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Review

Viral interferon regulatory factors and their role in modulating the immune response to gamma2-herpesvirus infection

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ABSTRACT

Herpesviruses result in life-long infections within their hosts. All herpesviruses must therefore contend with both the innate and adaptive immune responses, in order to persist. A novel immune evasion strategy employed by gamma2-herpesviruses such as Kaposi's sarcoma-associated herpesvirus (KSHV) and rhesus macaque rhadinovirus (RRV) is the viral interferon regulatory factors (vIRFs). Cellular interferon regulatory factors (IRFs) play important roles in regulating the interferon (IFN)-signaling pathway and are often targeted by invading pathogens. The vIRFs are viral proteins, which share homology with cellular IRFs. Research in the field of vIRFs has uncovered multi-faceted roles for these unique viral factors. Importantly, multiple studies have demonstrated that the vIRFs are involved in regulating both the innate and adaptive immune responses to viral infection. This report will provide a comprehensive review on the immune-modulatory effects of KSHV and RRV viral interferon regulatory factors.

KEYWORDS: viral interferon regulatory factors, vIRF, innate, adaptive, RRV, KSHV, *in vivo*

1. Introduction

The myriad of defensive schemes that the immune system employs to combat viral infections is only rivaled by the different ways viruses can evade and antagonize this response. Viral infections are first met with the fast acting and nonspecific innate immune response. Pattern recognition receptors, encoded by the host, detect pathogen-associated molecular patterns and trigger a wide array of often inter-connected signaling cascades leading to the production of antiviral molecules, changes in cellular functions, or programmed cell death. Critical factors in the innate response are interferons (IFNs) of which there are three types: type I (includes IFNα and IFNβ), type II (IFNγ), and type III (includes IFNλ) [1]. IFNs are peptides secreted from cells that act in autocrine and/or paracrine fashions to elicit antiviral states within cells. The regulation of IFNs is largely controlled by interferon regulatory factors (IRFs), a family of transcription factors involved in the regulation of IFNs and IFN-stimulated genes, and as such, activation of IRFs is central to the development of a successful IFN and innate immune response [2]. If the innate immune response does not clear a viral infection, then the adaptive immune response is activated and provides a specialized attack on the foreign invader and also provides an immunological memory in case the invader returns.

The *Herpesviridae* family consists of enveloped, double stranded DNA viruses that are further divided into *Alphaherpesvirinae*, *Betaherpesvirinae*, and *Gammaherpesvirinae* subfamilies. Herpesviruses are renowned for their ability to infect hosts and establish latent, life-long infections. As such, herpesviruses encode a plethora of macromolecules

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designed to evade both the innate and adaptive immune responses (Figure 1) [3-15]. An example of this is Kaposi's sarcoma-associated herpesvirus (KSHV), a gamma-herpesvirus that naturally infects humans [16]. While infection with KSHV is generally asymptomatic in healthy individuals, the virus can promote the development of Kaposi's sarcoma (KS), primary effusion lymphoma (PEL), multi-centric Castleman's disease (MCD), and some non-Hodgkin's lymphomas (NHL) in immune-compromised individuals, including AIDS patients [16-19]. Unfortunately, establishing animal models for KSHV have proven difficult [20]. Various attempts have been made to move KSHV research into animal models, with varying degrees of success [20-23]. Additionally, *in vitro*, KSHV produces a predominantly latent infection in cell culture, making the study of virus replication and expression of viral genes difficult [24, 25]. A viable alternative is to study an animal virus that can induce similar disease manifestations in its natural host, such as Rhesus macaque Rhadinovirus (RRV), which naturally infects rhesus macaques (RMs) and shares a high level of genetic similarity to KSHV [26, 27] (Figure 2). *In vitro*, RRV grows well in cell culture, and can generate a robust lytic infection in primary rhesus fibroblast cells [26, 28-30]. Importantly, RRV infection of RMs provides a powerful animal model with which to study KSHV-like infection and associated disease, as RRV infection of simian immunodeficiency virus (SIV)-infected RMs can lead to the development of MCD, NHL and retroperitoneal fibromatosis (a mesenchymal proliferative lesion that possesses cellular features that resemble KS) [31, 32]. Thus, the RRV/RM model provides an ideal system with which to study gamma-herpesvirus infections, dissect the roles and contributions of viral open reading frames (ORFs) and non-coding RNAs, and elucidate mechanisms of viral-mediated pathogenesis.

