Inflammasomes and viruses: cellular defence versus viral offence

Anna M. Gram,1 Joost Frenkel2 and Maaike E. Ressing1

1Department of Medical Microbiology, University Medical Center Utrecht, Utrecht, The Netherlands
2Department of General Paediatrics, Paediatric Division, University Medical Center Utrecht, Utrecht, The Netherlands

Pro-inflammatory cytokines are important mediators in immune responses against invading pathogens, including viruses. Precursors of the pro-inflammatory cytokines interleukin (IL)-1β and IL-18 are processed by caspase-1. Caspase-1 is activated through autocleavage, but how this is regulated remained elusive for a long time. In 2002, an intracellular multimeric complex was discovered that facilitated caspase-1 cleavage and was termed ‘inflammasome’. To date, different inflammasomes have been described, which recognize a variety of ligands and pathogens. In this review, we discuss the role of inflammasomes in sensing viral infection as well as the evasion strategies that viruses developed to circumvent inflammasome-dependent effects.

Introduction

During invasion by pathogens the innate arm of the host immune system is activated first. Recognition of pathogens by germline-encoded pattern-recognition receptors (PRR) initiates immediate defence mechanisms and orchestrates adaptive immunity. PRRs identify conserved pathogen-associated molecular patterns (PAMP), expressed by the intruders. Amongst others, PRRs comprise Toll-like receptors (TLRs), RIG-I-like receptors (RLRs), NOD-like receptors (NLRs) and AIM2-like receptors (ALRs) (Fig. 1). In general, TLRs are present on the plasma membrane or in endosomes, while RLRs, NLRs and the ALR absent in melanoma 2 (AIM2) reside in the cytoplasm of the host cell. The other human ALR, interferon-inducible protein 16 (IFI16; p204 in mice), is localized in the nucleus, but can relocates to the cytoplasm upon stimulation (Schattgen & Fitzgerald, 2011; Veeranki & Choubey, 2012).

There are 10 different TLRs known in humans (TLR1–10), while 12 TLRs have been identified in mice (TLR1–9, -11, -12 and -13) (Kawai & Akira, 2010). TLRs are activated upon stimulation with distinct ligands, such as lipopolysaccharides, nucleic acids and conserved bacterial components like lipopolysaccharides. Activation results in NF-κB and IRF3/7 signalling initiating expression of the type I interferons (IFNs) IFN-α and IFN-β and of pro-inflammatory cytokines, including pro-IL-1β. RLRs RIG-I and MDA5 recognize cytoplasmic RNA, leading to transcription of type I IFN (Onoguchi et al., 2011). In contrast to RLRs, the ALRs AIM2 and IFI16 are DNA sensors comprising an N-terminal pyrin domain (PYD) and C-terminal HIN 200 domain(s) for dsDNA binding. Expression of both proteins is inducible by type II IFN, IFN-γ. IFI16 initiates transcription of type I IFNs via STING (Unterholzner et al., 2010).

