The inflammasome as a target of modulation by DNA viruses

Lisi Amsler‡[1], Daniel Malouli‡[1] & Victor DeFilippis*[1]
[1]Vaccine & Gene Therapy Institute, Oregon Health & Science University, 505, NW 185th Avenue, Beaverton, OR 97006, USA
*Author for correspondence: Tel.: +1 503 418 2757 ■ Fax: +1 503 418 2701 ■ defilipp@ohsu.edu
‡Authors contributed equally

The cellular innate immune response represents the initial reaction of a host against infecting pathogens. Host cells detect incoming microbes by way of a large and expanding array of receptors that react with evolutionarily conserved molecular patterns exhibited by microbial intruders. These receptors are responsible for initiating signaling that leads to both transcriptional activation of immunologically important genes as well as protease-dependent processing of cellular proteins. The inflammasome refers to a protein complex that functions as an activation platform for the cysteine protease caspase-1, which then processes inflammatory molecules such as IL-1β and IL-18 into functional forms. Assembly of this complex is triggered following receptor-mediated detection of pathogen-associated molecules. Receptors have been identified that are essential to inflammasome activation in response to numerous molecular patterns including virus-associated molecules such as DNA. In fact, the importance of cytoplasmic DNA as an immune stimulus is exemplified by the existence of at least nine distinct cellular receptors capable of initiating innate reactivity in response to this molecule. Viruses that employ DNA as genomic material include herpesviruses, poxviruses and adenoviruses. Each has been described as capable of inducing inflammasome-mediated activity. Interestingly, however, the cellular molecules responsible for these responses appear to vary according to host species, cell type and even viral strain. Secretion of IL-1β and IL-18 are important components of antimicrobial immunity and, as a result, pathogens have evolved factors to evade or counteract this response. This includes DNA-based viruses, many of which encode multiple redundant counteractive molecules. However, it is clear that such phenotypes are only beginning to be uncovered. The purpose of this review is to describe what is known regarding the activation of inflammasome-mediated processes in response to infection with well-examined families of DNA viruses and to discuss characterized mechanisms of manipulation and neutralization of inflammasome-dependent activity. This review aims to shed light on the biologically important phenomena regarding this virus–host interaction and to highlight key areas where important information is lacking.

Infection of host cells by viruses and other microbial pathogens stimulates rapid intracellular innate immune responses. These are largely initiated by pattern recognition receptors (PRRs) that detect evolutionarily conserved pathogen-associated molecular patterns (PAMPs). Innate immune reactivity leads to initiation of cellular signaling that culminates in the expression or processing of directly antiviral or otherwise immunologically active molecules. These include antiviral effector molecules, interferons, and pro-inflammatory cytokines and chemokines. Such proteins are essential to the antiviral immune response and are involved directly by creating cellular and tissue environments refractory to virus replication, and indirectly by coordinating adaptive cell-mediated immune processes.

PRR-dependent signaling can lead to the activation of transcription factors including IRF 3 and 7, NF-κB, ATF2 and Jun. These proteins are involved in transcriptional upregulation of numerous immunologically important genes including type I IFNs (IFN-β and IFN-α subtypes), antiviral effectors (e.g., PKR, Viperin and ISG56) and proinflammatory molecules (e.g., TNF-α, IL-6 and IL-8). Stimulation of specific PRRs can also lead to activation of the cysteine protease caspase-1 (previously known as IL-1β-converting enzyme) by way of assembly of a protein complex termed the 'inflammasome' [1]. The inflammasome is commonly composed of an individual PRR, an adaptor molecule termed ASC, as well as caspase-1 that, following assembly, is cleaved from an inactive zymogen to an active protease. However, an ASC-independent inflammasome has also been described, in which the PRR is able to directly bind and activate pro-caspase-1 [2–5]. Active caspase-1 processes IL-1β

Review
Future Virology

Keywords
■ adenovirus ■ caspase-1 ■ herpesvirus ■ IL-18 ■ IL-1β ■ immunomodulation ■ inflammasome ■ inflammation ■ innate immunity ■ poxvirus

Future Medicine part of fsg

and IL-18 from their immature forms (pro-IL-1β and pro-IL-18) into secretable molecules. IL-33 has also been shown to be a target of caspase-1 [6], although its function in an antiviral context remains unclear. In some cell types (e.g., undifferentiated monocytes), IL-1β processing first requires an initial 'priming' event involving PRR-triggered, NF-κB-dependent expression of pro-IL-1β [7,8], although pro-IL-18 is constitutively expressed [9]. In addition to IL-1β and IL-18 processing, inflammasome-mediated caspase-1 activation is also required for cleavage of other, less well-defined substrates that ultimately lead to 'pyroptosis', a process of caspase-1-mediated apoptosis [10]. As such, inflammasome-associated activities constitute a microbe-triggered innate immune response involved in cell-intrinsic and paracrine-directed antimicrobial activity.

The immunological effects of IL-1β and IL-18 secretion are diverse and entail both innate and adaptive antiviral functions. Both cytokines are involved in activation of immune cells, such as monocytes, macrophages and neutrophils, and control development of $CD4^+$ T-cell activity toward Th17 (IL-1β) or Th1 (IL-18) responses [11–13]. IL-1β induces strong pyrogenic and inflammatory effects in numerous tissues [14], and the protein can affect nearly every cell type due to the constitutive expression of the IL-1 receptor (IL-1R) with which it interacts. Once stimulated, IL-1R leads to the activation of NF-κB, p38 and JNK transcription factors that trigger expression of numerous proinflammatory genes and endothelial adhesion molecules facilitating the migration of immune cells to sites of infection [15]. IL-18, by contrast, does not confer pyrogenic activity and signals via a receptor (IL-18R) that exhibits a more restricted expression pattern on Th1 cells, some myeloid cells and intestinal epithelial cells [16,17]. IL-18R signaling triggers activation of transcription factors that are similar to those triggered by IL-1R signaling due to functionally related intracellular domains of the two receptors. IL-18 strongly contributes to the induction of the Th1 response involving production and secretion of IFN-γ [12]. Organismal regulation of the effects of IL-1β and IL-18 involves at least five levels: control of transcription, mRNA stability, protein processing, secretion and receptor antagonism [17]. As will be discussed below, viral evasion of these cytokines' effects can also involve multiple, redundant mechanisms perhaps acquired evolutionarily from host cells.

The PRRs involved in microbial detection comprise a large and expanding group of molecules [18]. These include cell surface and endosomal proteins, such as Toll-like receptors (TLRs), of which there are ten human and 12 mouse members [19]. TLRs react with diverse ligands including lipopolysaccharide, nucleic acids and glycoproteins. Their stimulation induces activation of IRF3/7 or NF-κB and subsequent expression of antiviral effectors, type I IFNs or proinflammatory cytokines (including pro-IL-1β). Cytoplasmic PRRs include the RIG-I-like receptors, such as LGP2, RIG-I and MDA5, which are involved in sensing dsRNA species and signaling to IRF3 and NF-κB. RIG-I has also been shown to be capable of triggering virus-induced caspase-1 activation [20,21]. Another class of cytosolic receptors, termed nucleotide oligomerization domain-like receptors (NLR), consists of 23 human and at least 34 mouse proteins [22]. These are mostly categorized by their effector domains, which include PYRIN domains (NLRP) and caspase activation and recruitment domains, among others [23]. NLRP1 and NLRP3 (cryopyrin) are known to stimulate inflammasome assembly and caspase-1 activation in response to microbial molecules including anthrax lethal toxin (NLRP1) and muramyl dipeptide, bacterial RNA, poly(I:C), lipopolysaccharide and microbial lipopeptide, as well as numerous nonmicrobial stimuli (NLRP3) [24]. The broad diversity of NLRP3-stimulatory molecular structures suggests that the protein is not a ligand-interacting receptor, but rather a requisite intermediary for upstream sensors.

In recent years a surprising number of PRRs required for the detection of cytoplasmic dsDNA have emerged [25]. These include many that are involved in stimulating production of type-I IFNs such as ZBP1/DAI [26], DDX41 [27], RNA polymerase III [28,29], DHX9 and DHX36 [30] and LRRFIP1 [31]. Two other proteins, AIM2 and γ-IFI16, are dsDNA-sensing hematopoietic interferon-inducible nuclear proteins with a 200-amino acid repeat. These have both been shown to stimulate inflammasome assembly and caspase-1 activation in response to cytoplasmic DNA and infection with large DNA viruses [32–34]. Interestingly, IFI16 appears unique in that it has also been shown to be important for IRF3-mediated induction of type I IFN in response to infection with DNA viruses [35]. The large number of cellular receptors found to be capable of and required for innate immune activation in response to dsDNA overtly highlights the emerging importance of this molecule as a PAMP. Importantly, while DNA-based genomes are universal among cell-based microbial parasites

(e.g., bacteria, fungi and eukaryotes), they are employed by only a subset of viral pathogens that exhibit highly diverse morphology and replication strategies themselves.

