

Intrinsic host restriction factors of human cytomegalovirus replication and mechanisms of viral escape

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Author contributions: The authors made a substantial contribution to the conception and design of the study, data interpretation, and drafting and critically revising the manuscript; all authors approved the final version.

Supported by Italian Ministry of Education, University and Research - MIUR (PRIN 2012) to S. Landolfo (2012SNMJRL) and V. Dell'Oste (20127MFYBR); University of Turin, Research Funding 2014 to S. Landolfo, M. De Andrea, and V. Dell'Oste; Regione Piemonte to S. Landolfo (PAR-FCS 2007/2013).

Conflict-of-interest statement: The authors declare that they have no conflict of interest.

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Manuscript source: Invited manuscript

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Received: March 18, 2016
Peer-review started: March 21, 2016
First decision: April 19, 2016
Revised: May 3, 2016
Accepted: July 11, 2016
Article in press: July 13, 2016
Published online: August 12, 2016

Abstract

Before a pathogen even enters a cell, intrinsic immune defenses are active. This first-line defense is mediated by a variety of constitutively expressed cell proteins collectively termed "restriction factors" (RFs), and they form a vital element of the immune response to virus infections. Over time, however, viruses have evolved in a variety of ways so that they are able to overcome these RF defenses *via* mechanisms that are specific for each virus. This review provides a summary of the universal characteristics of RFs, and goes on to focus on the strategies employed by some of the most important RFs in their attempt to control human cytomegalovirus (HCMV) infection. This is followed by a discussion of the counter-restriction mechanisms evolved by viruses to circumvent the host cell's intrinsic immune defenses. RFs include nuclear proteins IFN- γ inducible protein 16 (IFI16) (a Pyrin/HIN domain protein), Sp100, promyelocytic leukemia, and hDaxx; the latter three being the key elements of nuclear domain 10 (ND10). IFI16 inhibits the synthesis of virus DNA by down-regulating UL54 transcription - a gene encoding a CMV DNA polymerase; in response, the virus antagonizes IFI16 *via* a process involving viral proteins UL97 and pp65 (pUL83), which results in the mislocalizing of IFI16 into the cytoplasm. In contrast, viral regulatory proteins, including pp71 and IE1, seek to modify or disrupt the ND10 proteins and thus block or reverse their inhibitory effects upon virus replication. All in all, detailed knowledge of these HCMV counter-restriction mechanisms will be fundamental for the future development of new strategies for combating HCMV infection and for identifying novel therapeutic agents.

Key words: Human cytomegalovirus; Intrinsic immunity; Restriction factors; Viral escape mechanisms; DNA sensors

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Core tip: Cellular “restriction factors”, active before human cytomegalovirus (HCMV) enters the cells, form a component of the intrinsic resistance to virus infection. Examples of such factors are hDaxx, promyelocytic leukemia, Sp100 - components of ND10 - and IFN- γ inducible protein 16 (IFI16), an Interferon-inducible protein of the Pyrin/HIN domain protein family. Over time, viruses have developed mechanisms to counteract ND10 and IFI16 through viral proteins, such as IE1 and pp71, or UL97 and pp65, respectively. Detailed knowledge of these mechanisms will provide new competencies useful to control HCMV infection and, in turn, contribute to the development of novel therapeutic approaches.

Landolfo S, De Andrea M, Dell’Oste V, Gugliesi F. Intrinsic host restriction factors of human cytomegalovirus replication and mechanisms of viral escape. *World J Virol* 2016; 5(3): 87-96 Available from: URL: <http://www.wjgnet.com/2220-3249/full/v5/i3/87.htm> DOI: <http://dx.doi.org/10.5501/wjv.v5.i3.87>

