated form has been shown to be the active one [14].

These results raise the possibility that there is no pp71 equivalent in MCMV, and that a common protein, IE1, fulfills the Daxx-defeating function, suggesting that a specific host repressor and its detrimental effect can be suppressed by different viral proteins. These suggestions need proof, and a

rationale for how evolutionary pressures selected for such differences between the mouse and human systems. We tested one potential difference between the human and mouse host.

ND10 proteins such as PML and Sp100 are interferon upregulated. We tested whether hDaxx and mDaxx as ND10-associated proteins are also upregulated by this cytokine at the transcriptional and protein level. As shown in Fig. 3, there is no difference in hDaxx transcripts in the presence or absence of interferon stimulation in human cells. Mouse cells; however, showed a distinct increase in mDaxx transcripts and a strong increase in protein production. Mouse and man therefore differ in this aspect of the interferon pathway, and so do the viruses that need to overcome this strong transcriptional repressor. Since pp71 is not present for the reactivation from latency, it is important to test whether the HCMV IE1 has similar properties to MCMV IE1 to help in the reactivation process of latent HCMV by repressing hDaxx.

Fig. 3 Difference in interferon activation of Daxx in mouse and human fibroblasts. Mouse and human fibroblasts were incubated in the presence of the respective interferon β (1,000 U/ml) for 24 h. RT-PCR and western blot show Daxx upregulation by IFN β in mouse fibroblasts (a + b) but not human fibroblasts (c). GAPDH negative control, ISG15 positive control for IFN β upregulation

Differences in HCMV and MCMV major immediate early protein 1 (UL123 and M123) functions

HCMV IE1 and MCMV IE1 share only 12% amino acid homology, mostly in the highly acidic C-terminal region. Moreover, HCMV IE1 is 20% shorter than MCMV IE1, so these two proteins appear to have very little in common. However, they share the same genetic structure, as well as the ability to disperse ND10 and augment viral transcription. Although the primary structures of HCMV IE1 and MCMV IE1 differ significantly, their secondary structures are surprisingly similar if the spacing of helical regions is used for comparison. One unresolved aspect of the apparent structural similarity between HCMV IE1 and MCMV IE1 is at which position SUMO is added. In HCMV IE1, SUMO is at aa 450 [15] and in MCMV IE1 we find the SUMO consensus sequence at aa 223. These rather large covalent modifications could have a strong differential influence on the 3D structure of IE1, affecting the functional properties of the two different IE1s. Since SUMO modifies very small amounts of protein at any given time [16], these SUMO subsets may have additional functions. In HCMV IE1, deletion of the SUMO modification site reduces the levels of IE2 transcript and their translation products [17] and PML desumoylation [18]. In MCMV IE1, the putative SUMO modification site is within the histone deacetylase (HDAC) binding site (unpublished); its precise functions; however, remain unknown.

MCMV like HCMV IE1, reduces the sumoylation of PML, creating an apparent increase of PML during the first hours after infection (Fig. 2a). The retention of PML sumoylated forms after infection with an IE1 deleted virus is shown in Fig. 2b. Aside from dispersion of ND10, IE1 of each virus has the effect of desumoylation of PML, which might release the other PML-bound proteins such as Daxx. Wild-type MCMV does not seem to affect Daxx. However, an additional slower-migrating band forms in the absence of MCMV IE1 (Fig. 2b). This is slightly different for HCMV, where for a brief period shortly after infection with

enough virus to produce induction of IE1/2 in approximately 50% of cells, the amount of hDaxx decreases to recover immediately thereafter (Fig. 2c). This differs from findings by others who either found a total destruction of hDaxx [8] or a gradual but low level diminishment over 6 h p.i. [19]. For MCMV, we find an increase in the slower form in the absence of IE1, likely the phosphorylated form. If that can be verified, then IE1 will keep mDaxx from becoming phosphorylated and from repressing. Different binding and repressive capacities depending on the phosphorylation status of Daxx have been reported [14].

After the release of tegument proteins and their transport into the nucleus, tegument proteins help in the transcriptional activation of the major immediate early transcription of the common 122/123 gene locus, producing the essential transactivator for the next step in the transcriptional cascade. Transcription starts at ND10 from viral genomes that had been deposited there by chance or by some unknown active process (Fig. 1a) [4, 5]. Other viral genomes that had arrived in the nucleus seem to be shut down somewhere in nuclear space. It still seems incongruous that the highest apparent transcriptional rate takes place at sites with the highest concentration of viral transcriptional repressors such as PML and Daxx. In addition, the highest concentration of IE2 is present at these sites, the location of the active major immediate early promoter for which IE2 (or IE3 for MCMV) is a repressor. Why that is so is not known. We can only speculate that binding of Daxx to the promoter region has led to a higher incidence of PML binding where PML is highly concentrated (nuclear depot hypothesis), thus resulting in our observation that these genomes are associated with ND10. That still leaves a conundrum: why does the virus start its transcription only there, while the other viral genomes are suppressed?