Both KSHV and RRV encode ORFs with homology to cellular IRFs, termed viral interferon regulatory factors (vIRFs) (Table 1). KSHV encodes four vIRFs (vIRF-1 through vIRF-4) from four ORFs, while RRV encodes eight vIRFs (R6 through R13) from eight ORFs [27, 33, 34]. The vIRFs of KSHV

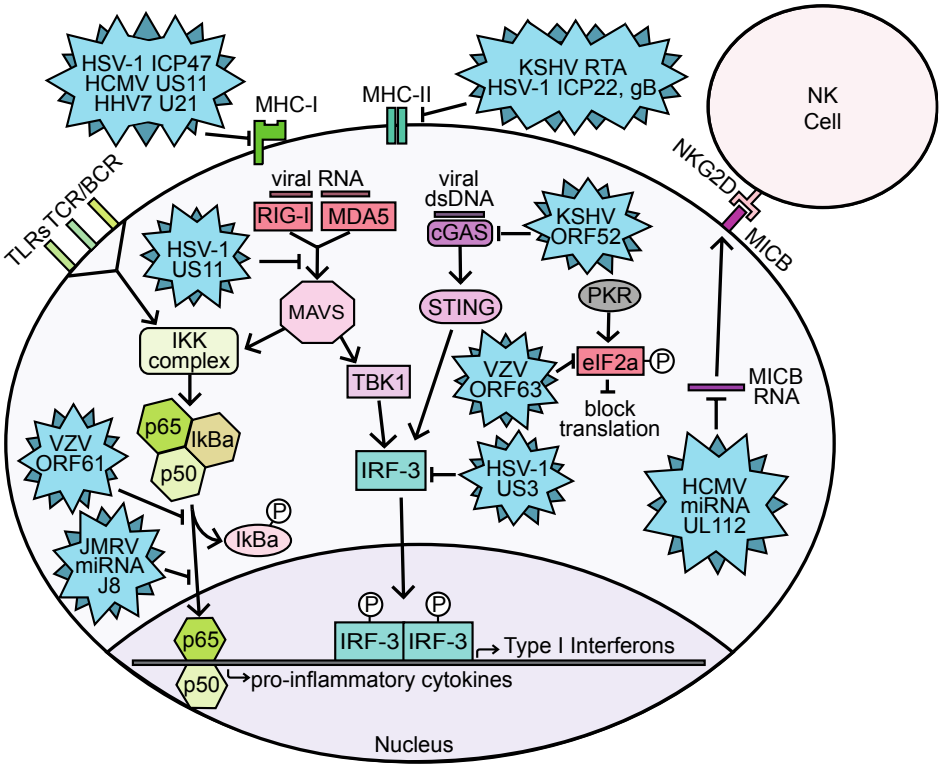

Figure 1. Herpesvirus evasion mechanisms. Examples of the diverse mechanisms employed by various primate herpesviruses to evade host immune defenses.

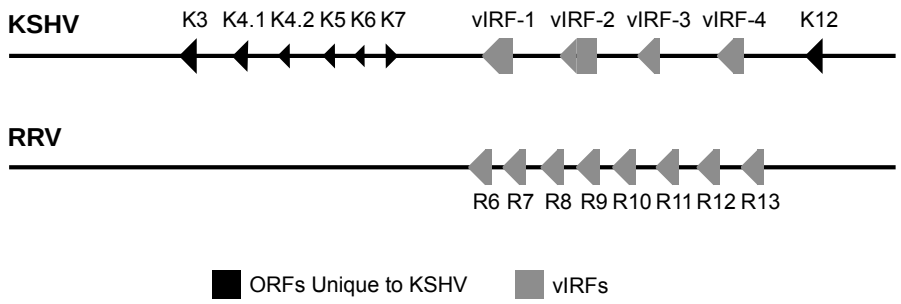

Figure 2. KSHV and RRV genomes. Diagram of the KSHV and RRV genomes. Black arrows denote ORFs that are only present in the KSHV genome. Gray arrows denote the vIRF ORFs. Diagram is not to scale.

Table 1. Comparisons of vIRFs and cellular IRFs.