INTRODUCTION

Viral replication in the infected cell is the result of complex interactions between host and viral proteins. Indeed, in the course of evolution, mammalian immune systems have evolved to respond *via* an array of cellular defense mechanisms, which include both innate and adaptive immune responses, designed to protect against and remove invading pathogens^[1]. The innate immune system, mediated by specialized cells such as natural killer cells (NK), dendritic cells, and macrophages^[2], is the first to respond, but it is not very specific and does not lead to a long-lasting memory of the response to the pathogen. A specific form of innate immunity, termed “intrinsic immunity”, has also been identified of late, thus generating a third branch of the immune system that was until now considered a bipartite system. Intrinsic immunity involved a set of defense mechanisms that operate on the cellular level^[3], realized by cellular proteins known as “restriction factors” (RFs), as they can interfere with various steps of the virus replication cycle^[4,5]. The word restriction factor was first coined by research groups studying the murine immune response to retroviruses. Work conducted over 40 years ago revealed that “friend virus susceptibility factor-1” was responsible for conferring resistance to infection by retroviruses^[6]. Retroviruses consequently became a model system for investigating intrinsic immunity and have been instrumental in deepening our knowledge of the interaction between viruses and their hosts^[7,8]. Over time, the notion of “intrinsic immunity” got up from the finding that the cells attacked by primate lentiviruses are able to resist infection, despite the fact that no signaling event appeared to be necessary for this form of defense, and from the finding that these cells constitutively express prototype human antiretroviral RFs, including the

APOBEC3 family of cytidine deaminases^[9,10], TRIM5a^[11], Tetherin^[7], SAMHD1^[12], and BST-2^[13,14]. RFs are thus germline-encoded proteins mediating the intrinsic cellular immune response against viral replication. Type I interferons (IFN) have been demonstrated to increase the expression of RFs, however cells targeted by IFN do not rely on its activity for constitutive antiretroviral activity^[15].

INTRINSIC IMMUNITY

The sensing of “pathogen-associated molecular patterns” (PAMPs) - typically microbe nucleic acids and proteins (usually absent from healthy hosts and thus hallmarks of infection) - by germline encoded proteins serving as “pattern recognition receptors” (PRRs) constitutes the earliest step in the innate immune response^[2,16]. Viral nucleic acid including DNA containing CpG motifs, and RNA species, including both double-stranded and single-stranded RNA, can be detected by Toll-like receptors TLR3, TLR7-8 and TLR9, or PRR in the cytoplasmic or the nuclear compartment^[17,18]. Two different innate immunity signaling cascades are triggered by detecting exogenous nucleic acid. In the first, transcription factors (TFs) are activated, such as NF- κ B and IRF3, culminating in the production of chemokines, cytokines, and IFN-type I^[19,20]. The second signaling cascade leads to inflammasome complex formation; this activates caspase-1, an enzyme that generates active cytokines set for secretion by proteolytically cutting pro-IL-1 β and pro-IL-18^[21-23]. Whereas PRRs activate signals that inhibit infection indirectly, RFs provide front-line defense by interfering directly with the activity of genes essential for the virus’s replication. Indeed, this is often computed before the production of antiviral cytokines has even been activated. Thus, the properties of RFs are clearly distinct to those of PRRs. First and foremost, while RFs are basally expressed in many cell types, their expression may be increased by IFN signaling. Second, isolated RFs have been shown to exhibit antiviral activity in cells, maintaining their capacity to inhibit precise steps in the viral life cycle. Third, viral proteins have evolved to antagonize certain RFs. Finally, genetic selection driven by host vs pathogen coevolution has undoubtedly operated on the genes for RFs^[3]. Thus, according to the concept of intrinsic immunity, we can define cell as either “restrictive” or “permissive” depending on viruses ability to replicate efficiently within them^[5,24]. Retroviruses have presented a model that has played a pivotal role in the development of our understanding of virus-host interactions^[4,8,14,15,18]. However, evidence now shows that several other viruses are also counteracted by intrinsic immunity, including herpesviruses^[25,26].

Here, we focus on the newest findings about human cytomegalovirus (HCMV), which belongs to the Herpesviridae family^[27,28], and provide a summary of the RFs that perturb its replication (Table 1). Interestingly, HCMV appears to have evolved a number of mechanisms to counteract the action of restriction factors, ultimately leading to the successful replication of viruses in cells^[29-32].

