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Interferon-Independent Innate Responses to Cytomegalovirus

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The critical role of interferons (IFNs) in mediating the innate immune response to cytomegalovirus (CMV) infection is well established. However, in recent years the functional importance of the IFN-independent antiviral response has become clearer. IFN-independent, IFN regulatory factor 3 (IRF3)-dependent interferon-stimulated gene (ISG) regulation in the context of CMV infection was first documented 20 years ago. Since then several IFN-independent, IRF3-dependent ISGs have been characterized and found to be among the most influential in the innate response to CMV. These include virus inhibitory protein, endoplasmic reticulum-associated IFN-inducible (viperin), ISG15, members of the interferon inducible protein with tetratricopeptide repeats (IFIT) family, interferon-inducible transmembrane (IFITM) proteins and myxovirus resistance proteins A and B (MxA, MxB). IRF3-independent, IFN-independent activation of canonically IFN-dependent signaling pathways has also been documented, such as IFN-independent biphasic activation of signal transducer and activator of transcription 1 (STAT1) during infection of monocytes, differential roles of mitochondrial and peroxisomal mitochondrial antiviral-signaling protein (MAVS), and the ability of human CMV (HCMV) immediate early protein 1 (IE1) protein to reroute IL-6 signaling and activation of STAT1 and its associated ISGs. This review examines the role of identified IFN-independent ISGs in the antiviral response to CMV and describes pathways of IFN-independent innate immune response induction by CMV.

Keywords: interferon, cytomegalovirus, IFN-independent, ISG, herpes, innate immunity

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INTRODUCTION

HCMV has a 236 kbp double stranded DNA (dsDNA) genome, 165 genes (1) encoding up to 751 protein products (2), a 45–100% seroprevalence in the adult population (3–7), and remains a significant human pathogen particularly in those with an underdeveloped or suppressed immune system. Just as HCMV infection can profoundly alter the overall adaptive immune response (8–13), it also generates a powerful innate response. Key mediators of this innate response are IFNs. There are three types of IFN: type I (α, β, κ, ω, τ, and ε), type II (γ), and type III (λ1, λ2, λ3, λ4). Type I and II IFNs are the best characterized in the context of HCMV and their induction, antiviral roles as well as the viral antagonism of these processes have been extensively reviewed (14–19). A role for type III IFNs, in the innate response to HCMV and murine CMV (MCMV), whose pathogenesis closely parallels that of HCMV (20), has recently been elucidated (21–27).

The innate response to both HCMV and MCMV infection is initiated when virus is detected by pattern recognition receptors (PRRs) including toll-like receptors (TLRs) TLR2 (28–31) and TLR9 (32–34). Once virus has bound and entered cells, HCMV and MCMV can be detected by cytosolic DNA sensors such as IFI16 (35, 36), ZBP1/DAI (37–39) and cGAS (32, 40) that signal through the stimulator of IFN genes (STING). Each of these pathways culminates in activation and dimerization of IRF3 resulting in production of type I IFN (41–44). Type I IFN production is subsequently enhanced by upregulation of IRF7, an ISG that is also capable of dimerizing and activating the type I IFN promoter (45). HCMV and MCMV infection both trigger production of type II IFN from $CD8^+$ T cells, $CD4^+$ T cells and natural killer (NK) cells (46–48). HCMV even remodels the IFNγ locus (IFNG) for sustained IFNγ expression in $NKG2C^{hi}$ NK cells (49, 50). IFNλ production is induced by HCMV and MCMV infection (22) and these type III IFNs are themselves ISGs with production stimulated by IFNα and IFNβ treatment (51).