Characterization of the proteins NOD1 and NOD2 resulted in identification of the NLR family, consisting of 22 proteins in humans and 34 in mice (Mason et al., 2012). NLRs are characterized by their tripartite structure consisting of a C-terminal leucine-rich repeat (LRR) domain, a central nucleotide-binding oligomerization (NACHT, also NOD) domain and an N-terminal effector domain (Martinson et al., 2009). The LRR domain consists of repeats of a characteristic leucine-rich sequence of 20–30 aa that facilitates protein–protein interactions. It is thought that the LRR domain might function as the ligand-binding site of NLRs, by analogy with the function of the LRR domain in TLRs. However, a receptor–ligand interaction has not yet been shown for any NLR. Upon nucleotide binding, the NACHT domain facilitates self-oligomerization and activation. Based on their different N-terminal effector domains, NLRs are divided into five subfamilies: the NLRAs (NLRs with acidic activation domain), NLRBs [NLRs with baculovirus IAP repeat (BIR) domain], NLCs [NLRs with caspase recruitment domain (CARD)], NLRPs (also termed NALPs; NLRs with PYD domain) and NLRXs (NLR family without strong homology to the N-terminal domain of any other NLR subfamily member) (Ting et al., 2008). Besides having roles in autophagy, reproduction and development (Mason et al., 2012), NLRs accomplish various functions in innate immunity such as inflammasome formation and pyroptosis. As discussed in this review, stimulation of some NLRs leads to their assembly into large multimeric protein complexes (~700 kDa), designated ‘inflammasomes’, which process precursors of the cytokines IL-1β and IL-18. Thereby,
Fig. 1. Pathogen-recognition receptors (PRRs): cellular localization and domain organization. Cellular localization of Toll-like receptors (TLRs), NOD-like receptors (NLRs), RIG-I-like receptors (RLRs) and AIM2-like receptors (ALRs) is shown. TLRs are localized on the plasma membrane and endosomes. TLRs contain leucine-rich repeat (LRR) domains and a Toll/interleukin-1 receptor (TIR) domain that mediates downstream signalling. NLRs are present in the cytosol and can be divided into five subfamilies based on their effector domain. The number of family members is indicated in parentheses. NLRs consist of an N-terminal effector domain, a NACHT domain, and LRRs. The effector domain can either be an acidic activation domain (AD), baculovirus IAP repeat (BIR) domain, caspase recruitment (CARD) domain, pyrin domain (PYD), or a domain without any homology to other NLR subfamily members. RLRs are expressed in the cytoplasm and consist of CARD domains and an RNA helicase domain, except for LPG2 that lacks CARD domains. ALRs are present in the cytoplasm and the nucleus; they consist of a PYD domain and a certain number of HIN 200 domains, which bind dsDNA.
interaction) and with pro-caspase-1 (CARD–CARD interaction), respectively (Fig. 2). RIG-I and ASC also associate, although it is not clear whether they interact directly or whether other adaptors are involved (Poeck et al., 2010). Cleavage of pro-caspase-1 in inflammasomes results in formation of the active caspase enzyme, which consists of two heterodimers of p20 and p10.

Inflammasomes are associated with two different functions, namely cytokine processing and pyroptosis, which are both caspase-1-dependent. In brief, pyroptosis is characterized as caspase-1-mediated cell death and differs to some extent morphologically from apoptosis (Lamkanfi, 2011). Thus far, pyroptosis has been observed in macrophages, dendritic cells (DCs) and neurons. Induction of pyroptosis occurs in response to infection with different intracellular bacteria, which trigger the NLR4 and NLRP1 inflammasomes (Miao et al., 2010). The relevance of pyroptosis to viral infection is at current unclear. The best-studied effector mechanism of inflammasomes is the processing of the cytokines pro-IL-1β and pro-IL-18 into mature and bioactive cytokines by caspase-1 (Dinarello, 2009). These are then exported by an ill-defined and unconventional secretion pathway.

IL-1β and IL-18 in antiviral immune responses

Pro-IL-1β is a 31 kDa protein that is cleaved by activated caspase-1 into mature IL-1β (18 kDa). IL-1β has a potent pyrogenic effect. It activates immune cells and promotes the upregulation of adhesion molecules on endothelial cells, thereby helping activated immune cells, such as neutrophils, to migrate to the site of infection. Mature IL-1β signals through the IL-1 receptor (IL-1R). IL-1R is constitutively expressed on many cell types. IL-1R has a cytosolic Toll/interleukin-1 receptor (TIR) domain, which is homologous to the TIR domain found in TLRs. Similar to TLR signalling, IL-1R stimulation results in the activation of NF-κB and the mitogen-activated protein kinases p38 and JNK. These transcription factors induce expression of multiple genes, including pro-inflammatory cytokines, which contribute to an immune response towards various pathogens (Dinarello, 2009). In view of its strong effects, IL-1β release is tightly controlled at multiple levels, such as transcription, mRNA stability, protein cleavage by inflammasomes and secretion (Sims & Smith, 2010). Furthermore, downstream signalling of the cytokine is regulated by IL-1Rα, a soluble antagonist of IL-1R.