		Cellular IRF	KSHV vIRF	RRV vIRF
KSHV	vIRF-1	IRF8[a] 23% / 15%	--	R10 28% / 15%
	vIRF-2	IRF4[a] 31% / 21%	--	R11 27% / 11%
	vIRF-3	IRF4[a]* 36% / 16%	--	R9 25% / 19%
	vIRF-4	none	--	R12* 26% / 16%
RRV	R6	IRF8[b] 21% / 16%	vIRF-1 25% / 15%	R10 45% / 12%
	R7	IRF8[b] 22% / 16%	vIRF-1 23% / 16%	R11 45% / 18%
	R8	IRF8[b] 23% / 14%	vIRF-1 21% / 15%	R12 52% / 17%
	R9	IRF8[b] 25% / 19%	vIRF-3* 25% / 16%	R13 53% / 11%
	R10	IRF8[b] 23% / 14%	vIRF-1 28% / 13%	--
	R11	IRF8[b] 24% / 16%	vIRF-1 24% / 15%	--
	R12	IRF8[b] 23% / 16%	vIRF-2 23% / 20%	--
	R13	IRF8[b] 21% / 16%	vIRF-1* 23% / 14%	--

Protein-protein BLAST comparison using NCBI database. Results presented as % identity/% similarity. [a]denotes comparison with human genome, [b]denotes comparison with macaca mulatta genome, *denotes those comparisons that required a position-specific iterated BLAST (PSI-BLAST) algorithm to produce a result.

have been extensively studied and have been shown to possess transcriptional regulatory, oncogenic, cell survival, and immune evasion functions [35-39]. While the RRV vIRFs have thus far been studied mainly for their role in immune evasion [40-42]. This review will discuss the current knowledge of the function of vIRFs in modulating the immune response to viral infection.

2. KSHV vIRF-1

2.1. Innate immune modulation by vIRF-1

vIRF-1 interacts with two adaptor molecules of the innate immune system, stimulator of interferon signaling genes (STING) and mitochondrial antiviral signaling protein (MAVS). When cyclic GMP-AMP synthase (cGAS) binds to dsDNA in the cytosol it synthesizes the second messenger cyclic GMP-AMP (cGAMP). cGAMP subsequently binds to STING resulting in conformational changes to STING and the phosphorylation of STING by tank binding kinase 1 (TBK1) [43-46]. IRF-3 is then able to bind to STING, and TBK1 can then phosphorylate and activate IRF-3, leading to transcription of IFNβ [46, 47]. The interaction of vIRF-1 and STING (demonstrated by transient expression of vIRF-1 *in vitro*) prevents the phosphorylation of STING and the ability of TBK1 to bind to STING [48].

RIG-I and MDA5 are cytosolic proteins capable of binding and detecting viral dsRNA in the cytosol [49]. Once bound to RNA, RIG-I and MDA5 interact with MAVS leading to the multimerization and phosphorylation of MAVS, which results in the activation of NFκB or IRF-3/7 (through the IKK complex and TBK1, respectively) to induce the transcription of type I IFNs and pro-inflammatory cytokines [46, 49]. While a role for RIG-I/MAVS signaling in the inhibition of KSHV infection and reactivation has been reported, it is currently unknown how KSHV is able to activate MAVS [50]. Regardless, vIRF-1 was recently reported to inhibit MAVS signaling during reactivation of KSHV [51]. MAVS is membrane bound on the surface of mitochondria and vIRF-1 was shown to also localize to mitochondria in a MAVS-dependent manner, in reactivated KSHV-infected PEL and BCBL-1 cells. Additionally, vIRF-1 was found to co-immunoprecipitate with MAVS. vIRF-1 expression during KSHV reactivation was able to inhibit the aggregation (and activation) of MAVS and disrupt the antiviral activity of MAVS (e.g., IFNβ production and induction of apoptosis) [51].

Several studies have also confirmed that vIRF-1 does interact with or otherwise inhibit cellular IRFs, as was hypothesized due to their homology to cellular IRFs. While two reports presented conflicting data as to whether *in vitro*-translated vIRF-1 was able to bind to *in vitro*-translated IRF-1, both reports demonstrated that exogenous expression of vIRF-1 was able to inhibit IRF-1 transactivating functions and antiviral functions [52, 53]. In addition to IRF-1, vIRF-1 also inhibits the functions of IRF-3. When protein levels of vIRF-1 were reduced (using peptide-conjugate phosphorodiamidate morpholino oligomers which block translation of target RNA sequences) in KSHV-infected and 12-o-tetradecanoylphorbol 13-acetate (TPA)-induced BCBL-1 cells, protein levels of both IRF-3 and STAT1 increased [54].

vIRF-1 has also been shown to interfere with the CBP/p300 transcriptional coactivator complex [55, 56]. Specifically, when vIRF-1 was over-expressed in Sendai virus-infected 293 cells and immunoprecipitated, vIRF-1 was found to bind to CREB binding protein (CBP) and p300 and reduce the ability of IRF-3 to bind the CBP/p300 complex. Decreased levels of IRF-3, in conjunction with impaired binding to transcriptional coactivators, results in the specific block of IRF-3 signaling by vIRF-1 following viral infection [56].