Table 1 Overview of host restriction factors for human cytomegalovirus

Host restriction factors PYHIN family	Regulation	CMV inhibition	HCMV counter measure	Ref.
IFI16	Type I IFN inducible	HCMV-DNA sensing in the nucleus Interaction with HCMV pp65 to inhibit UL54 promoter Antiviral cytokine expression	Sequestration by HCMV pp65 for MIEP activation Protection from proteasome degradation by pp65 Delocalization upon phosphorylation by HCMV UL97	[42,43,46,60,72]
AIM2	Type I IFN inducible	MCMV-DNA sensing in the cytoplasm Inflammasome activation	Not known	[66]
ND10 family PML hDaxx Sp100	Cell cycle dependent	Transcriptionally inactive chromatin state of MIEP induction	Targeting HCMV IE1 for degradation hDaxx binding by pp71 for proteasome degradation	[93-116]
KDMs	Cell cycle dependent	Inhibition of HCMV latency	Prevention of KDM association with the MIEP by HCMV UL138	[117]

CMV: Cytomegalovirus; HCMV: Human cytomegalovirus; MCMV: Murine cytomegalovirus; IFI16: IFN- γ inducible protein 16; PYHIN: Pysin/HIN domain; AIM2: Absent in melanoma 2; KDMs: Lysine-specific demethylases; MIEP: Major immediate early promoter.

THE IFN- γ INDUCIBLE PROTEIN 16 PROTEIN

The IFN inducible IFI16 protein is a member of the Pysin and HIN domain containing proteins (PYHIN) family; it is coded by an IFN-inducible group of genes residing on chromosome 1q23^[33-35]. In humans, this family includes five PYHIN proteins: The recently discovered "Pysin domain only protein 3", "Pysin and HIN domain family member 1" (PYHIN1), "absent in melanoma 2" (AIM2), "myeloid cell nuclear differentiation antigen" (MNDA), and "IFN- γ inducible protein 16" (IFI16)^[36,37]. All five of these proteins possess an N-terminal PYRIN domain, and at least four possess a conserved domain of 200-amino acid repeats (HIN-200) within the C-terminal region (in single or tandem copies), thus they are collectively known as PYHIN. The PYD (or PAAD or DAPIN) domain is a member of the death domain family and consists of an α -helical motif that interacts with other PYD-containing proteins^[38]. The HIN domain contains consensus motifs encompassing the 200-amino acid repeats, according to which it is classified into 3 subtypes, designated A, B, C^[39,40]. PYHIN1, MNDA, and IFI16 all contain nuclear localization sequences located within their N-termini, and as such are primarily expressed in the cell nucleus^[35,39,41]. However, in response to environmental stimuli, such as viral infection, they undergo post-translational modifications, *i.e.*, acetylation, and translocate into the cytoplasm^[42,43]. Alternative splicing of the *IFI16* gene produces three isoforms^[39]; each isoform is made up of two domains, designated A and B, each 200-amino acid long. These domains are divided by a spacer region that may vary in its length. The B isoform is the most predominant and has been detected in an array of histologically distinct cell types (*i.e.*, immune, endothelial, and epithelial cells^[44]). The IFI16 N-terminal region display a bi-partite "nuclear localization signal"^[45],

responsible for its nuclear subcellular localization in quiescent cells, such as: Fibroblasts^[46], endothelial cells, and keratinocytes (for a review see^[47]). It is of interest that IFI16 protein has also been identified within the nucleolus^[34]. However, in fibroblasts, macrophages, and keratinocytes, IFI16 is able to relocate from the nucleus to the cytoplasm. In fibroblasts and macrophages, this occurs following infection by herpesvirus^[42,43]; while in keratinocytes, exposure to ultraviolet B light is able to trigger this redistribution^[48,49]. In herpesvirus infection, IFI16 redistribution is associated with inflammasome, and after UVB exposure, it is associated with apoptosis. IFI16 is able to form homodimers or bind to other proteins to form heterodimers; its partners include: p53^[38,50,51], RB^[52], BRCA1^[53], ASC^[54] and STING^[55]. Indeed, protein-protein interactions are now thought to determine the subcellular localization of proteins; however, the molecular mechanisms regulating the redistribution of IFI16 from the nuclear to the cytoplasmic compartment remain unknown. Finally, a role of viral DNA sensor has also been attributed to cytoplasmic IFI16^[47,56]. Indeed, the capacity of IFI16 to bind to viral DNA has been confirmed both *in vitro* and *in vivo*^[40,55-60]. It is now believed that IFI16 may actually tune the innate immune response by stimulating IFN-type I release^[47,56]. Thus, in addition to the various types of protein-protein interactions involving IFI16, another factor that may lead or contribute to IFI16 redistribution within the cell is its binding to microbial DNA.