The importance of regulated IL-1β secretion is underscored by the severe clinical symptoms in patients with auto-inflammatory disorders, which are characterized by uncontrolled IL-1β release. Examples of these rare auto-inflammatory disorders include cryopyrin-associated periodic syndrome (CAPS), familial Mediterranean fever (FMF) and TNF receptor-associated periodic syndrome (TRAPS), which are caused by mutations in NLRP3, pyrin (a regulator of caspase-1), or inflammasome components.
of ASC) and p55 TNF receptor, respectively (Dinarello, 2011). Patients present symptoms ranging from fever, fatigue, and rash to joint, bone, and skin inflammation, serositis and deafness, and mental retardation in severe cases (Goldbach-Mansky et al., 2006; Leslie et al., 2006; Ross et al., 2008). Another genetic auto-inflammatory disorder is deficiency for IL-1Rz (DIRA). Patients with DIRA lack IL-1Rz, which has a crucial role in regulating the activity of IL-1β in vivo. DIRA patients exhibit similar symptoms as CAPS patients, but with much greater severity (Aksenjievich et al., 2009; Reddy et al., 2009). Patients can be treated effectively with either recombinant IL-1Rz (anakinra), recombinant IL-1R-Fcγ1 fusion protein (rilonacept) or mAbs neutralizing IL-1β (canakinumab) (Dinarello, 2011). So far, knowledge is missing concerning susceptibility to infectious diseases in these patients.

For in vivo studies on the role of IL-1β in viral infection, various knockout mice are available that are either deficient in the IL-1β cytokine or its receptor IL-1R. When challenged with influenza virus, IL-1R−/− mice exhibited augmented morbidity and mortality compared with wild-type mice, indicating that IL-1β is involved in the immune control of influenza virus (Schmitz et al., 2005). Another study using IL-1R−/− mice infected with herpes simplex virus type 1 (HSV-1) showed that viral loads were increased, while levels of inflammation were reduced; this resulted in enhanced occurrence of HSV-1-induced encephalitis (Sergerie et al., 2007). Taken together, these studies support the view that IL-1β is necessary for a protective response to viral infection.

IL-18 (17 kDa; pro-IL-18 22 kDa) is the second cytokine being processed into its bioactive form by inflammasomes. In contrast to IL-1β, IL-18 has no pyrogenic activity; induction of de novo transcription of pro-IL-18 is not needed, as it is constitutively expressed in multiple cell types (Puren et al., 1999). Similar to IL-1β, IL-18 signals through a heterodimeric IL-18 receptor (IL-18R) that contains a TIR domain activating NF-κB, p38 and JNK. Expression of IL-18R has been reported in Tfh1 cells, some myeloid cells, as well as intestinal epithelial cells (Dinarello, 2009; Sims & Smith, 2010). IL-18, in conjunction with IL-12, is a potent inducer of the type II IFN response via activation of natural killer cells (Chaix et al., 2008). However, it was suggested that IL-18 only contributes to increased levels of systemic and splenic, but not hepatic IFN-γ. IL-18−/− mice did not show reduced survival following infection with murine cytomegalovirus (Pien et al., 2000). Infection with influenza virus, however, did result in increased mortality and viral load in IL-18−/− mice (Liu et al., 2004). In other studies, administration of IL-18 prior to or early during infection with HSV-1 or vaccinia virus led to increased survival rates of HSV-infected mice or decreased pock formation, respectively (Fujioka et al., 1999; Tanaka-Kataoka et al., 1999). Taken together, there is evidence that IL-1β and IL-18 play a role in antiviral immunity, although the importance of IL-18 in antiviral responses may vary for viruses due to different roles and sites of action.