The focus of this review is a presentation of what is currently known regarding the molecular basis of activation of inflammasome-mediated processes by infection with DNA-based viruses, and the mechanisms used by these agents to impair or otherwise tolerate these responses. We chose to examine DNA viruses for two key reasons. First, as exemplified by the long and expanding list of DNA-associated PRRs, it is clear that detection of microbial DNA, whether genomic or otherwise, is an extremely important sentinel mechanism for innate immune activation and subsequent antiviral responses. Second, DNA viruses (especially large DNA viruses such as herpesviruses and poxviruses) exhibit potent, often redundant strategies for modulating inflammasome-dependent processes. These strategies are only beginning to come to light and, thus, numerous virus-encoded phenotypes directed at inflammasome-mediated activity remain to be characterized. Ultimately, it is our hope that this work provides a useful condensing of the key phenomena, both known and unknown, related to the interaction between the inflammasome and DNA viruses that facilitate and direct future hypothesis formulation and experimental endeavors. We present summaries of our current understanding of the molecular bases of induction and modulation of inflammasome-mediated processes phylogenetically, according to virus family, type and strain to allow evolutionary contrasts to be made evident.

Herpesviruses

The *Herpesviridae* comprise a highly diverse family of large, enveloped, dsDNA viruses that utilize the host cell nucleus for viral gene expression [36]. Herpesviruses are categorized into α, β and γ subfamilies according to evolutionary history and key aspects of tissue tropism and persistence strategy. Herpesviral infection triggers numerous and diverse innate and adaptive immune responses [37–40], yet despite these processes, all herpesviruses are able to persist in an infected host for life through the use of sophisticated mechanisms of immune evasion [41–43]. Activation and inhibition of the inflammasome by herpesviral species is still evolving as a field of research, yet important discoveries have been made, with implications for understanding viral pathogenesis and immune evasion (Table 1). The following sections will discuss what is currently known regarding the inflammasome-terminal molecular sensors involved in herpesvirus detection and strategies employed to block these responses.

α-herpesviruses

HSV-1 is the prototypic α-herpesvirus member and is responsible for mild herpetic lesions (cold sores), but can also lead to more serious conditions in immunocompromised hosts. Despite its prominence in herpesvirus research efforts, surprisingly little is known regarding HSV-1-mediated activation or inhibition of inflammasome-associated processes. Two separate groups have investigated HSV-1 in this context and concluded that the virus induces IL-1β processing and secretion in mouse thioglycollate-elicited macrophages [44] or human promonocytic THP-1 cells [45], and that this is an AIM2-independent process. Intriguingly, while IFI16 is known to be important for inflammasome activation by Kaposi's sarcoma-associated herpesvirus (KSHV) [32] (see below), to be essential for HSV-1-induced IFN synthesis [35,46,47] and even to promote resistance to HSV-1 infection in epithelial cells [48], the receptor has yet to be examined for its importance in inflammasome activation by HSV-1. Contrasting with these reports, global secretome analysis of HSV-1-infected human primary macrophages found no caspase-1, IL-1β or IL-18 release, even when cells were primed to express all necessary inflammasome components [49]. These results suggest that HSV-1 infection either does not activate inflammasome function in these cells or does so, but encodes a potent inhibitor(s) that subsequently blocks activation. It is worth noting that the reports mentioned here employ disparate multiplicities of infection (MOI) during HSV-1-induced inflammasome activation analysis ranging from MOI = 40 [44] to MOI = 1 [49]. Thus, the discrepancy in observed phenotype between species, cell lines and experimental procedures implies that further exploration including standardization of HSV-1-associated inflammasome activity is needed.

Varicella-zoster virus (VZV; human herpes virus 3) is also a member of the α-herpesvirus subfamily and is responsible for varicella (chickenpox) during primary infection and herpes zoster (shingles) following reactivation from viral latency later in life [50]. Nour *et al.* demonstrated that VZV infection is capable of stimulating caspase-1 activation and IL-1β secretion in three permissive human cell lines (promonocytic THP-1, primary lung fibroblasts and melanoma cells) [45]. Using a cross-linked anticaspase-1 antibody column, the authors showed that VZV infection led to

Table 1. List of virus-specific inflammasome-activating pattern recognition receptors and corresponding virus-encoded inhibitors.

Virus type	Detecting pattern recognition receptor	Inhibitor(s)	Ref.
Herpesvirus			
HSV-1	Unknown	Unknown	[44,45,49]
VZV	NLRP3?	Unknown	[45]
MCMV	AIM2	Unknown	[44]
KSHV	IFI16	Orf63	[32,56]
EBV	RIG-I, AIM2	miR-BART15	[64,71]
Poxvirus			
VV (WR)	AIM2	B13R (CrmA/SPI-2), B15R (vIL-1βR), C12L (vIL-18BP)	[33,93,105,115]
VV (MVA)	NLRP3	Loss of CrmA/SPI-2; retained vIL-1βR, vIL-18BP	[78]
CPXV	Unknown	CrmA/SPI-2, vIL-1βR, vIL-18BP	[80.106.115]
ECTV	Unknown	CrmA/SPI-2, vIL-1βR, vIL-18BP	[97,107,130]
MCV	Unknown	MC54L (vIL-18BP)	[115]
MYXV	NLRP3	Serp-2 (CrmA/SPI-2), M13L (ASC inhibitor)	[79,98,100]
SFV	Unknown	gp013L (ASC inhibitor)	[101]
Adenovirus			
Ad5	NLRP3	Unknown	[135,136,138]

Ad5: Adenovirus 5; CPXV: Cowpox virus; ECTV: Ectromelia virus; KSHV: Kaposi's sarcoma-associated herpesvirus; MCMV: Mouse CMV; MCV: Molluscum contagiosum virus; MVA: Modified vaccinia virus Ankara; MYXV: Myxoma virus; SFV: Shope fibroma virus; VV: Vaccinia virus strain; VZV: Varicella-zoster virus; WR: Western reserve.

the formation of an inflammasome complex that includes caspase-1, ASC and NLRP3. Furthermore, VZV-mediated inflammasome activation did not involve free radical reactive oxygen species (ROS), described as a microbe-induced trigger for other NLRP3-dependent responses [51]. Increased NLRP3 expression was also demonstrated in VZV-infected human skin xenografts in a SCID mouse model. Importantly, as with HSV-1, VZV-induced IL-1β secretion occurred independently of AIM2, a receptor shown to be necessary for inflammasome activation by other DNA viruses, such as the herpesvirus MCMV and vaccinia virus (VV) (discussed below) [33,44,45]. These results further illustrate the lack of consistency in host cell molecules involved in innate reactions to evolutionarily related viruses, a phenomenon likely associated with patterns of PRR expression across cell types and differences in tissue/cell tropism between related virus types (Table 1).

β-herpesviruses

The β-herpesvirus subfamily includes various CMVs, as well as human herpesvirus (HHV) 6A, 6B and 7. Human CMV (HCMV) is a genomically large (235 kb; ~170 open reading frames) pathogen infecting up to 80% of adults by age 40 in the USA [201]. The virus establishes a latent infection in myeloid progenitors and is generally asymptomatic in immunologically healthy individuals. However, it is a leading infectious cause of developmental abnormalities and can cause serious disease under conditions of immunocompromise [52]. CMVs are highly species-specific and, thus, animal models require infection with CMVs that have coevolved with the model species – a disadvantage when considering highly divergent mammals such as mice and humans. While IL-1β secretion has been observed during monocyte infection with HCMV [53], mouse CMV (MCMV) represents the most studied β-herpesvirus in the context of the molecular basis of inflammasome activation. Infection of macrophages harvested from wild-type mice with MCMV was found to activate caspase-1, as well as IL-1β processing and release [44]. By contrast, inflammasome activation is abolished during MCMV infection of macrophages from *AIM2*$^{-/-}$ mice. During MCMV infection *in vivo*, IL-18 plays an important role since it induces NK cells to produce IFN-γ [54,55]. In mice infected with the virus, the absence of AIM2 significantly diminished the serum concentration of IL-18 and similar results were seen

using mice lacking ASC. Moreover, absence of AIM2 and ASC led to a decrease in IFN-γ-producing NK cells in the spleen, determined *ex vivo* by intracellular staining, as well as a significant increase in splenic MCMV titers [44]. Thus, MCMV-induced inflammasome activity confers important downstream effects on the adaptive antiviral immune response.

To date it has been shown that HCMV induces IL-1β secretion during infection of primary myeloid cells [53]. However, unlike other human herpesviruses [32,45,56], little is known about the molecular basis of this secretion. Interestingly, the inflammasome receptor IFI16 has been investigated in the context of HCMV replication and viral gene expression and somewhat conflicting results are evident. Gariano *et al.* [57] recently showed that the receptor acts as a restriction factor for viral replication by sequestering a necessary host transcription factor. In contrast, another report demonstrated that IFI16 actually promotes viral replication in association with the viral protein pp65 [58]. While these reports imply different roles for IFI16, they suggest that the receptor might be important for the HCMV life cycle and its effect on inflammasome activation invites further investigation.

γ-herpesviruses

Members of the γ-herpesvirus subfamily are grouped in their potential to transform infected host cells [59]. The most clinically prominent γ-herpesviruses include EBV (HHV-4) and KSHV (HHV-8). EBV primarily infects B cells and is associated with Burkitt's lymphoma, Hodgkin lymphoma and nasopharyngeal carcinoma (NPC) [59]. KSHV is associated with Kaposi's sarcoma, primary effusion lymphoma and multicentric Castleman's disease – conditions that are most prevalent under conditions of immunosuppression such as with AIDS [59]. Animal-associated γ-herpesviruses that are employed as models for human disease and immunology include murine herpesvirus strain 68 and rhesus macaque rhadinovirus.