Inhibition of HCMV replication by IFI16 and viral evasion

AIM2 and IFI16 are the two PYHIN members that have been demonstrated to act as PRRs of intracellular DNA of virus origin^[51,60-66]. In particular, in cells infected with Kaposi Sarcoma Associated herpesvirus, IFI16 was revealed to form a functional inflammasome by interacting with ASC together with procaspase-1^[54]. Moreover, this virus triggered NF- κ B and IRF3 expression and activation [TFs routinely observed to be activated

after DNA transfection or the infection with herpes simplex virus type 1 (HSV-1)] could be inhibited by reducing IFI16 expression (or its mouse counterpart p204) using siRNA^[67-70]. Besides its role as a PRR, IFI16 had previously been recognized to carry out a variety of other functions in the cell, although none in relation to antiviral activity (reviewed in^[35]). However, our understanding of the functions of IFI16 in the cell has dramatically changed over modern years; this is largely due to the results gained from the application of two different experimental approaches (reviewed in^[42]). The first involves IFI16 knockdown through the use of specific siRNA or IFI16 inactivation achieved by transfecting cells with a lentivirus carrying a dominant negative mutant form of the protein^[71]. For example, eliminating functional IFI16 protein in fibroblasts isolated from human embryo lung (HELFs), *via* either methodology, was shown to significantly increase herpesvirus replication, including HCMV. The second approach involves augmenting the quantity of IFI16. The overexpression of IFI16 in HELFs infected with HCMV was associated with a 2.5 log reduction in viral yield. However, light had yet to be shed on the molecular mechanisms responsible for the antiviral role of IFI16, prompting an investigation into the consequences of overexpression on the distinct phases of virus replication. Exploiting the luciferase reporter gene methodology, transfection experiments were used to study the effects of deleting the viral polymerase (UL54) or UL44 promoters or introducing mutated forms of the two^[72]. These studies indicated the IR-1 locus (inverted repeat element 1), located upstream of the polymerase transcription start-site, to be the object of IFI16-induced virus suppression. Chromatin immunoprecipitation and EMSA revealed that Sp1-like factors were effectively blocked by IFI16 and that this in turn led to UL54 suppression. This result was confirmed by deleting the element within the UL44 promoter responsive to Sp1, which accordingly eliminated the suppressive effect of IFI16 on HCMV replication (UL44 protein associates with UL54 during viral DNA replication). Thus, in addition to confirming IFI16's role as a DNA sensor, for the first time IFI16 had also been demonstrated to act as a restriction factor of herpesvirus replication^[72] (Figure 1).

HCMV is nevertheless able to replicate in host cells despite the restrictive capacity of IFI16. This suggests that HCMV has developed evasion strategies to respond to the effects of IFI16^[42,43]. The first evidence sustaining a plausible HCMV evasion strategy was obtained by infecting fibroblasts with a BAC mutant unable to express UL97 phosphoprotein^[42]. Early on during infection, IFI16 binds to virus DNA, but at a later time point during viral DNA synthesis, IFI16 undergoes relocalization from the nucleus to the cytoplasm. It was also revealed that this virus-induced movement of IFI16 out of the nucleus required that UL97 (a viral protein kinase) bound to IFI16. Upon binding to UL97 phosphoprotein, IFI16 undergoes phosphorylation, which in turn promotes its nucleo-cytoplasmic relocalization. IFI16's ensuing transfer into the virus assembly complex is regulated by

the endosomal sorting complex required for transport machinery. Finally, IFI16 becomes integrated into newly assembled virions during the process of virus maturation and budding, effectively expelling IFI16 from the infected host^[42]. However, recent studies have revealed that IFI16 phosphorylation by UL97 is not the only mechanism for HCMV escape from IFI16 restriction activity. Using a BAC mutant virus unable to express the tegument protein pp65 (pUL83) Biolatti *et al*^[73] (2016, unpublished results) have demonstrated that IFI16 interacts with pp65 targeting early gene promoters including that of the viral DNA polymerase pUL54. The capability of IFI16 to downregulate virus growth was found to depend on its interaction with pp65 at the UL54 promoter, as shown by the growth properties of the HCMV mutant v65Stop in IFI16 knockdown cells. Interestingly, at later time points of HCMV infection, IFI16 was not degraded, as observed in HSV-1 - infected cells, but it was protected by its interaction with pp65. These data reveal a dual role for pp65. Initially it modulates IFI16 activity at the promoter of immediate-early and early genes, and subsequently, it delocalizes IFI16 from the nucleus, thereby protecting it from proteasomal degradation. Overall, these data identify a novel activity displayed by the pp65/IFI16 interactome in the regulation of *UL54* gene expression and IFI16 protein stability during HCMV replication.