**Activation of inflammasomes during viral infections**

To date, NLRP3-, AIM2-, IFI16- and RIG-I-dependent inflammasomes are known to be activated during viral infection. Below, the different viral activation mechanisms for these four inflammasomes are discussed in detail. Widely used read-outs for inflammasome activation include pro-caspase-1 or pro-IL-1β cleavage, as detected by Western blot analysis, or IL-1β secretion, measured by ELISA. In general, primary murine DCs or macrophages as well as the human monocytic cell line THP-1 are utilized to investigate inflammasome activation. Finally, knockout mice are available that lack single inflammasome components, such as NLRP3, ASC or caspase-1. Of note, caspase-1 knockout mice were recently discovered to also lack caspase-11. Therefore, previous findings concerning the relevance of caspase-1-containing inflammasomes need to be revisited (Kayagaki et al., 2011).

**NLRP3 inflammasome**

The NLRP3 inflammasome is the best-studied inflammasome. It was found to require at least two signals to acquire full activation in certain cell types, such as macrophages and DCs (Bauernfeind et al., 2009). As NLRP3 and pro-IL-1β protein expression is limited in resting cells, a first signal is needed to increase their expression. This first priming signal is triggered by PRRs (TLRs and NLRs) and cytokine receptors. A second signal then activates NLRP3, resulting in inflammasome assembly and recruitment of pro-caspase-1. The nature of this second signal is broad with a wide range of activators (e.g. crystalline structures, bacterial toxins and nucleic acids). Due to the structural variety of the known activators, it is assumed that NLRP3 senses them indirectly. There are several review articles discussing the various possibilities in detail (Bauernfeind et al., 2011; Latz, 2010). Amongst the different activators of NLRP3 inflammasome are several viruses. As viruses can also trigger e.g. TLR signalling, they might be able to provide combined priming and activating signals for the activation of the NLRP3 inflammasome.

**RNA viruses.** The first evidence that viral infections might activate the NLRP3 inflammasome came from an in vitro study reporting that addition of viral dsRNA (flock house virus and rotavirus) and infection with RNA viruses (Sendai virus and influenza A virus) activated caspase-1 in murine macrophages (Kanneganti et al., 2006). As active caspase-1 was not detected in murine NLRP3−/− macrophages in response to Sendai and influenza virus, the inflammasome initiator NLRP3 appeared to be crucial for caspase-1 activation.

Subsequently, other RNA viruses have been described to activate the NLRP3 inflammasome. Encephalomyocarditis virus (ECMV) and vesicular stomatitis virus (VSV) both stimulated NLRP3-dependent IL-1β release in murine DCs and macrophages (Rajan et al., 2011). Infection with
UV-treated virions did not result in the production of IL-1b, indicating that viral replication was required for inflammasome activation. Interestingly, caspase-1-deficient mice did not show decreased survival rates upon ECMV and VSV infection compared to wild-type mice, pointing towards a minor role for IL-1b in the host defence against these viruses. However, these findings contrast with another study (Poeck et al., 2010), which suggests RIG-I as a sensor for inflammasome activation as discussed below.

Measles virus (MV) infection activates several PRRs including TLRs and RLRs, resulting in type I IFN production (Ikegame et al., 2010; Schlender et al., 2005). Additionally, MV-infected THP-1 cells secreted IL-1b in an NLRP3-dependent, but RIG-I-independent manner (Komune et al., 2011). MV-mediated inflammasome activation required viral replication.

Recently, the hepatocyte cell line Huh7.5 was shown to secrete IL-1b upon infection with hepatitis C virus (HCV; Burdette et al., 2012). Inflammasome activation was NLRP3- and ASC-dependent, as shown by small interfering RNA-mediated silencing.