KSHV was recently shown to activate the inflammasome via the DNA receptor IFI16 [32]. KSHV infection of human endothelial cells was found to trigger the formation of a protein complex containing IFI16, ASC and caspase-1 in the nucleus. This result is the first demonstration of nuclear operation of inflammasome activity, a particularly relevant finding in light of the importance of nuclear localization of herpesvirus replication events. In addition, KSHV genomic DNA was found to colocalize with IFI16 and depletion of this receptor (but not AIM2) eliminated KSHV-induced caspase-1 activation. These results strongly argue that KSHV-associated DNA is responsible for triggering inflammasome-dependent responses via its detection by nuclear IFI16 and is consistent with the high significance of viral DNA as an immunostimulatory molecule.

KSHV exhibits a well-defined latent state involving the expression of few viral proteins [60,61], but during this period the virus downregulates the expression of immune response genes, including proinflammatory cytokines such as IL-1β [62]. Moreover, among herpesviruses, KSHV was the first for which an inflammasome inhibitor was described [56]. Orf63 protein was shown to interact directly with NLRP1 and inhibit its association with and activation of procaspase-1. Importantly, when primary human monocytes are infected with KSHV in the presence of shRNA directed against Orf63, an increase in IL-1β secretion and a decrease in lytic viral gene expression (Orf49, Orf50 and Orf57) is observed. In KSHV-infected BCBL-1 primary effusion lymphoma cells, the absence of Orf63 also decreased lytic viral gene expression and virus production. These data indicate that Orf63 is able to inhibit NLRP1-dependent inflammasome function, and that this is critical for viral gene expression and reactivation from latency. Interestingly, the authors also demonstrate that Orf63 can interact with NLRP3 and inhibit inflammasome activation induced by different NLRP3 agonists in THP-1 cells. This observation, along with bioinformatics analysis suggesting that Orf63 is likely not a structural NLR homolog [63], indicates that the protein evolved to behave as an inhibitor of multiple NLR-dependent responses. Importantly, the relationship between IFI16-dependent inflammasome induction by KSHV- and ORF63-mediated inhibition has not been examined [56]. While Orf63 was shown to inhibit NLRP3 inflammasome activation in THP-1 cells [56], NLRP3 was not found to be relevant for KSHV-induced inflammasome activity in endothelial cells [32], although its activity against KSHV in other cell types is not currently known. Thus, many questions remain regarding the exact basis and physiological relevance of Orf63's inhibitory ability. Furthermore, the relevance of NLR inflammasomes to KSHV infection appears to differ between cell types, a phenomenon that remains to be appropriately addressed.

EBV has also been studied in the context of inflammasome modulation. Extensive work in EBV-associated NPC cells has shown that they present upregulation of the inflammasome-related

components ASC, caspase-1, IL-1β, AIM2, RIG-1 and NLRP3, and that this correlates with better survival in NPC patients [64]. Importantly, by silencing the different receptors with specific siRNAs, investigators demonstrated that AIM2 and RIG-I activate the inflammasome in NPC cells upon detection of transfected EBV dsDNA and small RNAs, respectively. The involvement of a RIG-I inflammasome is not surprising, since it has been shown that EBV also induces IRF3 activation via RIG-I-mediated detection of virus-encoded RNAs [65,66]. Interestingly, activation of NLRP3 inflammasome by factors from the tumor microenvironment has also been demonstrated [63]. This is the first report implying a role for specific inflammasomes in an EBV-relevant context.

EBV also illustrates a potentially novel mechanism of inflammasome inhibition. Recently, it was shown that cellular *miR-223* is capable of controlling NLRP3 levels in a differentiation-dependent manner through post-transcriptional effects targeting the 3′-untranslated region (UTR) of NLRP3 mRNA, and that this can subsequently control inflammasome-dependent processes [67]. The virus is known to express multiple miRNA species [68], many of which are actually released from infected cells in exosomes [69,70]. Recently Haneklaus *et al.* demonstrated that the EBV-encoded *miR-BART15* could target the NLRP3 3′-UTR using the same binding site as *miR-223* [71]. In addition, exosomes containing *miR-BART15* released from EBV-infected B cells were found to be capable of targeting 3′-UTRs in proximal uninfected cells and even diminishing NLRP3-dependent inflammasome activity in those cells. While additional studies will be needed to characterize the biological importance of this mechanism, exosome-associated miRNA represents a potentially paradigm-shifting viral immune-evasion strategy. This also points to a role for NLRP3 in EBV-induced inflammasome activation.

Poxviruses

Poxviruses comprise an ancient family of closely related viruses that include a linear, dsDNA genome and that infect both vertebrate and invertebrate hosts [72]. The poxvirus genome is generally 150–200 kb, but can be >300 kb, and encodes >200 open reading frames [73]. Within the vertebrate-infecting subfamily *Chordopoxvirinae*, 90 genes are centrally conserved and comprise those most essential to virus replication [74]. The remaining genes, especially in the 5′ and 3′ flanking ends, encode numerous immunomodulating proteins that facilitate species-specific replication and transmission [75]. Compared with other DNA viruses, poxvirus replication is unusual in that no nuclear localization or processes occur. As such, poxvirus-sensing PRRs trigger signaling that initiates exclusively in the cytoplasm. The most thoroughly examined poxviruses, with respect to innate immune detection and inflammasome interaction, include VV (the agent used as a vaccine to eradicate smallpox), as well as the attenuated modified VV Ankara (MVA; a strain of passaged VV lacking many genes involved in immunomodulation [76,77]), cowpox virus (CPXV) and myxoma virus (MYXV), causative agent of myxomatosis in rabbits.

Inflammasome induction by poxviruses is represented by highly curious observations. While ASC dependence during poxvirus-mediated induction is clearly based on multiple studies, a role for NLRP3 in IL-1β processing and secretion was uncovered in a study using the human promonocytic cell line THP-1 during infection with MVA [78]. This detection of NLRP3-mediated inflammasome activation was likely facilitated by the absence of many immunomodulatory proteins in the MVA vaccine strain compared with other VV isolates (Table 1). NLRP3 was also shown to be necessary for inflammasome activation in these cells by a MYXV mutant lacking the ASC inhibitor M13L and to be dependent on infection-associated ROS and cathepsin B [79]. Unfortunately, these studies did not examine potential roles for DNA-associated PRRs such as AIM2 and IFI16. Conversely, using the virulent VV strain Western Reserve (WR), Hornung and colleagues observed normal caspase-1 activation following infection of macrophages harvested from *NLRP3*$^{-/-}$ mice [33]. However, AIM2 and ASC were found to be necessary for IL-1β secretion and caspase-1 activation by this strain. Thus, it is possible that the relative contributions of inflammasome components are influenced by poxvirus strain (especially regarding the presence or absence of immunomodulatory genes, for instance when comparing the highly attenuated VV strain MVA to a virulent VV isolate like WR), infected cell type or species, and this requires more thorough investigation.

Poxviral serine protease inhibitors impair caspase-1 activity

The first virally encoded protein described to interfere with constituents of the inflammasome was actually discovered in CPXV. CrmA is an abundant 38-kDa protein expressed early in infection with sequence homology to cellular

serine protease inhibitors (serpins) [80]. CrmA directly inhibits the enzymatic activity of caspase-1 (as well as other caspases) [81–84] by binding and behaving as a pseudosubstrate for protease activity [85]. Interestingly, as a cysteine proteinase, caspase-1 inhibition by CrmA represents one of the first described cross-class inhibitors within the serpin family [85,86]. CrmA-mediated caspase-1 inhibition has also been shown to be effective at preventing pyroptosis [87–90]. Viral serpin-like inhibitors were also identified in other poxvirus species such as VV and ectromelia virus and were subsequently termed serpin-like protease inhibitors (SPIs). Interestingly, CPXV and VV encode multiple SPIs exhibiting different functions during infection [91]. In CPXV, only the deletion of CrmA (SPI-2) led to attenuation in mice, whereas deletion of any other serpin gene resulted in a wild-type phenotype [92]. This suggests that CrmA/SPI-2 is the dominant serpin involved in immune evasion *in vivo*. Moreover, the CPXV model demonstrates a connection between the efficiency of inflammasome inhibition in relation to the absence of an immune evasion gene and loss of virulence, underscoring the *in vivo* importance of inflammation control by the virus. By contrast, deletion of either SPI-1 or SPI-2 in VV both led to wild-type phenotypes *in vitro* and *in vivo*, showing no signs of attenuation in the mouse [93]. In recent years, multiple studies have been conducted to elucidate the effects of CrmA deletion in VV, CPXV or rabbitpox virus *in vivo* using mice, and most of the resulting data showed attenuation of the deletion mutants compared with the wild-type [92,94,95], although other studies indicated no *in vivo* effect of the deletion [93] or even increased virulence in the absence of the viral serpin [96]. These substantial differences in outcome can most likely be explained by the different routes of infection used, ranging from subcutaneous and intraperitoneal to intratracheal infections, and the different mouse strains used by the separate groups. In cases where *in vivo* attenuation was observed, this was probably the result of enhanced viral clearance via increased inflammatory responses and an influx of inflammatory cells at the site of replication [92]. An aspect all these studies have in common is that the virus species probably does not use the mouse as its natural host. When ectromelia virus (also known as mousepox virus and a natural pathogen of mice) was deleted of its CrmA homolog, subcutaneous infection of BALB/c mice showed severe attenuation of the mutant [97]. In the absence of the viral serpin, more effective inflammasome signaling could be observed, leading to increased secretion of IL-18 and, subsequently, to increased NK-cell activation. Additionally, in MYXV, deletion of Serp-2 (MYXV homolog of CrmA) resulted in severe attenuation *in vivo*, as indicated by lack of secondary lesions in infected rabbits and a substantially higher survival rate. This also led to increased inflammation, with an enhanced influx of mononuclear cells into infected primary lesions resulting in an improved virus control and clearance [98]. In addition, rabbits infected with the deletion mutant showed a high degree of lymphodepletion due to increased apoptosis of lymphocytes in lymph nodes in the infected animals, underscoring a secondary function of the viral serpin in blocking the initiation of pyroptosis through caspase-1.