Key antiviral mediators of all IFN types are ISGs (52). Interferome, a database dedicated to chronicling all genes significantly regulated by IFN (changes $\geq$ 2-fold), identifies 12614 ISGs (53). Type I IFNs alone can trigger expression of more than 2,000 genes in humans, many of which are antiviral (54). Canonical induction of ISGs by type I, II, and III IFNs occurs by JAK/STAT signaling downstream of the type I IFN receptor (IFNAR1 + IFNAR2), the IFNγ receptor (IFNGR1 + IFNGR2) and the IFNλ receptor (IFNLR1 + IL10R2), respectively. The type I and II IFN receptors are widely expressed but type III IFN receptor expression is limited to epithelial cells (55, 56). ISGs stimulated by type I and III IFN contain an IFN stimulated response element (ISRE) in their promoter region that is bound by the activated transcription factor IFN stimulated gene factor 3 (ISGF3), comprised of phosphorylated STAT1 and STAT2 with IRF9 (55, 57–62), or by STAT2 homodimers associated with IRF9 (63–65). IFNγ induced ISG promoters contain γ-activated sequences (GAS) that are bound by STAT1 homodimers (66–70). However, upregulation of some ISG mRNAs in the early stages of HCMV infection (prior to DNA replication) are not inhibited by IFN neutralization (71, 72). Since this discovery, the body of literature demonstrating ISG induction independent of canonical IFN signaling pathways has been steadily expanding and those discussed in this review are summarized in **Figure 1**.

IFN-INDEPENDENT ISG PRODUCTION

Initial differential display analyses compared the susceptibility of genes upregulated early vs. late in infection to inhibition by IFN neutralizing antibodies and/or protein synthesis inhibitor cyclohexamide (CHX) (72). Three of these genes: IFIT2/ISG54/p54/cig42, IFIT3/ISG60/p60/cig49 and viperin/cig6, were upregulated by HCMV at 8 h post infection (hpi) and even accumulated following exposure to replication-incompetent ultraviolent-irradiated HCMV (UV-HCMV) (72). Blocking type I IFN with neutralizing antibodies failed to inhibit IFIT2, and IFIT3 induction, demonstrating that their upregulation was both IFN-independent and could be triggered by viral binding entry alone (72). A subsequent, broad mRNA analysis using oligonucleotide arrays found that levels of 258 mRNAs were altered more than 4-fold prior to initiation of HCMV DNA replication (71). IFIT2 and IFIT3 were among these quickly detected ISGs as were MxA, MxB, and ISG15 (71). The immediacy of this induction suggests a direct mechanism requiring few intermediary steps, indeed IFIT2, IFIT3, ISG15 (73) and viperin (72) upregulation can be detected 6 hpi with HCMV in the absence of *de novo* host and viral protein synthesis (cyclohexamide (CHX) treatment). This is also the case for IFIT1/ISG56/p56 (73) and indicates that this subset of ISGs may be induced/upregulated independently of IFN during HCMV infection.

IFN-Independent, IRF3-Dependent ISG Production

When searching for a mechanism underpinning IFN-independent ISG induction during CMV infection, initial studies turned to the powerful transcriptional regulator involved in IFN production, IRF3. Expression of constitutively active IRF3 in the absence of any viral stimulus could induce transcription of a subset of ISGs including IFIT1, IFIT2, IFIT3, ISG15, and viperin (74). IRF3-independent expression of these same ISGs was also observed during infection with other viruses: single stranded RNA (ssRNA) Newcastle disease virus (NDV) upregulated IFIT1, IFIT2 and ISG15 in cells that could respond to but were unable to produce type I IFN (75) and IFIT1 expression could be induced during ssRNA Sendai virus (SeV) infection by IRF3 nuclear translocation in cells unable to respond to type I IFN (76).