Influenza virus is the best-studied RNA virus regarding its interaction with and activation of the NLRP3 inflammasome (Owen & Gale, 2009; Pang & Iwasaki, 2011). Following up on the results of Kanneganti et al. (2006) describing in vitro inflammasome activation, Ichinohe et al. (2009) investigated the role of inflammasome activation in response to influenza A virus in vivo using wild-type and NLRP3- mice. They found that IL-1b secretion was NLRP3-dependent in alveolar macrophages and DCs, while in resident haematopoietic cells in lung tissue its secretion in response to influenza virus was NLRP3-independent. Interestingly, development of an adaptive immunity largely relied on inflammasome activation, but was NLRP3-independent. Therefore, it remains elusive whether another inflammasome initiator is involved in the recognition of influenza A virus. Two further studies investigated the role of NLRP3 inflammasome in antiviral immunity towards influenza virus in vivo (Allen et al., 2009; Thomas et al., 2009). Both studies show that, compared with wild-type mice, NLRP3-deficient mice have a higher mortality and produce fewer chemokines attracting leukocytes to the site of infection when challenged with influenza A virus. Allen et al. (2009) further report that an intact lysosomal pathway, active lysosomal enzymes, and production of reactive oxygen species (ROS) are required to activate the NLRP3 inflammasome. Thomas et al. (2009) propose that the NLRP3 inflammasome contributes to tissue repair during influenza virus infection. Moreover, they demonstrate that induction of NLRP3 inflammasome signalling does not play a major role in the establishment of an adaptive immune response, which is in contrast to the study by Ichinohe et al. cited above. Recently, a mechanism was suggested for the activation of the NLRP3 inflammasome during influenza virus infection: recognition of viral RNA by TLR7 induces pro-IL-1b production, thereby serving as signal one. Signal two could then be triggered by insertion of the viral ion channel protein M2 into the trans-Golgi network. Thereby, the intracellular ionic milieu is disturbed leading to K+ efflux and ROS production, which in turn are sensed by the NLRP3 inflammasome (Ichinohe et al., 2010). In a more recent paper, Ichinohe et al. (2011) showed that intestinal bacteria are essential for NLRP3 inflammasome function during influenza A virus infection. In normal mice, commensal bacteria provided a trigger, resulting in baseline expression of pro-IL-1b and NLRP3, while mice treated with antibiotics displayed decreased expression of the pro-inflammatory cytokine precursors and NLRP3 as well as reduced adaptive immune responses to influenza A virus infection. It appears that NLRP3 inflammasomes are most likely activated by ROS production during influenza virus infection. However, the role of NLRP3 inflammasomes in the development of adaptive immunity remains controversial.

DNA viruses. DNA viruses have also been found to provide ligands for NLRP3 inflammasome activation. When studying the small, non-enveloped DNA virus adenovirus, conflicting results were obtained. Initially, inflammasome activation in response to transfected adenovirus DNA was found to be NLRP3-independent. However, adenovirus infection or transduction of DNA did activate the NLRP3 inflammasome in murine macrophages (Muruve et al., 2008). This indicated that NLRP3 activation depended on the entry route of the viral DNA and that an additional NLRP3-independent inflammasome should exist, which sensed DNA following transfection. In contrast, Di Paolo et al. (2009) denied a role for inflammasomes in vivo, since neither NLRP3, nor caspase-1 or IL-1b, but rather IL-1z and b3 integrins were required to trigger an early pro-inflammatory immune response upon adenovirus infection in mice. Recently, it was demonstrated that release of cathepsin B induced by membrane penetration of adenovirus was sensed by the NLRP3 inflammasome in human, but not murine macrophages (Barlan et al., 2011a, b). It appears that adenovirus can activate the NLRP3 inflammasome via cathepsin B release and ROS production. In addition, species differences between human and mouse macrophages may exist with regard to NLRP3 activation in vivo, in particular by a cathepsin B-dependent mechanism.