PYD-protein family member, M13L, acts as an ASC-1 inhibitor

MYXV also encodes the first viral protein identified as an inhibitor of ASC-dependent processes. The *M13L* gene product was first described when the complete MYXV genome was sequenced as a viral protein with low homology to the mouse interferon-inducible protein 203 [99], but was later shown to be more closely related to PYRIN domain-containing proteins [100]. M13L was found to interact with ASC and, thereby, inhibit its function and prevent virus-induced caspase-1 activation as well as IL-1β and IL-18 secretion [100]. *In vivo* experiments in rabbits infected with a mutant lacking M13L-mediated ASC inhibition demonstrated strong attenuation compared with wild-type virus [100]. Here, the mutant virus was no longer able to control the inflammation induced by infection and the increased secretion of proinflammatory cytokines led to enhanced killing of virus-infected cells and viral control. Dissemination of virus to secondary sites likely involves lymphatic mobility of infected leukocytes, and M13L deletion led to diminished viral spread due to low productive infection of monocytes and lymphocytes. Interestingly, PYRIN domain-containing proteins have only been described in the genera *Leporipoxvirus*, *Yatapoxvirus* and *Suipoxvirus* [101]. The poxvirus shope fibroma virus encodes gp013L, a PYRIN domain-only protein capable of blocking ASC-dependent activity likely through competitive PYD–PYD binding [101]. Intriguingly, cellular PYRIN domain-only proteins exist and are capable of regulating ASC function [102], perhaps representing the evolutionary source of virus-encoded ASC effectors. It is also highly likely that additional PYD-containing immunomodulators will be characterized.

A virally encoded soluble IL-1β receptor abrogates IL-1β signaling

It is worth noting that genes encoding secreted neutralizing inhibitors of IL-1β and IL-18 have been characterized in poxviral genomes. While not exhibiting anti-inflammasome effects in the technical sense via direct inhibition of inflammasome components, these molecules effectively impair inflammasome-dependent physiological responses by impairing the downstream effects of inflammasome activation and, thus, likely evolved to counteract IL-1β- and IL-18-mediated antiviral functions. VV WR genes *B15R* and *B18R* encode immunoglobulin superfamily members with high homology to human and murine IL-1R [103,104]. When supernatants from VV-infected cells were examined for the ability to bind murine IL-1β, a secreted 33-kDa protein corresponding to *B15R* was identified [105]. These supernatants were also capable of inhibiting the proliferative response of B and T lymphocytes to IL-1β *in vitro*. Functional homologs of this protein have so far been described for CPXV and ectromelia virus [105–107]. Intracranial infection of weanling mice showed substantially increased LD_{50} for the *B15R*-knockout mutant, thus implicating this immune evasion function as important for virus-induced pathogenesis [105]. Contrastingly, intranasal infection of mice with VV lacking *B15R* resulted in significantly increased virulence [106]. This result might indicate a relationship between inflammation (otherwise controlled by the virus) and pathological effects mediated by excessive IL-1β production. In fact, IL-1β was shown to be a key inducer of fever, and the appearance of fever as well as increased weight loss are major differences between infection with VV strains that encode or lack the viral IL-1R [108].

Soluble poxvirus IL-18-binding proteins mimic host proteins in modulating the host antiviral response

In addition to IL-1β, poxviruses also counteract secreted IL-18 [109], which is important for NK- and T-cell stimulation, as well as Th1 development [12,110]. When the full genome sequence of the molluscum contagiosum virus (MCV; genus *Molluscipoxvirus*) was determined and analyzed, three viral homologs (MC51L, MC53L and MC54L) to cellular IL-18-binding proteins (IL-18BPs) were discovered [111]. Host IL-18BPs regulate IL-18 activity by efficiently competing with the IL-18 receptor for cytokine binding [112,113], although they show no significant homology to either IL-18 receptor subunit [112]. The isolated MCV IL-18BPs, MC53L and MC54L, showed murine IL-18 binding affinities similar to their cellular homologs [114], yet further experiments demonstrated that only MC54L-bound human IL-18, indicating that it was the only functional viral IL-18BP [115]. In spite of low overall levels of protein homology, MC54L and cellular IL-18BPs share similar IL-18 binding sites [115–117], and experiments with VV and ectromelia virus IL-18BPs revealed competition between the viral protein and the cellular IL-18 receptor due to an overlap of IL-18 binding sites [118–120]. Viral IL-18BPs are maintained in many poxviruses [121–124] and functionality has been shown for several of them [125–129]. *In vivo*, an ectromelia virus IL18BP deletion mutant was clearly attenuated and virus titers in tissues of infected mice were decreased relative to wild-type due to NK-cell- and cytotoxic T-lymphocyte-mediated control of virus infection [130]. Similarly, infection of BALB/c mice by a VV mutant lacking the IL-18BP *C12L* led to a generally weaker infection compared with the wild-type, with milder signs of illness, loss of body weight and reduced mortality [128]. The basis of this attenuation was found to be elevated levels of secreted IFN-γ due to abundant functional IL-18 and, as a direct consequence, heightened NK-cell and VV-specific cytotoxic T-lymphocyte killing during viral infection [131]. In light of these results, it is possible that viral proteins directed against the secreted products of inflammasome activation actually confer the majority of anti-inflammatory activity. Overall, these results indicate the importance of inhibiting IL-18-dependent effects for poxviral *in vivo* replication and pathogenesis.

Adenoviruses

Adenoviruses (Ads) are nonenveloped, dsDNA viruses that consist of seven subgroups, A–G, depending on their hemagglutination properties and sequence homology [132]. These viruses possess characteristics, such as manipulability and ability to infect dividing and nondividing cells, that make them suitable as vectors for ectopic gene therapy strategies, and which are a key reason they have been broadly studied with respect to their innate immune properties [133,134]. While no studies to date have described the inhibition of inflammasome-mediated activities by Ad, activation of the inflammasome by Ad molecules and virus-induced processes has been well-studied.

Several reports support a role for NLRP3 during Ad-induced inflammasome activation [135–138]. Muruve *et al.* first demonstrated that infection with Ad serotypes 3 and 5 activates

inflammasome-mediated IL-1β processing in human THP-1 cells, and that this response also occurs when Ad DNA is transfected into these cells, but not when DNA-free viral capsids are used as inoculum – implicating virus-associated DNA as a pivotal PAMP [138]. Using *NLRP3*$^{-/-}$ mice, it was shown that this molecule has an essential role in IL-1β secretion during Ad infection *in vitro* and *in vivo*. However, the absence of NLRP3 had no effect on general DNA-induced IL-1β processing (although Ad DNA specifically was not investigated in this context). Ad-induced NLRP3 inflammasome activation was then hypothesized to be mediated by endosomal membrane disruption generated by the virus during cytoplasmic entry. The NLRP3 inflammasome can be activated by endogenous danger signals, including lysosomal membrane damage and associated release of cathepsin B and ROS [139–142]. Barlan and colleagues demonstrated that Ad-induced IL-1β secretion was absent when cells were infected with an Ad mutant incapable of rupturing endosomal membranes [136]. Furthermore, chemical inhibition of cathepsin B or ROS also prevented Ad-induced IL-1β secretion. Surprisingly, the NF-κB-terminal DNA receptor TLR9 was found to be important to prime expression of inflammasome constituents NLRP3 and pro-IL-1β for Ad-induced activation in human but not mouse macrophage cells [136,138]. These studies highlight important realities regarding investigation into innate viral immune responses. First, the role of specific PAMPs (e.g., virus-associated DNA) may not be as relevant to innate immune induction as cell-derived danger-associated molecular patterns that appear as a physiological outcome of infection. This phenomenon is exemplified by the predominance of NLRP3 inflammasome activation following destabilization of cell membranes during Ad infection. Second, the molecules detected by specific PRRs may differ between species and cell types, and this should be taken into account when studying PRR-mediated processes.