Studies using herpes simplex virus type 1 (HSV-1) demonstrated that IFIT1 expression could be driven by infection even in the presence of CHX in human fibroblasts (HFs) but could not be detected in the human epithelial osteosarcoma cell line U2OS (77). U2OS cells can respond to IFN but have defects in the STING signaling pathway (78) involved in IRF3 activation and dimerization in response to DNA sensing by IFI16, ZBP1/DAI, and cGAS (79–82). Furthermore, HSV-1 infection of $IRF3^{-/-}$, $IRF3^{-/-}IRF9^{-/-}$, and $IRF1^{-/-}$ murine fibroblasts revealed that IRF3 was essential for generation of an antiviral state and IFIT2 expression in response to UV-HSV-1 (83). In the case of IFIT1, expression was directly induced by an IRF3-containing complex binding to its promoter region (77, 84).

In the context of HCMV infection, initiation of IFIT2 transcription was found to occur independently of STAT1 nuclear localization (85) and in the presence of CHX (86). Soon it emerged that expression of IFIT1, IFIT2, IFIT3 and ISG15 during HCMV could be IFN-independent but always required IRF3 activation (42, 73, 87). Subsequent studies revealed that viperin expression could be driven directly by HCMV glycoprotein B (gB), in an IFN-independent, IRF3/IRF1 dependent manner (88, 89). This aligns with data demonstrating that IRF3 translocation to the nucleus is a requirement for the IFN-independent induction of an antiviral state in response to UV-HCMV (87). In contrast, another transcription factor implicated in type I IFN production NFκB (90), remains cytosolic (91).