The latter problem may be a technical one. One can imagine that those viral genomes at ND10 transcribe, as do the others, but at ND10 a block in splicing causes the transcripts to accumulate to a detectable concentration. The other genomes would then transcribe fewer transcripts, producing no recognizable signal by in situ hybridization, but these are spliced and translated, producing enough IE1 to disperse all ND10 and release the ND10-associated proteins from this site. This hypothesis provides a possible explanation, but is difficult to test.

During latency of HCMV and MCMV, cells may multiply and divide. This should result in a random dilution of repressed virus genomes and eventually in a copy number per cell that makes reactivation unlikely. Epstein-Bar virus solved this problem by attaching viral genomes to the segregating chromosomes by EBNA1 after the S-phase single-round replication of both viral and host DNA. HCMV IE1 has been reported to attach to chromosomes [20], although this is difficult to observe because expression of the MIE

genes after infection blocks the cell cycle through the major immediate early transactivator IE2. In vivo, however, reactivation may not often cause HCMV IE2 expression as extrapolated from the mouse lungs where IE3 is rarely present. IE1 might be a candidate for such chromosome–viral genome interaction.

If such a genome attachment hypothesis were a common feature of β -herpesviruses, MCMV IE1 should also be attached to chromosomes. As shown in Fig. 4 it is not. Infected mouse cells divide, and the GFP-tagged IE1 and IE3 disperse in the cytoplasm and are excluded from chromosomes. HCMV and MCMV IE1 then differ in an as yet unknown function, exemplified by chromosome binding in cells that do not divide during infection and no attachment in host cells that divide quite readily.

Difference in cell cycle regulation by the major immediate early protein 2 or 3 (UL122 and M122)

IE3, the major transactivator of MCMV early genes, and its HCMV homologue IE2, are essential for replicative success [21, 22]. Whether these two proteins common to both viruses differ functionally has not been investigated. As with IE1, MCMV IE3 and HCMV IE2 lack much sequence similarity, except in the C-terminal portion of each molecule. These proteins interact with several proteins involved in transcription, such as the TATA-binding protein TBP [23] and the transcription-associated factor TAFIID [24] and function like TAFs [25]. Moreover, MCMV IE3 and HCMV IE2 are essential for transcriptional activation of early genes, such as those expressed from the UL/M112/113 locus [26, 27] even though these early proteins become recognizable at the same time as IE1 and IE2/IE3 at about 2 h p.i. [28, 29]. This suggests that very little IE3 is needed to activate the early promoter of M112/113. Although MCMV IE3 and HCMV IE2 are auto-repressors of MIEP,

they do not auto-repress at the same apparent concentration that activates the UL/M112/113 promoter. The highest concentration of IE2 in the region of the actively transcribing viral genome (the immediate transcript environment) should ensure repression despite the low concentration of HCMV IE2 or MCMV IE3 in the rest of the nucleus. Additional controls must block these auto-repressors from suppressing MIEP.

A mechanistic explanation for the differential repression/activation ability of IE2 is given by the recent finding that IE2 has different interaction sites for binding the MIEP and the early promoters [30]. This still leaves the problem of the large quantity of IE2 or MCMV IE3 at the site of highest rate of transcription. For HCMV, early proteins transcribed from the UL112/113 gene locus bind single- and double-stranded DNA and localize to nuclear inclusions [31] that reside with IE2 proteins and adjacent to ND10 [29]. Essentially the same has been observed for the MCMV equivalent M112/113. These domains expand and represent the prereplication domains. When replication starts within these domains, they become the replication compartment [32]. It is unknown why these prereplication domains expand and exist for an extended time before viral DNA synthesis begins.