Conclusion

Caspase-1-mediated processing and secretion of inflammatory cytokines represents an essential component of the antiviral immune response. Infection with diverse DNA virus types leads to inflammasome-mediated activities, such as IL-1β and IL-18 secretion, as well as pyroptosis. Intriguingly, evolutionarily and physiologically related virus types appear to trigger inflammasome activation via divergent receptors and processes, as in the case of herpesviral species (Table 1). Curiously, the importance of DNA sensors for inflammasome activation by related viruses (e.g., KSHV and MCMV) can vary significantly and is likely to be affected by infected cell type and PRR expression patterns therein. Moreover, the importance of NLRP3 to inflammasome activation owing to numerous and varied stimuli is perhaps an indication that this molecule is less likely to represent a PRR, but rather an adaptor for as-yet unidentified PRRs. The importance of inflammasome-mediated antiviral activity is clearly underscored by the diversity of viral phenotypes that have evolved to counteract the activation or physiological effects of the inflammasome (Table 1). This includes proteins directed at inflammasome assembly (KSHV Orf63; MYXV M13L), caspase-1 activity (poxvirus CrmA/SPI-2), IL-1β/IL-18 function (poxvirus MC54L, B15R), or even PRR mRNA stability (EBV *miR-BART15*). Given the diversity of viral systems and genes yet to be examined, it remains probable that additional, likely novel inhibitory genes will be identified.

Future perspective

Numerous questions remain regarding virus-associated stimulation and manipulation of inflammasome-mediated processes. For example, in the event that ligand-binding properties continue to be undetected for NLRP3, it will be necessary to identify the PRRs that specifically detect viruses such as Ad. In addition, the respective roles of NLRP3 and DNA sensors in innate immune stimulation by VV strains require greater elucidation, including the exploration of potentially contradictory observations, as described here. In addition, the importance of virus strain, host species and cell type to virus-induced inflammasome activation and function remains an important area with multiple questions yet to be answered. Ultimately, fundamental knowledge regarding the importance of viral infection in chronic inflammatory diseases, such as atherosclerosis, diabetes, cancers and autoimmune disorders, remains a largely unexplored area of research. Given the ability of viruses that exhibit chronic infection (e.g., herpesviruses) to stimulate inflammasome-mediated responses, it appears likely that this will play a role in diseases that involve inflammatory dysfunction. Characterizing the molecular basis of virus-induced inflammasome activity and the viral factors capable of impairing or manipulating these responses could lead to the identification of new therapeutic targets for multiple, seemingly unrelated diseases.

Executive summary

- The inflammasome is an intracellular, innate immune apparatus that is activated by receptor-mediated detection of pathogen-associated molecular patterns.
- The inflammasome is responsible for caspase-1-dependent processing of proinflammatory cytokines IL-1β and IL-18, in addition to less-well-characterized substrates that trigger pyroptosis.
- Inflammasome activity directly affects the adaptive immune response to viral infection and is important for viral clearance.
- Multiple pattern recognition receptors are known that are involved in infection-triggered initiation of innate immune responses, such as inflammasome activation.
- A large and expanding repertoire of DNA-sensing pattern recognition receptors exist that are involved in both transcriptional activation and processing of antiviral cytokines.
- Inflammasome activation in response to herpesvirus species is dependent on multiple virus-specific receptors, including IFI16, AIM2 and NLRP3.
- Thus far, the described inhibition of inflammasome-dependent activity by herpesviruses includes targeting of the inflammasome receptors NLRP1 and NLRP3 (Kaposi's sarcoma-associated herpesvirus Orf63) and reduced expression of the NLRP3 mRNA (EBV miBART15). It is highly likely that other herpesvirus-encoded inhibitory phenotypes will be identified.
- Vaccinia virus (a poxvirus)-mediated inflammasome activation requires either AIM2 or NLRP3, depending on the viral strain or host species. The molecular bases of these disparities require elucidation.
- Poxviruses encode multiple, redundant inflammasome-directed inhibitory phenotypes, including those targeting caspase-1 function (CrmA, serpin-like protease inhibitors), ASC-mediated assembly (PYRIN domain-only proteins) or soluble binding proteins of IL-1β and IL-18 (B15R and MC54L, respectively).
- Redundant inhibition of inflammasome-mediated physiological effects are essential for virus replication, but in some cases can lead to increased pathogenesis, as exemplified in animal models using deletion mutant viruses.
- Adenoviruses induce NLRP3-dependent inflammasome activation.
- While adenoviral DNA is capable of triggering inflammasome activation, infection-associated activation involves reactive oxygen species and cathepsin B release in response to endosomal rupture.
- The role of viral DNA in inflammasome activation requires more thorough investigation, with consideration of the potential role of infection-associated cellular stress responses.
- The importance of infection-dependent inflammasome activity in chronic inflammatory disease remains an important area of inquiry.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

References

Papers of special note have been highlighted as:
▪▪ of considerable interest

1. Franchi L, Eigenbrod T, Munoz-Planillo R, Nunez G. The inflammasome: a caspase-1-activation platform that regulates immune responses and disease pathogenesis. *Nat. Immunol.* 10(3), 241–247 (2009).
2. Poyet JL, Srinivasula SM, Tnani M, Razmara M, Fernandes-Alnemri T, Alnemri ES. Identification of Ipaf, a human caspase-1-activating protein related to Apaf-1. *J. Biol. Chem.* 276(30), 28309–28313 (2001).
3. Broz P, Von Moltke J, Jones JW, Vance RE, Monack DM. Differential requirement for Caspase-1 autoproteolysis in pathogen-induced cell death and cytokine processing. *Cell Host Microbe* 8(6), 471–483 (2010).
4. Mariathasan S, Newton K, Monack DM *et al.* Differential activation of the inflammasome by caspase-1 adaptors ASC and Ipaf. *Nature* 430(6996), 213–218 (2004).
5. Zhao Y, Yang J, Shi J *et al.* The NLRC4 inflammasome receptors for bacterial flagellin and type III secretion apparatus. *Nature* 477(7366), 596–600 (2011).
6. Schmitz J, Owyang A, Oldham E *et al.* IL-33, an interleukin-1-like cytokine that signals via the IL-1 receptor-related protein ST2 and induces T helper type 2-associated cytokines. *Immunity* 23(5), 479–490 (2005).
7. Bauernfeind FG, Horvath G, Stutz A *et al.* Cutting edge: NF-κB activating pattern recognition and cytokine receptors license NLRP3 inflammasome activation by regulating NLRP3 expression. *J. Immunol.* 183(2), 787–791 (2009).
8. Netea MG, Nold-Petry CA, Nold MF *et al.* Differential requirement for the activation of the inflammasome for processing and release of IL-1β in monocytes and macrophages. *Blood* 113(10), 2324–2335 (2009).
9. Puren AJ, Fantuzzi G, Dinarello CA. Gene expression, synthesis, and secretion of interleukin 18 and interleukin 1β are differentially regulated in human blood mononuclear cells and mouse spleen cells. *Proc. Natl Acad. Sci. USA* 96(5), 2256–2261 (1999).
10. Fink SL, Cookson BT. Apoptosis, pyroptosis, and necrosis: mechanistic description of dead and dying eukaryotic cells. *Infect. Immun.* 73(4), 1907–1916 (2005).
11. Chung Y, Chang SH, Martinez GJ *et al.* Critical regulation of early Th17 cell differentiation by interleukin-1 signaling. *Immunity* 30(4), 576–587 (2009).
12. Dinarello CA. IL-18: a Th1-inducing, proinflammatory cytokine and new member of the IL-1 family. *J. Allergy Clin. Immunol.* 103(1 Pt 1), 11–24 (1999).
13. Lasiglie D, Traggiai E, Federici S *et al.* Role of IL-1 beta in the development of human T(H)17 cells: lesson from NLPR3 mutated patients. *PLoS One* 6(5), e20014 (2011).
14. Dinarello CA. Blocking IL-1 in systemic inflammation. *J. Exp. Med.* 201(9), 1355–1359 (2005).
15. O'Neill LA. The interleukin-1 receptor/Toll-like receptor superfamily: 10 years of progress. *Immunol. Rev.* 226, 10–18 (2008).