In summary, these experiments point toward IFI16 nuclear egression, subsequent to its binding to UL97 and pp65, as the mechanism through which HCMV is successfully able to evade IFI16 restriction activity; this removal of IFI16 from its site of restriction activity is finalized with its incorporation into newly formed virions and expulsion from the cell altogether^[42]. This is most likely the event that, to all intents and purposes, underlies the HCMV's successful evasion of IFI16 antiviral activity (Figure 1, Table 1).

NUCLEAR DOMAIN 10

The nuclear matrix, hypothesized by some to organize and regulate a number of nuclear functions within the nucleus of eukaryotes^[74,75], contains discrete bodies designated "nuclear domain 10" (ND10), "promyelocytic leukemia (PML) nuclear bodies", or PODS. These bodies appear as sphere-like, measuring between 0.1-1 μm in diameter, and in some circumstances they present a granular center. ND10 can be found within the nucleoplasmic domains collectively termed the interchromosomal space, often next to proteinaceous bodies. Sp100, hDaxx, and PML protein are three of the protein constituents of ND10. These proteins recruit additional proteins that are SUMOylated^[76,77]. One of these additional proteins is SUMO, a protein related to ubiquitin, and its conjugation to PML is implicated in the further recruitment of yet more binding partners^[76,77]. ND10 are devoid of RNA or DNA and they typically gather into clusters of 5 to 15^[78]. PML protein forms the outer "casing" of the structure, and its protein partners are usually concentrated inside. Functionally, ND10 play a regulatory role, influencing