The overall effect of vIRF-1 modulation of cellular IRFs and transcriptional co-activators is the inhibition of the transcription of type I IFNs, as well as other IFN-stimulated genes. Activation of both the IFNA and IFNB gene promoters was shown to be greatly diminished by transient transfection of vIRF-1 [51, 52]. Additionally, in response to Sendai virus infection, exogenous vIRF-1 inhibited the transcription of IFNα, IFNβ, and the IFN-stimulated genes ISG15, RANTES and IP-10 [56-58]. Interestingly, one report questioned the biological relevance of vIRF-1 during lytic KSHV replication. Elevated levels of vIRF-1 following reactivation in BCBL-1 cells were only transient and occurred early during the lytic cascade. While high levels of vIRF-1 could inhibit IFNα-induced gene expression, this was not sustained once levels of vIRF-1 dropped resulting in the inhibition of infectious virus production [59].

Aside from the inhibition of transcriptional activation, exogenous vIRF-1 has also been shown to co-immunoprecipitate with HERC5, an E3 ligase responsible for conjugating the ubiquitin-like ISG15 protein onto target proteins [60]. Overexpression of vIRF-1 reduced the levels of ISG15 conjugation to cellular proteins, perhaps through its interaction with HERC5. Because knockdown of ISG15 by short hairpin RNA

(shRNA) resulted in increased KSHV reactivation, it has been suggested that vIRF-1 may play a role in the reactivation of latent KSHV infections [60].

2.2. Adaptive immune modulation by vIRF-1

T cells are important in the adaptive immune response, and function by recognizing specific pathogens, clearing them either directly or indirectly, and remaining in circulation to patrol the host for subsequent infections by the same pathogen. In order to recognize peptides derived from pathogens, the peptides must be presented in the context of major histocompatibility complex (MHC) class I or MHC class II molecules on the surface of infected cells. Lagos *et al*. demonstrated that overexpression of vIRF-1 results in reduced transcription and cell surface expression of MHC I, thereby preventing recognition of KSHV-infected cells by CD8+ T cells *in vitro*. The authors went on to show that inhibition of MHC class I expression was mediated through the interaction of vIRF-1 with the transcriptional coactivator p300, as removal of the p300 binding sequence from vIRF-1 prevented vIRF-1-mediated downregulation of MHC class I in transfected cells [61]. Thus, vIRF-1 is capable of regulating the adaptive immune response directly, in addition to its roles in modulating the innate immune response.

3. KSHV vIRF-2

3.1. Innate immune modulation by vIRF-2

Like vIRF-1, vIRF-2 is able to interact with cellular IRFs. One report demonstrated that vIRF-2 interacts with IRF-1, IRF-2, and IRF-8 in *in vitro* pull-down assays, while a second report discovered an interaction between vIRF-2 and IRF-3 as well [62, 63]. However, only a down regulation of the IRF-1- and IRF-3-induced transactivation of the IFNA4 and IFNB gene promoters has been demonstrated [62-64]. The vIRF-2 inhibition of IRF-3 was shown to occur through the simultaneous binding of vIRF-2 to both activated IRF-3 and procaspase-3 [63]. Exogenous expression of vIRF-2 was able to induce the loss of IRF-3 even in the presence of the proteasome inhibitor MG132, while treatment with a general caspase inhibitor was able to inhibit the vIRF-2-induced loss of IRF-3 protein. This implicates caspase 3 (and perhaps other caspases) in the vIRF-2-induced degradation of IRF-3. Interestingly, this same study found a caspase 3-independent mechanism for vIRF-2 inhibition of IRF-3 function; however, the mechanism has not been fully elucidated [63].

Additionally, a GST-vIRF-2 fusion protein was found to bind to the transcriptional coactivator p300 as well as to the p65 component of NFκB. In fact, His_6-vIRF-2 homodimers were found to bind to the NFκB consensus-binding site and exogenous expression of vIRF-2 was able to inhibit NFκB transactivation of IFNβ [62].

vIRF-2 is also able to inhibit signaling through the interferon stimulated response element (ISRE) as induced by IFNα or the IFNλ family members IL-28A and IL-29. vIRF-2 is able to block ISRE signaling by reducing protein levels of IRF-9 and phosphorylated STAT1, which prevents the formation of ISGF-3 [65]. ISGF-3 is a complex composed of STAT1, STAT2, and IRF9, that translocates to the nucleus and acts upon ISRE promoters to induce transcription of IFN-stimulated genes [66].