16. Dinarello CA. Immunological and inflammatory functions of the interleukin-1 family. *Annu. Rev. Immunol.* 27, 519–550 (2009).
17. Sims JE, Smith DE. The IL-1 family: regulators of immunity. *Nat. Rev. Immunol.* 10(2), 89–102 (2010).
18. Brennan K, Bowie AG. Activation of host pattern recognition receptors by viruses. *Curr. Opin. Microbiol.* 13(4), 503–507 (2010).
19. Kawai T, Akira S. The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors. *Nat. Immunol.* 11(5), 373–384 (2010).
20. Poeck H, Bscheider M, Gross O *et al.* Recognition of RNA virus by RIG-I results in activation of CARD9 and inflammasome signaling for interleukin 1 beta production. *Nat. Immunol.* 11(1), 63–69 (2010).
21. Rintahaka J, Wiik D, Kovanen PE, Alenius H, Matikainen S. Cytosolic antiviral RNA recognition pathway activates caspases 1 and 3. *J. Immunol.*180(3), 1749–1757 (2008).
22. Franchi L, Warner N, Viani K, Nunez G. Function of Nod-like receptors in microbial recognition and host defense. *Immunol. Rev.* 227(1), 106–128 (2009).
23. Ting JP, Lovering RC, Alnemri ES *et al.* The NLR gene family: a standard nomenclature. *Immunity* 28(3), 285–287 (2008).
24. Franchi L, Munoz-Planillo R, Nunez G. Sensing and reacting to microbes through the inflammasomes. *Nat. Immunol.* 13(4), 325–332 (2012).
25. Barber GN. Cytoplasmic DNA innate immune pathways. *Immunol. Rev.* 243(1), 99–108 (2011).
26. Takaoka A, Wang Z, Choi MK *et al.* DAI (DLM-1/ZBP1) is a cytosolic DNA sensor and an activator of innate immune response. *Nature* 448(7152), 501–505 (2007).
27. Zhang Z, Yuan B, Bao M, Lu N, Kim T, Liu YJ. The helicase DDX41 senses intracellular DNA mediated by the adaptor STING in dendritic cells. *Nat. Immunol.* 12(10), 959–965 (2011).
28. Ablasser A, Bauernfeind F, Hartmann G, Latz E, Fitzgerald KA, Hornung V. RIG-I-dependent sensing of poly(dA:dT) through the induction of an RNA polymerase III-transcribed RNA intermediate. *Nat. Immunol.* 10(10), 1065–1072 (2009).
29. Chiu YH, Macmillan JB, Chen ZJ. RNA polymerase III detects cytosolic DNA and induces type I interferons through the RIG-I pathway. *Cell* 138(3), 576–591 (2009).
30. Kim T, Pazhoor S, Bao M *et al.* Aspartate-glutamate-alanine-histidine box motif (DEAH)/RNA helicase A helicases sense microbial DNA in human plasmacytoid dendritic cells. *Proc. Natl Acad. Sci. USA* 107(34), 15181–15186 (2010).
31. Yang P, An H, Liu X *et al.* The cytosolic nucleic acid sensor LRRFIP1 mediates the production of type I interferon via a beta-catenin-dependent pathway. *Nat. Immunol.* 11(6), 487–494 (2010).
32. Kerur N, Veettil MV, Sharma-Walia N *et al.* IFI16 acts as a nuclear pathogen sensor to induce the inflammasome in response to Kaposi Sarcoma-associated herpesvirus infection. *Cell Host Microbe* 9(5), 363–375 (2011).

▪▪ **First demonstration that a pattern recognition receptor responsible for IFN-terminal signaling could also be required for inflammasome activation. In addition, it shows nuclear localization of a herpesviral pattern recognition receptor.**

33. Hornung V, Ablasser A, Charrel-Dennis M *et al.* AIM2 recognizes cytosolic dsDNA and forms a caspase-1-activating inflammasome with ASC. *Nature* 458(7237), 514–518 (2009).
34. Rathinam VA, Jiang Z, Waggoner SN *et al.* The AIM2 inflammasome is essential for host defense against cytosolic bacteria and DNA viruses. *Nat. Immunol.* 11(5), 395–402 (2010).

▪▪ **First description of a cytoplasmic DNA receptor (AIM2) as being important for virus-induced inflammasome activation.**

35. Unterholzner L, Keating SE, Baran M *et al.* IFI16 is an innate immune sensor for intracellular DNA. *Nat. Immunol.* 11(11), 997–1004 (2010).
36. Philip E, Pellett BR. The family *Herpesviridae*: a brief introduction. In: *Fields Virology*. Knipe DM, Howley PM, Griffin DE *et al.* (Eds). Lippincott Williams & Wilkins, PA, USA, 2480–2499 (2007).
37. Koelle DM. HSV-1 and 2: Immunobiology and host response. In: *Human Herpesviruses. Biology, Therapy, and Immunoprophylaxis*. Arvin A, Campadelli-Fiume G, Mocarski E *et al.* (Eds). Cambridge University Press, Cambridge, UK, 616–641 (2007).
38. Arvin A, Abendroth A. VZV: immunobiology and host response. In: *Human Herpesviruses. Biology, Therapy, and Immunoprophylaxis*. Arvin A, Campadelli-Fiume G, Mocarski E *et al.* (Eds). Cambridge University Press, Cambridge, UK, 700–712 (2007).
39. Moss DJ, Khanna R. EBV: immunobiology and host response. In: *Human Herpesviruses. Biology, Therapy, and Immunoprophylaxis*. Arvin A, Campadelli-Fiume G, Mocarski E *et al.* (Eds). Cambridge University Press, Cambridge, UK, 904–914 (2007).
40. Boshoff DLaC. Immunobiology and host response to KSHV infection. In: *Human Herpesviruses. Biology, Therapy, and Immunoprophylaxis*. Arvin A, Campadelli-Fiume G *et al.* (Eds). Cambridge University Press, Cambridge, UK, 915–928 (2007).
41. Means RE, Jung JU. Human gammaherpesvirus immune evasion strategies. In: *Human Herpesviruses. Biology, Therapy, and Immunoprophylaxis*. Arvin A, Campadelli-Fiume G, Mocarski E *et al* (Eds). Cambridge University Press, Cambridge, UK, 559–586 (2007).
42. Powers C, Defilippis V, Malouli D, Fruh K. Cytomegalovirus immune evasion. *Curr. Top. Microbiol. Immunol.* 325, 333–359 (2008).
43. Vandevenne P, Sadzot-Delvaux C, Piette J. Innate immune response and viral interference strategies developed by human herpesviruses. *Biochem. Pharmacol.* 80(12), 1955–1972 (2010).
44. Rathinam VA, Jiang Z, Waggoner SN *et al.* The AIM2 inflammasome is essential for host defense against cytosolic bacteria and DNA viruses. *Nat. Immunol.* 11(5), 395–402 (2010).
45. Nour AM, Reichelt M, Ku CC, Ho MY, Heineman TC, Arvin AM. Varicella-zoster virus infection triggers formation of an interleukin-1β (IL-1β)-processing inflammasome complex. *J. Biol. Chem.* 286(20), 17921–17933 (2011).
46. Orzalli MH, Deluca NA, Knipe DM. Nuclear IFI16 induction of IRF-3 signaling during herpesviral infection and degradation of IFI16 by the viral ICP0 protein. *Proc. Natl Acad. Sci. USA* 109(44), E3008–E3017 (2012).
47. Li T, Diner BA, Chen J, Cristea IM. Acetylation modulates cellular distribution and DNA sensing ability of interferon-inducible protein IFI16. *Proc. Natl Acad. Sci. USA* 109(26), 10558–10563 (2012).
48. Conrady CD, Zheng M, Fitzgerald KA, Liu C, Carr DJ. Resistance to HSV-1 infection in the epithelium resides with the novel innate sensor, IFI-16. *Mucosal Immunol.* 5(2), 173–183 (2012).
49. Miettinen JJ, Matikainen S, Nyman TA. Global secretome characterization of herpes simplex virus 1-infected human primary macrophages. *J. Virol.* 86(23), 12770–12778 (2012).
50. Mueller NH, Gilden DH, Cohrs RJ, Mahalingam R, Nagel MA. Varicella zoster virus infection: clinical features, molecular pathogenesis of disease, and latency. *Neurol. Clin.* 26(3), 675–697 (2008).
51. Zhou R, Tardivel A, Thorens B, Choi I, Tschopp J. Thioredoxin-interacting protein links oxidative stress to inflammasome activation. *Nat. Immunol.* 11(2), 136–140 (2010).
52. Britt W. Manifestations of human cytomegalovirus infection: proposed

mechanisms of acute and chronic disease. *Curr. Top. Microbiol. Immunol.* 325, 417–470 (2008).

53. Yurochko AD, Huang ES. Human cytomegalovirus binding to human monocytes induces immunoregulatory gene expression. *J. Immunol.* 162(8), 4806–4816 (1999).
54. Szomolanyi-Tsuda E, Liang X, Welsh RM, Kurt-Jones EA, Finberg RW. Role for TLR2 in NK cell-mediated control of murine cytomegalovirus *in vivo*. *J. Virol.* 80(9), 4286–4291 (2006).
55. Pien GC, Satoskar AR, Takeda K, Akira S, Biron CA. Cutting edge: selective IL-18 requirements for induction of compartmental IFN-γ responses during viral infection. *J. Immunol.* 165(9), 4787–4791 (2000).
56. Gregory SM, Davis BK, West JA *et al.* Discovery of a viral NLR homolog that inhibits the inflammasome. *Science* 331(6015), 330–334 (2011).