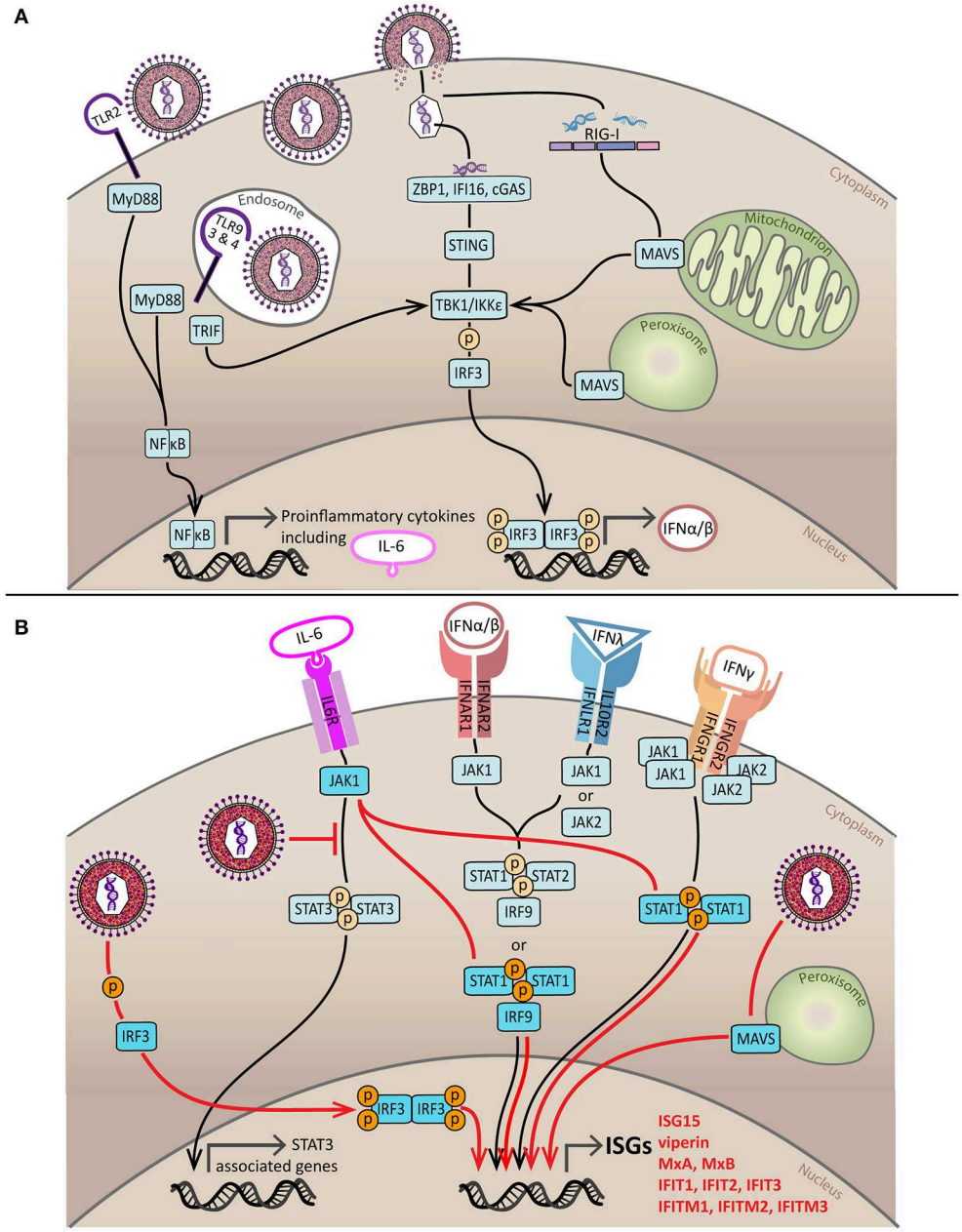

FIGURE 1 | Induction and subversion of the innate IFN response by HCMV. **(A)** Sensing of HCMV by components of the innate immune response initiates production of IFNs and proinflammatory cytokines. HCMV is sensed by PRRs on the cell surface (TLR2) and in endosomes (TLR3, TLR4, and TLR9). Signaling from TLR2, TLR3, and TLR4 is through MyD88 and results in the activation and nuclear translocation NFκB, a transcription factor that stimulates expression of proinflammatory cytokines such as TNF, IL-8, IL-12, and IL-6. TLR9 and TLR4 signal through TRIF which causes activation by phosphorylation of IRF3 via TBK1/IKKε, activated IRF3 dimerizes and enters the nucleus to stimulate production of type I IFNs. HCMV infection can also be recognized by viral nucleic acid detectors in the cytoplasm; DNA sensors ZBP1, IFI16 and cGAS signal through ER-resident STING to activate TBK1/IKKε whilst the viral RNA sensor RIG-I activates TBK1/IKKε by signaling via MAVS located on the mitochondria or peroxisomes. The end result of both of these pathways is IRF3 phosphorylation, dimerization, nuclear translocation and production of type I IFNs. **(B)** IFN-dependent and IFN-independent pathways of ISG induction during HCMV infection. For IFN-dependent induction of ISGs to occur type I, type II and type III IFNs must bind to their cell surface receptors. Type I and III IFN receptors signal through various combinations of JAK proteins to phosphorylate STAT1 or STAT1 and STAT2 which form a complex referred to as ISGF3 with IRF9. ISGF3 then translocates to the nucleus where it binds to the ISRE to induce ISG production. The type II IFN receptor utilizes both JAK1 and JAK2 to phosphorylate STAT1, leading to its dimerization and nuclear translocation. Once in the nucleus, activated STAT1 dimers bind to GAS and stimulate ISG production. The three key pathways of HCMV-mediated IFN-independent ISG induction are indicated in red. Firstly, HCMV can directly activate IRF3; additionally, HCMV can sequester STAT3 and redirect the activated JAK1, created by IL-6 receptor binding, to phosphorylate STAT1; and finally peroxisomal MAVS may be able to trigger IFN-independent ISG expression at early times following infection. Black line = canonical IFN-dependent ISG induction pathway, red line = HCMV-induced, IFN-independent ISG induction pathway.