Lastly, it has been demonstrated that ectopically expressed vIRF-2 can bind to and inhibit the kinase activity of protein kinase R (PKR), resulting in the inhibition of the IFNα-induced translational block [67]. PKR expression is induced upon IFN stimulation and when PKR binds to dsRNA it is activated and can phosphorylate eIF2α leading to a block in cellular translation. This block in translation can lead to programmed cell death as a means to clear viral infection [68]. The binding of vIRF-2 to PKR inhibited the phosphorylation of eIF2α by PKR following dsRNA treatment [67]. However, eIF2α is not the only target of PKR. Under certain circumstances PKR can also phosphorylate IκBα, the negative regulator of NFκB resulting in degradation of IκBα and activation of NFκB [69]. However, there is no direct evidence that vIRF-2 can inhibit the PKR-mediated activation of NFκB.

4. KSHV vIRF-3

4.1. Innate immune modulation by vIRF-3

Similar to vIRF-1 and vIRF-2, vIRF-3 is also able to interact with cellular IRFs. The outcome of these interactions, however, is not as clear as with vIRF-1 or -2. Both exogenously and endogenously expressed IRF-5 protein co-immunoprecipitates with endogenous

vIRF-3 protein in multiple KSHV-infected cell lines [70, 71]. In reporter cell lines, exogenous vIRF-3 expression inhibits IRF-5 mediated activation of IFNA and IFNB promoters. In this same system, it was found that overexpression of vIRF-3 also results in reduced type I IFN production following Newcastle disease virus infection, indicating that vIRF-3 can prevent virally-induced production of IFN [70]. vIRF-3 was found to inhibit the transactivating function of IRF-5 by preventing IRF-5 from binding to DNA promoters [70, 71].

Additionally, vIRF-3 has been shown to modulate the activities of IRF-3 and IRF-7. One report demonstrated a repressive function for vIRF-3, in which co-transfection of vIRF-3 with either IRF-3 or IRF-7 (in mouse NIH3T3 cells) resulted in reduced activation of the IFNA4 promoter following virus-stimulation, when compared to transfection of either IRF-3 or IRF-7 alone [72]. However, a second report from the same group presented data that vIRF-3 enhances activation of IFNA and IFNB promoters in human cell lines [73]. The authors of this study showed that exogenous vIRF-3 complexes with endogenous IRF-3 and IRF-7; this interaction does not inhibit these cellular IRFs from binding to CBP/p300, and the presence of vIRF-3 increases the DNA binding affinity of this complex. When vIRF-3 was over-expressed, the transactivation of IFNA and IFNB promoters by IRF-3 and IRF-7 following Sendai virus infection were increased compared to IRF-3 and IRF-7 alone [73].

Like vIRF-2, vIRF-3 has also been found to be capable of modulating NFκB signaling. As stated above, NFκB must first dissociate from IκBα before it can translocate and act in the nucleus. This dissociation is partly regulated by the IκB Kinase (IKK) complex, of which IKKβ is a member [74]. Transiently expressed vIRF-3 selectively binds to and inhibits IKKβ kinase activity leading to repression of the NFκB transactivating functions [75].

Additionally, vIRF-3 is able to inhibit PKR signaling. Ectopic expression of vIRF-3 inhibited the PKR-mediated block in translation as well as apoptosis; however, no direct interaction between vIRF-3 and PKR was found [76]. Therefore, vIRF-3 likely utilizes a different mechanism than vIRF-2 to inhibit PKR signaling. Interestingly, exogenous expression of vIRF-3 was not able to inhibit PKR activation of NFκB [76].

4.2. Adaptive immune modulation by vIRF-3

In addition to its innate immune evasion functions, vIRF-3 also plays a role in the evasion of the adaptive immune response. While vIRF-1 functions to down modulate MHC class I molecules, vIRF-3 has been found to play a role in the inhibition of MHC class II expression [77, 78]. MHC class II transcription is induced by the MHC class II transactivator protein, CIITA, and CIITA transcription is induced by IFNγ [79]. Overexpression of vIRF-3 inhibits the transcription of CIITA by inhibiting the production of IFNγ, which results in the reduced levels of MHC class II transcripts and protein [77, 78]. Additional data also suggests a CIITA-independent mechanism for the downregulation of MHC class II transcription. While overexpression of vIRF-3 in a KSHV-negative B cell line resulted in the reduction of both CIITA and MHC class II transcripts, the knock down of vIRF-3 in a KSHV-positive B cell line resulted in a reduction in MHC class II transcripts without a change in CIITA transcript levels [78]. The CIITA-independent mechanism of vIRF-3 regulation of MHC class II expression has not yet been elucidated.