The large enveloped DNA viruses, pox- and herpesviruses, also activate inflammasomes. The orthopoxvirus ‘modified vaccinia virus Ankara’ (MVA) activates the NLRP3 inflammasome upon infection of both human and murine macrophages (Delaloye et al., 2009). The leporipoxvirus myxoma virus stimulates NLRP3 inflammasome in a ROS- and cathepsin B-dependent way (Rahman & McFadden, 2011).

Additionally, the NLRP3 inflammasome is activated by varicella-zoster virus (VZV), an alpha-herpesvirus. VZV induces NLRP3, but not AIM2, inflammasome formation and IL-1b release, which was mediated by caspase-1 in macrophages, primary lung fibroblasts and melanoma cells (Nour et al., 2011).
Last but not least, a single nucleotide polymorphism (SNP) in the 3'-UTR of NLRP3 has been associated with susceptibility to the retrovirus human immunodeficiency virus 1 (HIV-1) (Pontillo et al., 2010). The G allele of this SNP appeared to be protective, suggesting that NLRP3 inflammasome-dependent production of IL-1β may play a role against viral infection in vivo. However, little further is known about how polymorphisms in inflammasome components might relate to susceptibility to viral infections.

Evidence accumulates that infection with either RNA or DNA viruses is sensed by the NLRP3 inflammasome. The exact mechanism of NLRP3 inflammasome activation and the in vivo relevance of its response to most viral infections still require further investigation (Fig. 3).

**AIM2 inflammasome**

The idea that an inflammasome would exist capable of recognizing bacterial, viral, mammalian and synthetic DNA was originally proposed by Muruve et al. (2008). Their idea was based on observing IL-1β secretion in an ASC-dependent, but NLRP3-independent way (Muruve et al., 2008). More recently, the sensor molecule AIM2 was identified by several groups and it was shown to form an ASC-dependent inflammasome (Fig. 3) (Bürckstümmer et al., 2009; Fernandes-Alnemri et al., 2009; Hornung et al., 2009; Roberts et al., 2009). AIM2 differs from members of the NLR family in that it lacks a NACHT domain. It contains a HIN 200 domain instead and a PYD domain, which interacts with ASC and thereby, recruits pro-caspase-1. In contrast to NLRP3, the ligand of AIM2, dsDNA, has been identified and direct binding of DNA to the HIN 200 domain was demonstrated (Hornung et al., 2009).

Thus far, infections by the orthopoxvirus vaccinia virus and by the beta-herpesvirus murine cytomegalovirus (mCMV) were found to be sensed by AIM2, but not by NLRP3, in murine macrophages (Hornung et al., 2009;...
Rathinam et al., 2010). The contribution of the AIM2 inflammasome in vaccinia virus infections in vivo still needs to be assessed. In macrophages, infected with mCMV in vitro AIM2 mediated both IL-1β processing and the expression of IFN-β, the latter occurring independently of inflammasome activation (Rathinam et al., 2010). In vivo studies with mCMV infections revealed that serum IL-18 levels as well as splenic IFN-γ+ natural killer cell numbers were reduced, while viral load in the spleen was increased in AIM2- or ASC-deficient mice compared with wild-type mice (Rathinam et al., 2010).

Interestingly, the AIM2 inflammasome did not recognize the alpha-herpesvirus HSV-1, although this virus is known to trigger IL-1β release (Muruve et al., 2008). This suggests the existence of alternative, yet unidentified, inflammasome initiators.