To interrogate the IFN-independent, IRF3-dependent response to HCMV HFs have been engineered (92, 93) to lack either IRF3 through expression of the nPro protein of bovine viral diarrhea virus (BVDV) (nPro/HFs) which binds and degrades IRF3 (94) or STAT1, by expression of the parainfluenza virus type 5 (PIV-5) V protein (V/HFs) which targets STAT1 for proteasomal degradation (95). These nPro/HFs and V/HFs were recently utilized, alongside IRF3 KO CRISPR/Cas9 HFs, to demonstrate that expression of viperin, ISG15, IFIT1, IFIT2, IFIT3, Mx1, and Mx2 mRNA during infection with HCMV can be induced in an IRF3-dependent, STAT1-independent manner (96). In fact, mRNA levels of IFIT1, IFIT2, and IFIT3 were as highly elevated in the absence of STAT1-mediated IFNAR signaling as in the parental HFs (96) underlining the capacity of such IFN-independent mechanisms to profoundly regulate ISG expression. Many of these IFN-independent, IRF3-dependent ISGs are among the most potently induced by CMV infection and examining the roles these genes play in the innate response to CMV is essential to understanding the ramifications of this non-canonical regulation.

Viperin

Viperin inhibits the egress and replication of many viruses (97–102). However, in the context of HCMV, viperin upregulation is proviral, initiated by infection to manipulate cellular metabolism and cause the accumulation of cytosolic lipids for use in production of the viral envelope (103). In trophoblasts, a cell type of particular clinical relevance due to their role in the transmission of congenital HCMV (104–106), viperin is required for efficient expression of immediate early viral genes (107). Viperin is also known to enhance type I IFN production in plasmacytoid dendritic cells (pDCs) by localizing to lipid rafts and acting as a scaffold for recruitment of interleukin-1 receptor-associated kinase 1 (IRAK1) and TNF receptor associated factor 6 (TRAF6) (108).

In addition, viperin has been identified to act in its capacity as a member of the radical *S*-adenosyl-L-methionine (SAM) superfamily of enzymes to facilitate conversion of cytidine triphosphate (CTP) to 3′-deoxy-3′,4′-didehydro-CTP (ddhCTP) (109). Thus far, ddhCTP is known to act as a terminator of RNA synthesis by viral (Dengue and Zika) RNA-dependent RNA polymerases (109) and so investigations into its interaction with the HCMV encoded viral DNA polymerase are warranted. The viperin gene (RSAD2) lies in close proximity to the gene encoding cytidylate monophosphate kinase 2 (CMPK2) in the genome, suggesting a potential functional link to this pathway (109). Expression of CMPK2 is so closely linked to viperin that, following stimulation by IFN, viperin, CMPK2 and a long non-coding RNA (lncRNA) called lncRNA-CMPK2 are all co-transcribed (110). Interestingly, lncRNA-CMPK2 acts as a negative regulator of ISG expression (including ISG15, IFIT3 and IFITM1) (110). If IFN-independent, CMV-induced viperin upregulation also enhances expression of lncRNA-CMPK2, this could be a novel mechanism utilized by the virus to dampen the antiviral ISG response.

Furthermore, viperin has been demonstrated to be important for replication of Kaposi's sarcoma-associated herpesvirus (KSHV), a function attributed to the ability of viperin to catalyze oxidation of methionine in the viral DNA helicase, enhancing its expression and function (111). In this context, IFN-independent viperin upregulation by HCMV may be a way to ensure viral replication proceeds with maximum efficiency and thus the potential of viperin to modify the HCMV viral helicase-primase complex should be considered for further study. Overall, IFN-independent upregulation of viperin by HCMV seems to be a process initiated by the virus very early in infection to prepare the cell for its role as a virus-producing factory.

ISG15

ISG15 is a small ubiquitin-like protein that exists in three forms: (1) unconjugated within the cell, (2) conjugated within the cell (112, 113), and (3) secreted into serum (mainly by granulocytes) where it promotes NK maturation and IFNγ production (114). During HCMV infection accumulation of both free and conjugated ISG15 can be partially inhibited by interfering with the canonical IFNAR signaling pathway with a JAK inhibitor (115) but some IFN-independent, IRF3-dependent expression remains (96). Whilst the mechanisms by which ISG15 regulates CMV infection are currently unknown, it appears to possess antiviral activity as blocking ISG15 accumulation enhances viral replication (115) and HCMV antagonizes both the production of unconjugated ISG15 and ISGylation (115–118).