5. RRV vIRFs

The eight RRV vIRFs (encoded by ORFs R6 through R13) are located in the same region of the genome as the KSHV vIRFs (Figure 2) [27, 80]. Six of the RRV vIRF protein sequences share some level of identity with KSHV vIRF-1 (21%-28%) while R9 and R12 share some level of identity with KSHV vIRF-3 (25%) and vIRF-2 (23%), respectively (Table 1). Unlike KSHV, RRV displays robust lytic replication *in vitro*, allowing for the unique opportunity to study the function of vIRFs during *de novo* infection [24, 25, 30]. A complete analysis of the function of all 8 RRV vIRFs is still forthcoming; however, much has already been learned about the function of several of the vIRFs both *in vitro* and *in vivo*.

6. RRV vIRF immune modulation *in vitro*

6.1. Analysis of individual vIRFs *in vitro*

Analysis of the RRV vIRF R6 has been performed using both *in vitro* overexpression systems and virological approaches [40]. Using a luciferase reporter rhesus fibroblast cell line and transient

transfection of RRV R6, it was shown that R6 could inhibit the activation of the ISRE promoter by poly(I:C). This inhibition correlated with the reverse transcription (RT)-PCR results showing that transient transfection of RRV R6 could reduce IFNβ transcripts by 50% in poly(I:C)-stimulated cells compared to empty vector. Further, it was found that R6 achieves this inhibition of the IFNβ response by binding to CBP and phosphorylated IRF-3. Due to this interaction, R6 prevents the CBP/p300/IRF-3 complex from binding to DNA, resulting in the shuttling of phosphorylated IRF-3 out of the nucleus, followed by proteasomal degradation. Finally, using both exogenously expressed R6 and an infectious bacterial artificial chromosome (BAC)-derived form of RRV encoding a form of R6 tagged with an HA epitope tag, R6 was shown to act early during infection, and was also demonstrated to be packaged within the virion. As a result, this virion-associated form of R6 can function immediately upon infection to inhibit IFNβ transcription [40].

Transient transfection of either R10 or R11 inhibited the poly(I:C)-stimulated secretion of type I IFN, although to a lesser extent than R6 [41]. Interestingly, transient transfection of R7 into a rhesus fibroblast luciferase reporter cell line, followed by transfection of poly(I:C) stimulation, revealed that R7 does not inhibit type I IFN transcription or secretion. In fact R7 appeared to enhance the stimulation by poly(I:C) compared to empty vector [41].

6.2. Analysis of the complete set of RRV vIRFs *in vitro*

Using an infectious BAC of RRV strain 17577, a recombinant virus was generated in which all eight vIRF-encoding ORFs were deleted from the viral genome, resulting in the production of a vIRF knockout virus (vIRF-ko RRV) [41]. Analysis of this recombinant vIRF-ko RRV virus indicated that it displayed similar growth kinetics in rhesus fibroblasts *in vitro* when compared to wild-type BAC-derived RRV (WT-BAC RRV). However, *in vitro* infection of RM peripheral blood mononuclear cells (PBMCs) or splenocytes with vIRF-ko RRV indicated that this virus was less efficient at infecting these cells than WT-BAC RRV, implying that vIRFs help RRV establish infection. It was suggested that this differential infection efficiency between the two viruses was due to differential inhibition of the IFN response. This was confirmed by the analysis of transcript levels in infected telomerized rhesus fibroblasts and rhesus PBMCs, where it was found that vIRF-ko RRV virus induced higher levels of both type I and type II IFNs compared to WT-BAC RRV between 6 and 72 hours post-infection [41]. One cell type that was found to display similar infection efficiencies for both vIRF-ko RRV and WT-BAC RRV was plasmacytoid dendritic cells (pDCs). In rhesus pDCs, intracellular cytokine staining showed that vIRF-ko RRV virus infection resulted in longer and sustained IFNα production compared to WT-BAC RRV [41]. These results suggest a role for the RRV vIRFs in the suppression of both the type I and type II IFN response, early during RRV infection. In addition, Western blot analysis and immunofluorescence microscopy showed that vIRF-ko RRV infection of rhesus fibroblasts resulted in increased levels of phosphorylated IRF-3 within the nucleus compared to WT-BAC RRV infection [41]. This implies that vIRF modulation of IRF-3 may be one mechanism by which RRV suppresses the type I IFN response. Taken together, these findings all clearly demonstrate that the vIRFs function in inhibiting the IFN production during RRV infection.