**IFI16 inflammasome**

Like AIM2, IFI16 belongs to the ALR family, but its cellular localization is different, whereas AIM2 acts strictly cytosolically, IFI16 is mainly localized in the nucleus due to its nuclear localization sequence (NLS). A recent publication presents IFI16 as a nuclear dsDNA sensor in Kaposi’s sarcoma-associated herpesvirus (KSHV)-infected endothelial cells (Kerur et al., 2011). Subsequently, IFI16 assembles a functional inflammasome in the nucleus, resulting in caspase-1 activation and processing of IL-1β in the cytoplasm, although IFI16-dependent IL-1β secretion was not studied. Formation of the nuclear inflammasome was assessed by co-immunoprecipitation and colocalization studies of different inflammasome components such as ASC and caspase-1. Caspase-1 activation and cytosolic IL-1β processing were decreased upon IFI16 knockdown during KSHV infection. Assembly of the IFI16 inflammasome in the nucleus is remarkable as all other known inflammasomes are activated and assembled in the cytoplasm. Activation of IFI16 and subsequent inflammasome formation might be restricted to DNA viruses, which replicate in the nucleus. Although IFI16 was earlier shown to sense HSV-1, thereby leading to IFN-β induction via STING (Unterholzner et al., 2010), previous studies could not demonstrate the formation of an IFI16 inflammasome in 293T cells or THP-1 macrophages (Fernandes-Alnemri et al., 2009; Hornung et al., 2009).

**RIG-I inflammasome**

Recently, the family of inflammasome initiators was extended with RIG-I (Fig. 3). RIG-I-induced pro-IL-1β production via NF-κB activation as well as caspase-1-dependent pro-IL-1β cleavage by forming an inflammasome in response to the rhabdovirus VSV upon infection of murine DCs (Pocek et al., 2010). DCs from RIG-I-/- mice did not secrete IL-1β in response to VSV. In this report, RIG-I was co-immunoprecipitated with ASC, the adaptor molecule that links caspase-1 and the initiator molecule. Conflictingly, Rajan et al. (2011) reported that VSV is recognized by NLRP3 (discussed above) and not by RIG-I. The reasons for this discrepancy remain unclear. However, it is still under debate whether RIG-I itself is involved in inflammasome formation or whether it regulates an unknown NLRP3-independent inflammasome (Pocek & Ruland, 2012).

**Viral evasion of inflammasome activation and effector functions**

To permit viral replication, viruses have evolved different mechanisms to counteract or evade both activation of inflammasomes and resulting effector functions. Studies on other antiviral immune response pathways revealed that viral interference occurs at different levels: viruses can (i) alter expression levels of key molecules inhibiting their detection by PRRs, (ii) inhibit activation and downstream signalling of the immune pathway, for instance by direct interaction with host proteins, and (iii) inactivate effector molecules by binding or cleaving them. Indeed, reports have recently been published showing that viruses employ all three strategies to counteract functions of inflammasomes.

Currently, there is only limited evidence for viral modulation of inflammasome component expression levels. Genome-wide examination of host gene expression profiles revealed that transcription of ASC was downregulated during infection of keratinocytes with the human papillomavirus types 16 and 18 (Karim et al., 2011). It is reasonable to speculate that host shut off mechanisms exhibited by many viruses such as gamma-herpesviruses, poliovirus and influenza virus affect the expression levels of inflammasome components (Covarrubias et al., 2009; Rowe et al., 2007; Sharma et al., 2004; Vreede & Fodor, 2010).

Here, we give a short overview of the known inflammasome evasion proteins and of their evasion strategies, in particular at the levels of inhibition of inflammasome assembly, caspase-1 activation and neutralization of effector molecules (Fig. 4).

**Inhibition of inflammasome assembly**

The large DNA genomes of pox- and herpesviruses allow these viruses to encode several immune evasion proteins (reviewed by Coscoy, 2007; Seet et al., 2003). Among these are proteins that inhibit the assembly of inflammasomes, for instance by preventing oligomerization of the inflammasome sensors or recruitment of the adaptor ASC.

Originally, KSHV ORF63 was selected as a candidate inflammasome inhibitor on the basis of presumed similarity to the LRR domains of NLRP1, although this is currently subject to debate (Boyle & Monie, 2012; Gregory et al., 2011). Still, KSHV ORF63 interacts with NLRP1, NLRP3 and NOD2 and inhibits IL-1β production during the viral life cycle by preventing NLRP1 oligomerization.