On the other hand, it is interesting to note that whilst in murine studies $ISG15^{-/-}$ mice are generally more sensitive to disseminated viral infections (119) human patients presenting with primary immunodeficiencies associated with defects in ISG15 expression are not (120). In fact, $ISG15^{-/-}$ fibroblasts isolated from such patients and primed with type I IFN were less susceptible to infection with HCMV than controls. This was attributed to the elevated levels of antiviral ISGs in these cells, a result of ISG15s ability to bind and stabilize the E3 ubiquitin ligase-like protein USP18, which acts as a negative regulator of the type I IFN response (120, 121).

It is also possible that HCMV manipulates levels of ISG15 to shift monocytes toward the mixed M1/M2 macrophage phenotype that is observed during infection (122) and hypothesized to enhance viral dissemination and persistence (123, 124). This is because in the absence of infection, ISG15 plays a role in the maintenance of mitochondrial homeostasis (125). Specifically, ISGylation of mitochondrial components can control mitochondrial function: reducing the rate of oxidative phosphorylation (OXPHOS) and causing a corresponding decrease in mitochondrial reactive oxygen species (ROS) (126). A reduction in levels of mitochondrial ROS alters macrophage polarization, shifting these cells toward a mixed M1/M2 phenotype (126).

IFITs

IFITs are ISGs with antiviral capabilities against flaviviruses, poxviruses, coronaviruses and papillomaviruses (127–130). A pan-viral mechanism of host defense mediated by IFITs is the sequestration of eukaryotic initiation factor (eIF3) by IFIT1 which slows the overall rate of cellular protein synthesis (76, 84, 131). A more specific strategy depends on the recognition

and binding of viral RNA lacking 2′-O methylation of the 5′ RNA cap by IFIT1 (132). This binding ability is enhanced by association with IFIT2 and IFIT3 (133). Despite the fact that CMV replication takes place wholly within the nucleus, export of viral mRNAs does occur (134) and these may be sensed by IFITs. Another possibility is that IFITs may directly bind essential CMV proteins in the cytoplasm, as IFIT1 does to inhibit human papilloma virus (HPV) infection (135, 136). Although the mechanisms of the IFIT-mediated antiviral response to HCMV are still unclear, a significant reduction in titer has been reported when the virus is grown in IFIT1 overexpressing fetal astrocytes (137).

IFITMs

IFITM proteins are also implicated in the antiviral response against a wide range of viruses: orthomyxoviruses, flaviviruses, filoviruses, and coronaviruses often by blocking membrane fusion (127, 138–140). However, overexpression of IFITM1, IFITM2 and IFITM3 does not inhibit HCMV infection but rather results in a modest increase in the percentage of infected cells (141, 142). Short hairpin RNA (shRNA) knockdown of IFITM1 alone or in combination with IFITM2 and IFITM3 inhibits HCMV infection as they are required for successful formation of the HCMV virion assembly complex (vAC) and production of infectious progeny virions (142). It is interesting to note that despite this proviral role, IFITM proteins are noticeably downregulated at later stages of infection (48–72 hpi) (142).

Direct induction of IFITMs by HCMV may also contribute to the severe consequences of congenital infection as IFITM expression can inhibit the fusion of cytotrophoblast cells into the multinucleated syncytiotrophoblast, a structure at the interface between maternal and fetal tissue, essential for placental development (143).