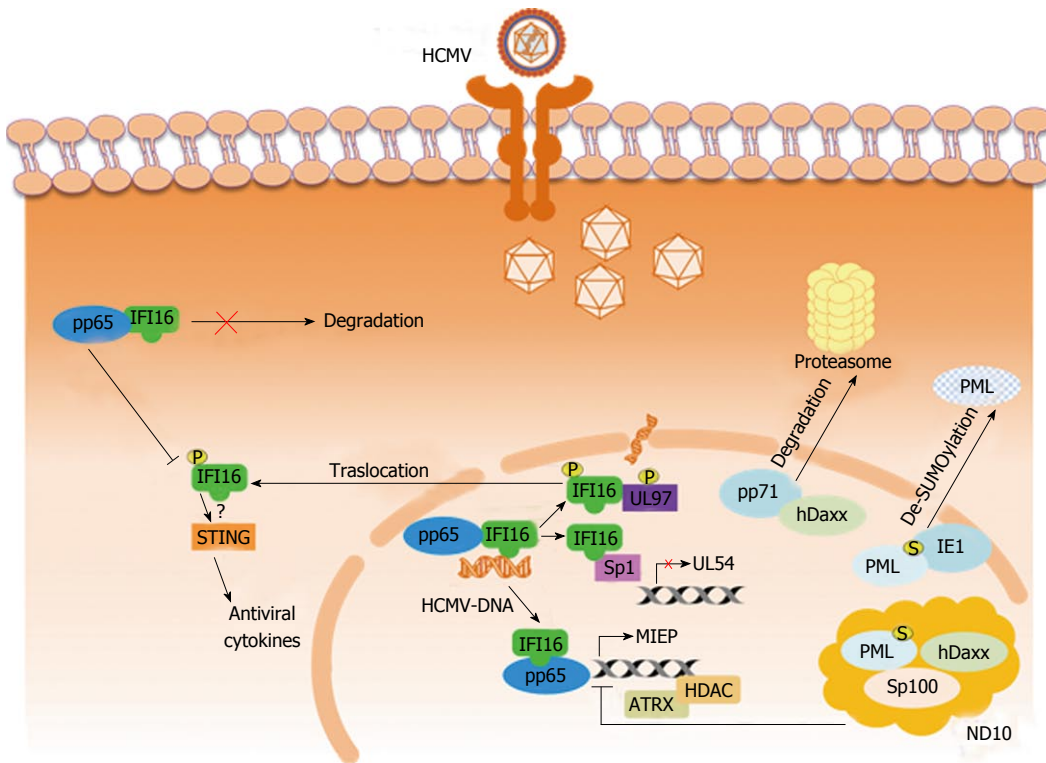


Figure 1 Simplified model of host restrictions against human cytomegalovirus and mechanisms of viral escape. IFI16 recognizes HCMV-DNA in the nucleus. In the early phases of infection, HCMV-pp65 hijacks IFI16 to activate MIEP expression; later on the complex IFI16-pp65 blocks HCMV replication by inhibiting the action of Sp1 on UL54 promoter. IFI16 is not degraded, but it was stabilized by its interaction with pp65. To evade IFI16 antiviral activity, HCMV induces nuclear translocation of IFI16 upon recruitment of HCMV kinase UL97 to IFI16. In the cytoplasm, IFI16 activates STING-mediated antiviral cytokine expression. ND10 components (hDaxx, Sp100, PML) limit viral replication by silencing viral *IE* genes, inducing a transcriptionally inactive chromatin state of the MIEP via recruitment of ATRX or HDACs to the viral DNA. The viral pp71 binds to hDaxx for proteasome degradation and relieves MIEP repression. Degradation of hDaxx is preceded by the release of ATRX from ND10. IE1 promotes the dispersion of the ND10, by de-SUMOylation, with the consequent inhibition of PML oligomerization. HCMV: Human cytomegalovirus; IFI16: IFN- γ inducible protein 16; PYHIN: Pyrin/HIN domain; AIM2: Absent in melanoma 2; MIEP: Major immediate early promoter; ND10: Nuclear domain 10; ATRX: Alpha thalassemia and mental retardation syndrome X-linked; PML: Promyelocytic leukemia.

diverse key processes, such as DNA damage repair^[79], oncogenesis^[80-82], apoptosis^[83-85], senescence^[86,87], and gene expression regulation^[75,76]. The fact that ND10 are subject to profound biochemical modification during virus infection is of particular interest^[81,87-90]. During the infection of quiescent cells, evidence indicates that ND10 accumulates viral DNA within their central core and/or at their periphery. Moreover, considering the fact that IFNs stimulate an increase expression of PML, hDaxx, and Sp100^[91-93], it would appear that ND10 play a key role in the innate antiviral response.

ND10 restriction of HCMV replication vs viral evasion tactics

Although aggregates of HCMV *IE* gene transcripts only form adjacent to ND10 - originally considered the ideal cellular location for HCMV to initiate its program of *IE* gene expression - it was soon realized that early during infection HCMV actually targets ND10 in order to destroy them^[94-97] (Figure 1, Table 1). The viral regulatory protein IE1 is responsible for this activity; moreover ND10 destruction by IE1 correlates with efficient lytic replication^[98]. ND10 are now understood to be key cellular restriction factors playing an effective biological role in the inhibition of virus replication. The silencing of PML

expression by siRNA and thus depletion of this ND10 constituent, for instance, was shown to increase the susceptibility of "human primary foreskin fibroblasts" (HFF) to HCMV infection^[92]. Indeed, the capacity of ND10 bodies to restrict HCMV is achieved by down-regulating *IE* gene expression^[93]. Of note, when PML-null HFF were used, HCMV infection resulted in the formation of Sp100 and hDaxx^[99]. It has since emerged that it is actually the virus itself that stimulates the recruitment of ND10 constituents to nucleoprotein complex of HCMV^[98]. What is more, studies suggest that this ND10-instigated intrinsic immune response probably entails other ND10 associated proteins, like Sp100 and hDaxx^[100-102].

In summary, the results of studies carried out by various groups together depict a scenario where all the three key ND10 proteins - *i.e.*, Sp100, hDaxx, and PML - are involved in restricting viral replication through the silencing of viral *IE* gene expression^[97,102-104]. In addition, infection experiments comparing the effects of single vs double knock-down of ND10 constituents found that HCMV gene expression was actually lower in the single knock-down cells compared with the respective double knock-down conditions^[101,102]. This undoubtedly points toward the individual proteins playing independent roles in HCMV restriction. Epigenetic mechanisms able to

block viral genome transcription and replication may be involved^[105,106]. During early infection, viral DNA exists in a repressive chromatin state, a result of posttranslational modifications of histones^[106]. Through its ability to recruit to the viral DNA chromatin modifying enzymes, including the histone deacetylases, or chromatin remodeling protein “alpha thalassemia and mental retardation syndrome X-linked” (ATRX)^[105,107], hDaxx has been demonstrated to convert the HCMV immediate-early enhancer/promoter (MIEP) into a transcriptionally inactive chromatin state^[108]. Sp100 and PML have similarly been confirmed to interact with enzymes that modify chromatin; once again implicating the contribution of possible epigenetic modifications in the intrinsic immune repression of *IE* gene expression^[109,110].