7. RRV vIRF immune modulation *in vivo*

Analysis of the effects of vIRF deletion on *in vivo* infection and immune regulation was accomplished by infecting RMs with vIRF-ko RRV virus and comparing this to RMs infected with WT-BAC RRV. In these studies, expanded specific pathogen-free RMs seronegative for RRV were infected intravenously with $5x10^6$ PFU of either WT-BAC RRV or vIRF-ko RRV [42]. These studies were the first to analyze the function of any viral vIRFs during *de novo* infection *in vivo*. The results of this work indicated that the RRV vIRFs aid in the initial infection, replication, and persistence of the virus. Specifically, infection of RMs with vIRF-ko RRV virus resulted in lower viral DNA loads (measured in whole blood) and less viremia compared to WT-BAC RRV infection. Additionally, CD20+ B cells isolated from vIRF-ko RRV-infected RMs harbored much lower levels of RRV genome compared to WT-BAC RRV-infected RMs [42].

7.1. Innate immune modulation *in vivo*

Following experimental intravenous infection of RMs with either WT-BAC RRV or vIRF-ko RRV, type I IFN was measured in the plasma by incubation with type I IFN reporter cells. This sensitive assay revealed that the vIRFs are important for inhibiting the type I IFN response early during infection (within the first two weeks). Specifically, 75% of vIRF-ko RRV-infected RMs had measurable levels of type I IFN in their plasma at one day post-infection, and all animals displayed sustained type I IFN levels in their plasma within the first two weeks of infection [42]. In comparison, only 50% of the WT-BAC RRV-infected RMs had any measurable type I IFN in their plasma during the first two weeks, and only 33% of RMs had sustained type I IFN levels in their plasma during the first two weeks of infection [42]. These results suggest the RRV vIRFs play a role in the suppression of the type I IFN response at early time-points during *in vivo* infection.

7.2. Adaptive immune modulation *in vivo*

In addition to their effects on innate immune responses *in vivo*, the RRV vIRFs were also found to inhibit the development of RRV-specific T cell responses, especially within the first two weeks of infection. vIRF-ko RRV infection of RMs resulted in the detection of RRV-specific CD4+ and CD8+ T cells 7 to 14 days earlier than with WT-BAC RRV-infected RMs [42]. In line with these differences in T cell responses, IFNγ and IL-12p40 (two cytokines important for development of a TH1 response) were detected in plasma of vIRF-ko RRV-infected RMs at elevated levels throughout the first week of infection (IFNγ), or became detectable two weeks earlier (IL-12p40) than in WT-BAC RRV-infected RMs [42]. IFNγ was detected in the plasma of vIRF-ko RRV-infected RMs during the