MxA and MxB

The Mx proteins MxA and MxB are a family of dynamin-like GTPases first reported for their antiviral activity against influenza and are now well characterized in response to other viruses (144, 145). MxA is found in the cytosol and inhibits influenza virus infection through retention of the viral genome (146). On the other hand, MxB localizes to the cytoplasmic face of nuclear pores (147) and is able to inhibit HIV-1 replication by blocking nuclear viral genome accumulation (148, 149). Both MxA and MxB are highly upregulated by HCMV infection (73, 150) and it has recently been discovered that MxB overexpression inhibits replication of HSV-1, HSV-2, Kaposi's sarcoma-associated herpesvirus (KSHV), MCMV, and HCMV (151, 152). HSV-1 and MCMV inhibition manifested in a similar way to that of HIV-1, a block in the delivery of viral genome to the nucleus (151). However, in terms of the regions of protein at play, this mechanism was found to differ substantially with a requirement for GTP binding but not GTP hydrolysis (152, 153). Knockdown of MxB has also been implicated in stalling cell cycle progression (147) and it has been suggested that the HCMV virion protein pUL69 that contributes to the cell cycle arrest (154) does so via an interaction with MxB (155).

ALTERNATE IFN-INDEPENDENT PATHWAYS OF INNATE RESPONSE INDUCTION

Direct ISG induction by IRF3 is not the only pathway associated with the IFN-independent response to CMV. In human monocytes, IFN-independent, biphasic activation of STAT1 with differential phosphorylation at early (30 min) compared to late (24 h) time points post-HCMV infection appears to influence motility, migration, differentiation and polarization (156).

Regulation of mitochondrial activity is emerging as another IFN-independent innate response mediator. A number of years ago it was discovered that HCMV DNA could induce ISG expression in an IRF3-dependent, TLR-independent manner that involved TANK-binding kinase 1 (TBK1), IκB kinase epsilon [IKKε; originally called IKK-inducible (IKKi)], and mitochondrial antiviral-signaling protein (MAVS) (157). More recently, peroxisomal MAVS has been implicated in rapid type-I IFN-independent ISG (viperin, Mx2, IFIT3, IFIT2) expression (158). Conversely, mitochondrial MAVS appears to be involved in IFN-dependent ISG production (158). HCMV actively impairs mitochondrial MAVS signaling through the viral mitochondria-localized inhibitor of apoptosis (vMIA) and reduces type I IFN production (159). vMIA has also been found to localize to peroxisomes and induce their fragmentation by interaction with the cytoplasmic chaperone protein Pex19, hijacking the transport machinery of peroxisomal membrane proteins (160). This suggests that disabling IFN-independent ISG transcription induced by peroxisomal MAVS contributes to efficient CMV infection.

The HCMV immediate early gene 1 (IE1) is also capable of inducing expression of ISGs in the absence of IFN production. HCMV IE1 induces expression of IL-6 (161) which usually signals through JAK and STAT3 (162). However, IE1 binds and sequesters STAT3 (163), leaving JAK, already activated by IL-6, free to phosphorylate STAT1. Thus IE1 re-routes IL-6 signaling to activate STAT1 resulting in transcription of ISGs independently of IFN (164).

FUNCTIONAL IMPORTANCE OF IFN-INDEPENDENT INNATE RESPONSES

Early studies examining IFN-independent induction of an antiviral state showed that treatment of human embryonic lung fibroblasts (HELFs) with UV-HCMV rendered these cells resistant to subsequent viral infection in the absence of detectable IFN production (91). Intriguingly, whilst high multiplicity of infection (MOI) UV-HCMV also induced an antiviral state in the HELFs, this required IFN production (91). Paladino et al. (91) proposed a model by which, when cells are exposed to limited numbers of virus particles (low MOI), induction of an internal antiviral state is sufficient to control infection, however, when many virus particles are present (high MOI), cells secrete IFN to protect neighboring

cells too. The ability to induce an antiviral state in the absence of IFN production may be important in cells such as neurons, where inflammation is undesirable. In this respect, neurotropic arboviruses have been shown to induce protective type I IFN-independent, IRF3-dependent responses (165).