▪▪ **Describes Kaposi's sarcoma-associated herpesvirus Orf63 as the first herpesviral inhibitor of ASC-dependent responses.**

57. Gariano GR, Dell'oste V, Bronzini M *et al.* The intracellular DNA sensor *IFI16* gene acts as restriction factor for human cytomegalovirus replication. *PLoS Pathog.* 8(1), e1002498 (2012).
58. Cristea IM, Moorman NJ, Terhune SS *et al.* Human cytomegalovirus pUL83 stimulates activity of the viral immediate-early promoter through its interaction with the cellular IFI16 protein. *J. Virol.* 84(15), 7803–7814 (2010).
59. Taylor GS, Blackbourn DJ. Infectious agents in human cancers: lessons in immunity and immunomodulation from γ–herpesviruses EBV and KSHV. *Cancer Lett.* 305(2), 263–278 (2011).
60. Chandriani S, Ganem D. Array-based transcript profiling and limiting-dilution reverse transcription-PCR analysis identify additional latent genes in Kaposi's sarcoma-associated herpesvirus. *J. Virol.* 84(11), 5565–5573 (2010).
61. Fakhari FD, Dittmer DP. Charting latency transcripts in Kaposi's sarcoma-associated herpesvirus by whole-genome real-time quantitative PCR. *J. Virol.* 76(12), 6213–6223 (2002).
62. Gregory SM, Wang L, West JA, Dittmer DP, Damania B. Latent Kaposi's sarcoma-associated herpesvirus infection of monocytes downregulates expression of adaptive immune response costimulatory receptors and proinflammatory cytokines. *J. Virol.* 86(7), 3916–3923 (2012).
63. Boyle JP, Monie TP. Computational analysis predicts the Kaposi's sarcoma-associated herpesvirus tegument protein ORF63 to be α-helical. *Proteins* 80(8), 2063–2070 (2012).
64. Chen LC, Wang LJ, Tsang NM *et al.* Tumour inflammasome-derived IL-1beta recruits neutrophils and improves local recurrence-free survival in EBV-induced nasopharyngeal carcinoma. *EMBO Mol. Med.* 4(12), 1276–1293 (2012).
65. Samanta M, Iwakiri D, Takada K. Epstein-Barr virus-encoded small RNA induces IL-10 through RIG-I-mediated IRF-3 signaling. *Oncogene* 27(30), 4150–4160 (2008).
66. Samanta M, Iwakiri D, Kanda T, Imaizumi T, Takada K. EB virus-encoded RNAs are recognized by RIG-I and activate signaling to induce type I IFN. *EMBO J.* 25(18), 4207–4214 (2006).
67. Bauernfeind F, Rieger A, Schildberg FA, Knolle PA, Schmid-Burgk JL, Hornung V. NLRP3 inflammasome activity is negatively controlled by *miR-223*. *J. Immunol.* 189(8), 4175–4181 (2012).
68. Forte E, Luftig MA. The role of microRNAs in Epstein-Barr virus latency and lytic reactivation. *Microbes Infect.* 13(14–15), 1156–1167 (2011).
69. Gourzones C, Gelin A, Bombik I *et al.* Extra-cellular release and blood diffusion of BART viral micro-RNAs produced by EBV-infected nasopharyngeal carcinoma cells. *Virol. J.* 7, 271 (2010).
70. Meckes DG, Jr., Shair KH, Marquitz AR, Kung CP, Edwards RH, Raab-Traub N. Human tumor virus utilizes exosomes for intercellular communication. *Proc. Natl Acad. Sci. USA* 107(47), 20370–20375 (2010).
71. Haneklaus M, Gerlic M, Kurowska-Stolarska M *et al.* Cutting edge: *miR-223* and EBV *miR-BART15* regulate the NLRP3 inflammasome and IL-1beta production. *J. Immunol.* 189(8), 3795–3799 (2012).
72. Moss B. *Poxviridae:* the viruses and their replication. In: *Fields Virology*, Knipe DM, Howley PM (Eds). Lippincott Williams & Wilkins, PA, USA, 2905–2946 (2007).
73. Gubser C, Hue S, Kellam P, Smith GL. Poxvirus genomes: a phylogenetic analysis. *J. Gen. Virol.* 85(Pt 1), 105–117 (2004).
74. Upton C, Slack S, Hunter AL, Ehlers A, Roper RL. Poxvirus orthologous clusters: toward defining the minimum essential poxvirus genome. *J. Virol.* 77(13), 7590–7600 (2003).
75. Kotwal GJ, Moss B. Analysis of a large cluster of nonessential genes deleted from a vaccinia virus terminal transposition mutant. *Virology* 167(2), 524–537 (1988).
76. Antoine G, Scheiflinger F, Dorner F, Falkner FG. The complete genomic sequence of the modified vaccinia Ankara strain: comparison with other orthopoxviruses. *Virology* 244(2), 365–396 (1998).
77. Blanchard TJ, Alcami A, Andrea P, Smith GL. Modified vaccinia virus Ankara undergoes limited replication in human cells and lacks several immunomodulatory proteins: implications for use as a human vaccine. *J. Gen. Virol.* 79(Pt 5), 1159–1167 (1998).
78. Delaloye J, Roger T, Steiner-Tardivel QG *et al.* Innate immune sensing of modified vaccinia virus Ankara (MVA) is mediated by TLR2-TLR6, MDA-5 and the NALP3 inflammasome. *PLoS Pathog.* 5(6), e1000480 (2009).
79. Rahman MM, Mcfadden G. Myxoma virus lacking the pyrin-like protein M013 is sensed in human myeloid cells by both NLRP3 and multiple Toll-like receptors, which independently activate the inflammasome and NF-κB innate response pathways. *J. Virol.* 85(23), 12505–12517 (2011).
80. Ray CA, Black RA, Kronheim SR *et al.* Viral inhibition of inflammation: cowpox virus encodes an inhibitor of the interleukin-1β converting enzyme. *Cell* 69(4), 597–604 (1992).
81. Tewari M, Quan LT, O'Rourke K *et al.* Yama/CPP32 beta, a mammalian homolog of CED-3, is a CrmA-inhibitable protease that cleaves the death substrate poly(ADP-ribose) polymerase. *Cell* 81(5), 801–809 (1995).
82. Srinivasula SM, Ahmad M, Fernandes-Alnemri T, Litwack G, Alnemri ES. Molecular ordering of the Fas-apoptotic pathway: the Fas/APO-1 protease Mch5 is a CrmA-inhibitable protease that activates multiple Ced-3/ICE-like cysteine proteases. *Proc. Natl Acad. Sci. USA* 93(25), 14486–14491 (1996).
83. Takahashi A, Hirata H, Yonehara S *et al.* Affinity labeling displays the stepwise activation of ICE-related proteases by Fas, staurosporine, and CrmA-sensitive caspase-8. *Oncogene* 14(23), 2741–2752 (1997).
84. Zhou Q, Snipas S, Orth K, Muzio M, Dixit VM, Salvesen GS. Target protease specificity of the viral serpin CrmA. Analysis of five caspases. *J. Biol. Chem.* 272(12), 7797–7800 (1997).
85. Komiyama T, Ray CA, Pickup DJ *et al.* Inhibition of interleukin-1 beta converting enzyme by the cowpox virus serpin CrmA. An example of cross-class inhibition. *J. Biol. Chem.* 269(30), 19331–19337 (1994).
86. Viswanathan K, Bot I, Liu L *et al.* Viral cross-class serpin inhibits vascular inflammation and T lymphocyte fratricide; a study in rodent models *in vivo* and human cell lines in vitro. *PLoS ONE* 7(9), e44694 (2012).

87. Gagliardini V, Fernandez PA, Lee RK *et al.* Prevention of vertebrate neuronal death by the crmA gene. *Science* 263(5148), 826–828 (1994).
88. Miura M, Zhu H, Rotello R, Hartwieg EA, Yuan J. Induction of apoptosis in fibroblasts by IL-1 beta-converting enzyme, a mammalian homolog of the *C. elegans* cell death gene *ced-3*. *Cell* 75(4), 653–660 (1993).
89. Wang L, Miura M, Bergeron L, Zhu H, Yuan J. *Ich-1*, an Ice/ced-3-related gene, encodes both positive and negative regulators of programmed cell death. *Cell* 78(5), 739–750 (1994).
90. Boudreau N, Sympson CJ, Werb Z, Bissell MJ. Suppression of ICE and apoptosis in mammary epithelial cells by extracellular matrix. *Science* 267(5199), 891–893 (1995).
91. Smith GL, Howard ST, Chan YS. Vaccinia virus encodes a family of genes with homology to serine proteinase inhibitors. *J. Gen. Virol.* 70 (Pt 9), 2333–2343 (1989).
92. Thompson JP, Turner PC, Ali AN, Crenshaw BC, Moyer RW. The effects of serpin gene mutations on the distinctive pathobiology of cowpox and rabbitpox virus following intranasal inoculation of Balb/c mice. *Virology* 197(1), 328–338 (1993).
93. Kettle S, Blake NW, Law KM, Smith GL. Vaccinia virus serpins B13R (SPI-2) and B22R (SPI-1) encode M(r) 38.5 and 40K, intracellular polypeptides that do not affect virus virulence in a murine intranasal model. *Virology* 206(1), 136–147 (1995).
94. Legrand FA, Verardi PH, Jones LA, Chan KS, Peng Y, Yilma TD. Induction of potent humoral and cell-mediated immune responses by attenuated vaccinia virus vectors with deleted serpin genes. *J. Virol.* 78(6), 2770–2779 (2004).
95. Macneill AL, Moldawer LL, Moyer RW. The role of the cowpox virus *crmA* gene during intratracheal and intradermal infection of C57BL/6 mice. *Virology* 384(1), 151–160 (2009).
96. Tscharke DC, Reading PC, Smith GL. Dermal infection with vaccinia virus reveals roles for virus proteins not seen using other inoculation routes. *J. Gen. Virol.* 83(Pt 8), 1977–1986 (2002).
97. Melo-Silva CR, Tscharke DC, Lobigs M *et al.* The ectromelia virus SPI-2 protein causes lethal mousepox by preventing NK cell responses. *J. Virol.* 85(21), 11170–11182 (2011).
98. Messud-Petit F, Gelfi J, Delverdier M *et al.* Serp2, an inhibitor of the interleukin-1beta-converting enzyme, is critical in the pathobiology of myxoma virus. *J. Virol.* 72(10), 7830–7839 (1998).
99. Cameron C, Hota-Mitchell S, Chen L *et al.* The complete DNA sequence of myxoma virus. *Virology* 264(2), 298–318 (1999).
100. Johnston JB, Barrett JW, Nazarian SH *et al.* A poxvirus-encoded pyrin domain protein interacts with ASC-1 to inhibit host inflammatory and apoptotic responses to infection. *Immunity* 23(6), 587–598 (2005).