As with early proteins expressed from UL112/113, those expressed from M112/113 also form nuclear inclusions that localize adjacent to a limited number of ND10. Later during the viral replication cycle, these nuclear inclusions house various stages of the replicated genomes and capsids, and exclude host DNA [32]. This expanding and PML- and Daxx-recruiting domain can be mimicked by transfecting cells with M112/113 alone or with both M112/113 and IE3. In cells transfected with M112/113 alone, M112/113 forms a space where cellular and viral proteins are segregated whereas IE3 segregates only in the presence of M112/113 [32] and accumulates to a higher level than when expressed alone, suggesting that M112/113 protects IE3 from degradation. Immunoprecipitation shows that in cells transfected with M112/113 and IE3, M112/113 binds IE3, and MIEP-driven luciferase reporter assays demonstrate that despite the M112/113-dependent higher accumulation of IE3, its repressive effect is substantially diminished. This may account for the paradoxical finding, demonstrated by in situ hybridization, that high concentrations of the auto-repressor IE3 accumulate at the site of viral immediate early transcription. Thus, despite the high concentrations of IE3 present early during infection around MIEP, MIEP is not repressed because binding with M112/113 products may make IE3 functionally unavailable. For HCMV the effect of the 112/113 gene products on IE2 has not been determined but the location of the transcripts, IE2 and UL112/113 suggests a similar function. The recent finding of the viral genome together with IE2 suggests that the viral genome

Fig. 4 Mouse embryonic fibroblasts (2 passage) infected with wild type MCMV GFP-IE1/3. Diffuse GFP represents IE1 and particulate or aggregated GFP represents IE3. Mitotic cell has no GFP attached to chromosomes (blue)

nucleates IE2 accumulation [33] although the large so called DNA signal in this publication suggests that no RNase treatment had been used and that therefore a similar observation was made as the one establishing the immediate transcript environment [5].

The colocalization of HCMV IE2 and MCMV IE3 with their respective 112/113 gene products, as well as the repression of the MCMV IE3, reveals a novel function for M112/113 proteins: their ability to block the interaction between an immediate early protein and its own promoter. Alternatively, after DNA binding to the viral genome at ND10, M112/113 may accumulate through heteromultimerization to form a dense matrix (similar to the PML-based ND10 matrix) that can trap incoming IE3. To act as an IE3 sink, this matrix would need sufficient binding sites for IE3. Once bound by this “sink,” IE3 is prevented from reaching MIEP and interacting with it. The determination of IE3 residency times in the prereplication domain may distinguish these possibilities.

Can M112/113 gene products suppress other IE3 functions? One function assumed to be similar to the HCMV IE2 is the block of cell cycle progression in HCMV infected cells [34, 35], which has been attributed to IE2 interaction with or inhibition of cyclins (see review [1]). If M112/113 gene products are highly efficient, the presumptive mouse cell cycle repression might be suppressed. A first hint of this was that many MCMV infected cells appeared as pairs of daughter cells. Proving that MCMV infected cells can pass through the full cell cycle, and not just to divide once, required direct observation of cells dividing in the presence of expressed IE3.

We constructed MCMV where GFP was added to the N-terminus of exon 2, thus expressing both IE1 and IE3 as a GFP-tagged protein. Time-lapse photography was used to follow the infected cells with activated MIEP over 4 days. We found that MCMV infected cells can progress through several cell cycles while the major immediate early proteins are expressed. HCMV infected human cells with a parallel construction cannot. The infected human cells enlarge in size, some fuse and eventually apoptose. Rarely did we see MCMV infected mouse cells die and leave the substrate within the observational time period. First round reinfection for the GFP-tagged wild type virus started to appear at 23 h. post infection. Allowing for the IE proteins to accumulate to recognizable concentrations one can assume that infectious virus was produced 21 h p.i (movie can be viewed at http://www.wistar.org/research_facilities/maul/timelaps_mov.htm).

We had previously shown that many infected cells do not proceed to full viral replication [7] and in vivo experiments had shown that in the lung most cells with an activated MIEP did not produce IE3 [36, 37]. We might therefore be observing division in cells that did not produce

IE3. To test this, we used a virus that had been constructed by fusing exon 3 and 5, thus deleting exon 4 and with it IE1. Infection of fresh mouse embryonic fibroblasts (second passage) with this IE1 deletion mutant showed IE3 expression in several aggregates, and cells with these aggregates divided as did the wild type virus infected cells (see http://www.wistar.org/research_facilities/maul/timelaps_mov.htm and Fig. 5). The simplest interpretation is that MCMV IE3 does not block the cell cycle. Another possibility is that the M112/113 gene products inhibit the cell cycle block by blocking IE3 interaction with any of the essential cyclins or cell cycle promoting proteins.

If IE3 activates early proteins, M112/113 might not be the only activated early protein while the mouse cell is cycling and dividing. We might therefore speculate that such early proteins necessary for viral replication are also activated with the potential for low-level replication of viral genomes but without viral progeny production. Genome carrying cells might therefore spread by cell division. This would maintain the viral genome in cell types that divide, similar to the γ -herpesvirus strategy. Infected cells may be maintained in reproductive latency but not be eliminated by cellular turnover or division based dilution.