Until now, the role of ND10 has largely been studied in the context of productive HCMV infection. Interestingly, Saffert and Kalejta^[110], using three different cellular settings, including NT2 and THP-1 cells, primary human CD34⁺ cells, and two myeloblastic cell lines (Kasumi-3 and KG-1), provided evidence that hDaxx is also involved in *IE* gene silencing in latent HCMV infections. By contrast, the group led by Sinclair provide evidence indicating that hDaxx protein is only marginally involved in MIEP regulation during latent infection, since its knockdown in NT2 cells block *IE* gene expression^[105]. To solve this apparent discrepancy, Stamminger’s group^[111] used the THP-1 monocytes recognized as reliable *in vitro* latency model of HCMV. In non-differentiated THP-1 monocytes, HCMV undergoes latency; while in THP-1 cells induced to undergo differentiation towards a macrophage-like phenotype, achieved using PMA, HCMV enters its lytic cycle^[111]. The results obtained showed that the silencing of PML, hDaxx, or Sp100 expression by small hairpin RNA in non-differentiated THP-1 monocytes did not have an effect on *IE* gene expression. In contrast, the silencing of ND10 in differentiated THP-1 significantly augmented cells positive for *IE* gene expression. Altogether, these conclusions indicate that hDaxx, PML, and Sp100 serve as restriction factors of *IE* gene expression, but are only marginally involved in the establishment of HCMV latency^[111].

Nevertheless, we know that HCMV is still able to undergo successful lytic replication in spite of the restrictive behavior of the ND10 constituent proteins; this tells us that HCMV has co-evolved to circumvent this aspect of the innate immune response. Indeed, we now know that shortly after virus penetration, pp71 (the viral transactivator tegument protein) moves to ND10 bodies where it interacts with ND10 constituent proteins^[112]; in particular, it associates with hDaxx, which, as discussed above, is capable of down-regulating *IE* gene expression by silencing MIEP^[113]. The interaction between pp71 and hDaxx results in the latter being directed down the path of proteasome degradation, thereby relieving MIEP repression^[112]. However, investigations by other groups have recently shown that the scenario is actually much more complex. Degradation of hDaxx is preceded by the

pp71-stimulated release of ATRX from ND10, and it is the displacement of ATRX that alleviates the repression of *IE* gene expression^[113].

While pp71 is fundamental to counteract the capacity of both ATRX and hDaxx to silence virus genes, it is the action of IE1 protein that seems to eliminate the restrictive effects of PML protein. Indeed, IE1 stimulates ND10 body dispersal, with the associated displacement of both PML and Sp100. At low MOI, IE1 synergizes with IE2 and promotes the activation of various viral gene expression^[114,115]. However, only IE1 is required for ND10 dispersal. Moreover, the disruption of PML by IE1 is not followed by degradation of PML *via* proteasome; instead it becomes de-SUMOylated; which effectively inhibits PML oligomerization and thus its ability to re-associate within ND10 bodies^[116] (Figure 1, Table 1).

LYSINE-SPECIFIC DEMETHYLASES

Lysine-specific demethylases (KDMs) inhibit the establishment of HCMV latency by getting rid of epigenetic “tags” present on histones associated with the repression of MIEP. Interestingly, the viral UL138 protein counteracts this defense by interfering with the association of KDMs with the MIEP^[117] (Table 1).

Thus, the presence of viral factors neutralizing cognate host restriction factors indicate that HCMV has developed multiple escape strategies over lifelong colonization at the cellular level.

CONCLUSION

In conclusion, frontline cell defense against HCMV replication is now known to be accomplished by different proteins through different pathways. Moreover, the viral countermeasures to overcome these restriction factors are now clearly understood to involve a number of viral proteins, including pp71, IE1, UL97, and pp65. Ongoing research is presently being focused at compiling a more in-depth picture of the molecular mechanisms involving ND10 that underlie the host cell’s restrictive response the viral evasion strategies.

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P- Reviewer: Chan CH, Roohvand F **S- Editor:** Qiu S

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