▪▪ **First description of a viral inhibitor of ASC function. The paper also nicely explores the phenotypic impact of this viral trait using animal and *ex vivo* models.**

101. Dorfleutner A, Talbott SJ, Bryan NB *et al.* A shope fibroma virus PYRIN-only protein modulates the host immune response. *Virus Genes* 35(3), 685–694 (2007).
102. Stehlik C, Krajewska M, Welsh K, Krajewski S, Godzik A, Reed JC. The PAAD/PYRIN-only protein POP1/ASC2 is a modulator of ASC-mediated nuclear-factor-kappa B and pro-caspase-1 regulation. *Biochem. J.* 373(Pt 1), 101–113 (2003).
103. Smith GL, Chan YS. Two vaccinia virus proteins structurally related to the interleukin-1 receptor and the immunoglobulin superfamily. *J. Gen. Virol.* 72(Pt 3), 511–518 (1991).
104. Mcmahan CJ, Slack JL, Mosley B *et al.* A novel IL-1 receptor, cloned from B cells by mammalian expression, is expressed in many cell types. *EMBO J.* 10(10), 2821–2832 (1991).
105. Spriggs MK, Hruby DE, Maliszewski CR *et al.* Vaccinia and cowpox viruses encode a novel secreted interleukin-1-binding protein. *Cell* 71(1), 145–152 (1992).
106. Alcami A, Smith GL. A soluble receptor for interleukin-1β encoded by vaccinia virus: a novel mechanism of virus modulation of the host response to infection. *Cell* 71(1), 153–167 (1992).
107. Smith VP, Alcami A. Expression of secreted cytokine and chemokine inhibitors by ectromelia virus. *J. Virol.* 74(18), 8460–8471 (2000).
108. Alcami A, Smith GL. A mechanism for the inhibition of fever by a virus. *Proc. Natl Acad. Sci. USA* 93(20), 11029–11034 (1996).
109. Ghayur T, Banerjee S, Hugunin M *et al.* Caspase-1 processes IFN-gamma-inducing factor and regulates LPS-induced IFN-gamma production. *Nature* 386(6625), 619–623 (1997).
110. Nakamura K, Okamura H, Wada M, Nagata K, Tamura T. Endotoxin-induced serum factor that stimulates gamma interferon production. *Infect. Immun.* 57(2), 590–595 (1989).
111. Senkevich TG, Bugert JJ, Sisler JR, Koonin EV, Darai G, Moss B. Genome sequence of a human tumorigenic poxvirus: prediction of specific host response-evasion genes. *Science* 273(5276), 813–816 (1996).
112. Novick D, Kim SH, Fantuzzi G, Reznikov LL, Dinarello CA, Rubinstein M. Interleukin-18 binding protein: a novel modulator of the Th1 cytokine response. *Immunity* 10(1), 127–136 (1999).
113. Aizawa Y, Akita K, Taniai M *et al.* Cloning and expression of interleukin-18 binding protein. *FEBS Lett.* 445(2–3), 338–342 (1999).
114. Xiang Y, Moss B. IL-18 binding and inhibition of interferon gamma induction by human poxvirus-encoded proteins. *Proc. Natl Acad. Sci. USA* 96(20), 11537–11542 (1999).
115. Xiang Y, Moss B. Correspondence of the functional epitopes of poxvirus and human interleukin-18-binding proteins. *J. Virol.* 75(20), 9947–9954 (2001).
116. Xiang Y, Moss B. Determination of the functional epitopes of human interleukin-18-binding protein by site-directed mutagenesis. *J. Biol. Chem.* 276(20), 17380–17386 (2001).
117. Esteban DJ, Buller RM. Identification of residues in an orthopoxvirus interleukin-18 binding protein involved in ligand binding and species specificity. *Virology* 323(2), 197–207 (2004).
118. Meng X, Leman M, Xiang Y. Variola virus IL-18 binding protein interacts with three human IL-18 residues that are part of a binding site for human IL-18 receptor α subunit. *Virology* 358(1), 211–220 (2007).
119. Kato Z, Jee J, Shikano H *et al.* The structure and binding mode of interleukin-18. *Nat. Struct. Biol.* 10(11), 966–971 (2003).
120. Krumm B, Meng X, Li Y, Xiang Y, Deng J. Structural basis for antagonism of human interleukin 18 by poxvirus interleukin 18-binding protein. *Proc. Natl Acad. Sci. USA* 105(52), 20711–20715 (2008).
121. Senkevich TG, Koonin EV, Bugert JJ, Darai G, Moss B. The genome of *molluscum contagiosum* virus: analysis and comparison with other poxviruses. *Virology* 233(1), 19–42 (1997).
122. Lee HJ, Essani K, Smith GL. The genome sequence of Yaba-like disease virus, a yatapoxvirus. *Virology* 281(2), 170–192 (2001).
123. Shchelkunov SN, Totmenin AV, Babkin IV *et al.* Human monkeypox and smallpox viruses: genomic comparison. *FEBS Lett.* 509(1), 66–70 (2001).
124. Afonso CL, Tulman ER, Lu Z *et al.* The genome of swinepox virus. *J. Virol.* 76(2), 783–790 (2002).

125. Smith VP, Bryant NA, Alcami A. Ectromelia, vaccinia and cowpox viruses encode secreted interleukin-18-binding proteins. *J. Gen. Virol.* 81(Pt 5), 1223–1230 (2000).

126. Esteban DJ, Nuara AA, Buller RM. Interleukin-18 and glycosaminoglycan binding by a protein encoded by Variola virus. *J. Gen. Virol.* 85(Pt 5), 1291–1299 (2004).

127. Calderara S, Xiang Y, Moss B. Orthopoxvirus IL-18 binding proteins: affinities and antagonist activities. *Virology* 279(1), 22–26 (2001).

128. Symons JA, Adams E, Tscharke DC, Reading PC, Waldmann H, Smith GL. The vaccinia virus C12L protein inhibits mouse IL-18 and promotes virus virulence in the murine intranasal model. *J. Gen. Virol.* 83(Pt 11), 2833–2844 (2002).

129. Nazarian SH, Rahman MM, Werden SJ *et al.* Yaba monkey tumor virus encodes a functional inhibitor of interleukin-18. *J. Virol.* 82(1), 522–528 (2008).

130. Born TL, Morrison LA, Esteban DJ *et al.* A poxvirus protein that binds to and inactivates IL-18, and inhibits NK cell response. *J. Immunol.* 164(6), 3246–3254 (2000).

131. Reading PC, Smith GL. Vaccinia virus interleukin-18-binding protein promotes virulence by reducing gamma interferon production and natural killer and T-cell activity. *J. Virol.* 77(18), 9960–9968 (2003).

132. Jones MS, 2nd, Harrach B, Ganac RD *et al.* New adenovirus species found in a patient presenting with gastroenteritis. *J. Virol.* 81(11), 5978–5984 (2007).

133. Arnberg N. Adenovirus receptors: implications for targeting of viral vectors. *Trends Pharmacol. Sci.* 33(8), 442–448 (2012).

134. Kamimura K, Suda T, Zhang G, Liu D. Advances in gene delivery systems. *Pharmaceut Med* 25(5), 293–306 (2011).

135. Barlan AU, Danthi P, Wiethoff CM. Lysosomal localization and mechanism of membrane penetration influence nonenveloped virus activation of the NLRP3 inflammasome. *Virology* 412(2), 306–314 (2011).

136. Barlan AU, Griffin TM, Mcguire KA, Wiethoff CM. Adenovirus membrane penetration activates the NLRP3 inflammasome. *J. Virol.* 85(1), 146–155 (2010).

137. Lee BH, Hwang DM, Palaniyar N, Grinstein S, Philpott DJ, Hu J. Activation of P2X(7) receptor by ATP plays an important role in regulating inflammatory responses during acute viral infection. *PLoS ONE* 7(4), e35812 (2012).

138. Muruve DA, Petrilli V, Zaiss AK *et al.* The inflammasome recognizes cytosolic microbial and host DNA and triggers an innate immune response. *Nature* 452(7183), 103–107 (2008).

139. Duncan JA, Gao X, Huang MT *et al.* Neisseria gonorrhoeae activates the proteinase cathepsin B to mediate the signaling activities of the NLRP3 and ASC-containing inflammasome. *J. Immunol.* 182(10), 6460–6469 (2009).

140. Hornung V, Bauernfeind F, Halle A *et al.* Silica crystals and aluminum salts activate the NALP3 inflammasome through phagosomal destabilization. *Nat. Immunol.* 9(8), 847–856 (2008).

141. Willingham SB, Bergstralh DT, O'Connor W *et al.* Microbial pathogen-induced necrotic cell death mediated by the inflammasome components CIAS1/cryopyrin/NLRP3 and ASC. *Cell Host Microbe* 2(3), 147–159 (2007).

142. Jin C, Flavell RA. Molecular mechanism of NLRP3 inflammasome activation. *J. Clin. Immunol.* 30(5), 628–631 (2010).

Website

201. CDC. Cytomegalovirus and congenital CMV infection. www.cdc.gov/cmv/overview.html