If viral DNA replicates in cycling cells, the combined daughter cells would have more viral genomes than the parental cell. This prediction is difficult to test, as cells replicating virus productively might swamp the minor increase in viral genomes in the dividing cells. Also, single cell evaluation does not allow the analysis of viral genome load over time. However, counting transcribing viral genomes may be possible assuming that viral genomes transcribe at the immediate transcript environment, and that these environments are outlined by IE3 aggregation in successive cell cycles. This experiment has been conducted as a preliminary test on two cells dividing at different times after infection. It showed that IE3 sites are reemerging after dispersal during mitosis (Fig. 5). Table 1 shows the number of IE3 sites over time derived from Fig. 5. The number of IE3 aggregates increases after mitosis over the maximally seen number in the parental cell, and each daughter cell initially displays an approximately equal number of IE3 sites. As in many other cells, the number of IE3 aggregates decreases during the cell cycle (though not equally in daughter cells) and only diffuse GFP-IE3 is seen, which eventually disappears also (Fig. 5 b–h; cell 1b). Here the assumption is that the viral genomes become transcriptionally silenced. The increase in apparent transcription sites (IE3 aggregates from 10 in cell 1 to the combined 14 in cell 1a and b at about 2 h after mitosis) may therefore indicate only a desilencing effect of genomes silenced, not viral genome replication, but this by itself is an interesting phenomenon of the G1 phase of the cell cycle.

Fig. 5 Selected images from time lapse recording of MCMV-GFP_{IE3}, i.e., the deletion IE1 mutant produced by fusing exon 3 and 5. Second passage mouse fibroblasts were infected for 1 h at room temperature then washed in new medium before incubation at 37°C. Time after 37°C incubation is indicated in the upper left corner. Infected cells producing IE3 are labeled 1 and 2 and following cell division by adding **a** and **b** to indicate the respective daughter cells as they move considerable distances. IE3 aggregates were counted from these images and are presented in Table 1

Testing whether CMV replicates during the cell cycle like γ -viruses would require developing a cell culture-based replicative latency model to measure viral genome increase quantitatively, or the technical advance of following single cells over many generations followed by induction of transcriptional reactivation and quantitation of transcription sites.

HCMV IE2 and the homologous MCMV IE3 show an obvious functional difference. HCMV IE3 can block the cell cycle, whereas the presence of MCMV IE3 in the viral context has no apparent effect on cell cycle progression. This assertion needs the caveat of a potential difference in the respective 112/113-gene product function. MCMV 112/113

gene products may modify the potential cell cycle blocking IE3 function whereas the HCMV 112/113 gene product may not do so. Regardless, the difference of these biological functions seems fundamental between the viruses, as a cell cycle block is not induced by another viral protein.

Outlook

The mouse system is the most economic small animal system for investigations into the cytomegalovirus-generated pathology. Because these viruses have coevolved with their respective hosts over very long times, they have become

Table 1 Number of IE3 aggregates were obtained from Fig. 5**IE3 aggregates in successive cell cycles**

	Cell 1		Cell 2	
	a	b	a	b
a	10		6	
b	Mitosis		6	
c	7	7	7	
d	7	4	9	
e	6	1	8	
f	3	0	Mitosis	
g	2	0	6	5
h	1	0	6	5
i	1	0	6	4
j	1	0	4	1
k	1	–	4	–
l	1	–	1	–
m	Plaque	–	Mitosis	
n	–	–	0	2 – –

Time intervals are not even but were selected for ease of counting. The actual mitosis of cell 2 was within the interval not shown, so one sees only the recently divided daughter cells in Fig. 5 g. For demonstrating the trend, cell pairs were selected where one eventually produced a plaque. This cell (1a) had moved out of the detailed field shown, that is, counts for j–m were made from the larger field and are cropped in Fig. 5. The second mitosis of cell 2 was not further analyzed as a nearby forming plaque from cell 3 might have led to secondarily new IE3 sites

highly species-specific and developed means to counteract the host's defenses, often with new genes that have no equivalents in other viral species of the same family. These genes are easily recognized from the sequence analysis. What is not easy is the determination of their respective functions in vivo. If our goal is to define viral functions that are similar or analogous in the small animal system to those in the humans, then concepts developed in the two systems have to be compared to find clinically relevant parallels. Even common or public genes may have developed private functions. If the private functions are dominant for viral replicative success, they are unlikely to be equivalent in investigations on interference. This is especially important for the essential and multifunctional proteins, such as the HCMV IE1 and IE2, which are likely target candidates for interference. Comparing the immediate early transcription unit of HCMV and MCMV should therefore receive priority.

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