Recently, the power of IFN-independent innate responses to CMV has been illustrated by the finding that human macrophages co-cultured with HCMV-infected retinal pigment epithelial cells (RPEs) can limit viral replication and spread in a cell-cell contact dependent manner that could not be blocked by vaccinia-derived type I IFN binding protein B18R, nor by neutralizing antibodies against either IFNγ or TNFα (166). It has also been shown that HCMV virus particles pre-treated with HCMV-specific antibodies that do not replicate, nor express IE antigens, can enter human macrophages and induce an antiviral state that renders these cells less susceptible to subsequent HCMV infection independently of IFN production (167).

IFN-independent ISG induction can also be used to regulate the development of cells key to viral persistence and dissemination. In human monocytes infected with HCMV, ISGs are upregulated independently of IFN (4 hpi) that function to enhance monocyte motility and migration (156). This occurs in a STAT1-dependent manner that also suppresses transcription of anti-inflammatory M2-associated cytokines (IL-10 and CCL18), promoting polarization of macrophages toward a mixed M1/M2 phenotype (156). ISG15 was among the ISGs found to be upregulated in monocytes 4hpi with HCMV (156). ISG15 may contribute both directly and indirectly to the mixed M1/M2 macrophage phenotype, causing monocyte-specific upregulation of IL-10 (168) whilst simultaneously inducing production of M1 macrophage-stimulating cytokine IFNγ by NK and T cells (114).

CONCLUDING REMARKS

When considering the innate response to CMV infection, IFN and the ISG-mediated induction of an antiviral state are important first elements. The intention of this review has been to highlight the substantial body of literature accumulating around IFN-independent innate responses to CMV. IFN-independent induction of ISGs is an important phenomenon and ISGs produced via this pathway appear to play both pro- and anti-viral roles during infection. This complicates direct interrogation of the IFN response during viral infection and necessitates careful consideration of kinetics, as particular ISG may be upregulated directly by the virus, independently of IFN, to play a proviral role early in infection but later on, when IFN-dependent expression dominates, may antagonize infection.

When examining plaque number and size at 7 days post infection, we reported no difference in rates of CMV replication nor spread between cells unable to produce IFN (IRF3 degraded) and those that could not respond to IFN (lacking STAT1) (92), even though the latter cells still allowed viral induction of IFN-independent ISGs (96). Focusing on earlier time points, before loss of IFN production/signaling becomes the overwhelming factor affecting infection efficiency, may reveal more subtle differences conferred by abrogation of either IRF3 or STAT1 signaling.

A deeper understanding of the various functions of IFN-independent ISGs may enable their relative abundance to serve as a predictor of disease progression. For example, high levels of IL-6 have been correlated with CMV reactivation and poor prognosis for transplant patients (169–171); perhaps this is because ISGs produced by IE1 re-routing the IL-6 response to enhance infection. If this were the case, interference with STAT1 homodimer-mediated ISG expression may improve prognosis.

Since CMV infected, polarized macrophages are key mediators of T cell activation and proliferation (172), if IFN-independent ISGylation influences macrophage polarization then levels of ISG15 induced directly by CMV early in infection may provide an indication as to whether or not a robust T cell response will be generated.

It is also important to note that many of these ISGs, including viperin, IFIT2, IFIT3, Mx1 and ISG15 are defined as part of the 28 core mammalian ISGs i.e., produced in all nine mammalian species tested (54). It would therefore be prudent to determine whether their IFN independence is also conserved across species especially since rhesus CMV does not induce IRF3 activation nor the associated ISG expression (173).

Finally, with the IFN-independent nature of these ISGs becoming clear, caution should be exercised when using these ISGs as surrogate readouts for interferon signaling, as it is clear that they are also induced directly by viral infection.

AUTHOR CONTRIBUTIONS

CA generated the initial draft of the manuscript. All other authors (BM, AA and BS) contributed to the subsequent writing and review of the manuscript.

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