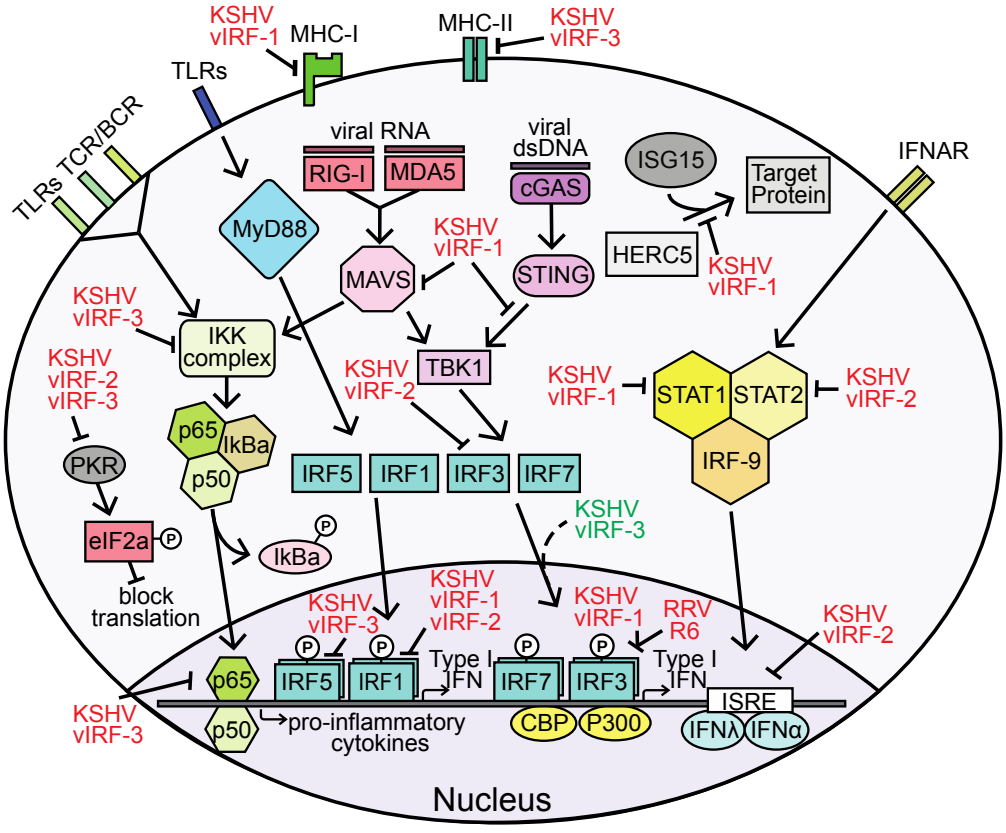

Figure 3. vIRF immune evasion strategies. Schematic depicting the known roles of each vIRF in host immune evasion. The vIRFs labeled in red represent those which inhibit the immune system, while the vIRF labeled in green represents a vIRF that enhances the designated immune signaling pathway.

Table 2. Effects of RRV vIRFs *in vitro* and *in vivo*.

	In vitro	*In vivo*	
Innate immune evasion	vIRFs reduce transcript levels of type I and type II interferons between 6-72 hpi in tRFs and PBMCs	vIRFs reduce IFNα levels in plasma during the first two weeks of infection	**Innate immune evasion**
	vIRFs inhibit the production of IFNα by pDCs	vIRFs inhibit the sustained production of IFNγ in plasma during the first two weeks of infection	**Adaptive immune evasion**
	vIRF R6 binds to CBP and phosphorylated IRF-3 preventing the complex from binding to DNA and inhibiting IFNβ production in tRFs	vIRFs inhibit the appearance of IL-12p40 in plasma during the first two weeks of infection	
	vIRFs R10 and R11 both inhibit the poly(I:C) stimulated secretion of type I IFN in HEK293 cells	vIRFs delay the RRV-specific CD4+ and CD8+ T cell response	

first 7 days post-infection, while WT-BAC RRV-infected RMs only displayed detectable levels of IFNγ in plasma at day 1 post-infection. IL-12p40 was detected in plasma of vIRF-ko RRV-infected RMs one day after infection, but was not detected at similar levels in the plasma of WT-BAC RRV-infected RMs until day 14 post-infection [42].

8. Summary

Analysis of the vIRFs encoded by KSHV and RRV has shown that these viral proteins act in multiple ways to affect the immune response to viral infection (Figure 3). Data obtained thus far on the RRV vIRFs suggests similar roles, and perhaps mechanisms, for immune evasion as compared with the proposed functions of the KSHV vIRFs (Table 2). *In vivo* studies of RRV have also demonstrated that vIRFs have a functional consequence on *de novo* viral infection, aiding in the establishment of infection in the host by suppressing both the innate and adaptive immune responses. Continued research on the RRV vIRFs will provide important information on vIRF functions in the context of viral infection both in cell culture and in the RM model, which may clear up some of the conflicting data obtained for the KSHV vIRFs in cell culture. Future studies in both the KSHV and RRV models will continue to elucidate the many functions and mechanisms of the vIRF ORFs.

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CONFLICT OF INTEREST STATEMENT

The authors state no conflict of interest.

ABBREVIATIONS

BAC : bacterial artificial chromosome
CBP : CREB-binding protein
cGAMP : cyclic GMP-AMP
cGAS : cyclic GMP-AMP synthase
IFN : interferon
IKK : IκB kinase
IRF : interferon regulatory factor
ISRE : interferon-stimulated response element
KS : Kaposi's sarcoma
KSHV : Kaposi's sarcoma-associated herpesvirus
MAVS : mitochondrial antiviral signaling protein
MCD : multi-centric Castleman's disease
NHL : non-Hodgkin's lymphoma
ORF : open reading frame
PBMCs : peripheral blood mononuclear cells
pDC : plasmacytoid dendritic cells
PEL : primary effusion lymphoma

PFU	:	plaque forming units
PKR	:	protein kinase R
RM	:	rhesus macaque
RRV	:	rhesus macaque rhadinovirus
SIV	:	simian immunodeficiency virus
STING	:	stimulator of interferon signaling genes
TBK1	:	tank-binding kinase 1
TPA	:	12-o-tetradecanoylphorbol 13-acetate
vIRF	:	viral interferon regulatory factor
vIRF-ko	:	vIRF knockout virus
WT	:	wildtype

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