Unlike KSHV ORF63, the V protein of MV does not share homologous or conserved sequences with its target protein...
NLRP3 (Komune et al., 2011). The V protein interacts with NLRP3 in an unknown fashion, thereby inhibiting inflammasome activation. Infection with MV lacking the V protein resulted in augmented IL-1β secretion. Further investigation of the oligomerization inhibition is needed to clarify this mechanism.

Inhibition of caspase-1 function has been reported for different genera of poxviruses, suggesting that it is an important and widely used evasion mechanism in different hosts. The leporipoxvirus myxoma virus, which infects rabbits, encodes the PYD-only protein M13L-PYD, which interacts with and inhibits ASC, thereby preventing caspase-1-dependent IL-1β production (Johnston et al., 2005). Of these, the PYD-only protein gp013L of SFV acts in a similar way as M13L-PYD of myxoma virus (Dorfleutner et al., 2007). This mechanism of inflammasome regulation is not unique for viruses, but is thought to resemble the working mechanism of host-encoded 'pyrin-only proteins' (POPs). POps interact with inflammasome sensors, thereby preventing recruitment of ASC and further assembly of the inflammasome (Stehlik & Dorfleutner, 2007). Additionally, another group of human proteins is known to regulate inflammasome activation. Characterization of the CARD-only proteins (COPs) COP, INCA and ICEBERG revealed that these proteins inhibit inflammasome assembly in vitro by homotypic CARD interactions (Stehlik & Dorfleutner, 2007). Due to these interactions, pro-caspase-1 cannot be recruited and incorporated into the inflammasome. It might
be interesting to investigate if viruses have also hijacked this pathway through homologues.

**Inhibition of caspase-1 function**

Downstream signalling of inflammasome activation is prevented by inhibition of caspase-1 (Lamkanfi & Dixit, 2011). Orthopoxviruses and influenza virus encode inhibitors of caspase-1. Cowpoxvirus (CPV) was first described to encode the caspase-1 inhibitory protein cytokine response modifier A (CrmA) (Ray et al., 1992). CrmA inhibits caspase-1 by binding rapidly and acts as a pseudosubstrate for this protease (Komiyama et al., 1994). In other orthopoxviruses, such as vaccinia virus, ectromelia virus (EV) and rabbit poxvirus (RPV, a vaccinia virus strain), the CrmA homologues were named serpin-like protease inhibitor (SPI)-1 and SPI-2 (Dobbelstein & Shenk, 1996; Turner et al., 2000). The role of different SPLs in CPV and RPV in vivo was examined in a mouse study. Mice infected intranasally or intratracheally with CPV or RPV lacking CrmA/SPI-2 showed attenuation compared with wild-type virus or virus without SPI-1 (MacNeill et al., 2009; Thompson et al., 1993). The basis for this phenotype remains unclear as no significant differences were found in virus titres or number of apoptotic cells. Interestingly, intranasal infection of mice with vaccinia virus deletion mutants lacking SPI-1 or SPI-2 did not reveal attenuation compared to wild-type virus (Kettle et al., 1995). These apparently conflicting phenotypes may relate to the presence of additional evasion molecules (see below).

Influenza virus, which is sensed by the NLRP3 inflammasome, appears to possess a caspase-1 inhibitor (Stasakova et al., 2005). The immune evasion protein NS1 of the PR8 strain (H1N1) was already reported to inhibit IRF3 and the IFN-β promoter (Kochs et al., 2007). An additional caspase inhibitory function of the NS1 protein was deduced from infection of primary human macrophages with mutant virus bearing a partly deleted N-terminal RNA-binding domain of NS1. This resulted in an increase in caspase-1 maturation compared to infection with wild-type virus, thereby leading to elevated IL-1β and IL-18 secretion. Details of the inhibition mechanism await further elucidation. The mechanism appears to differ from the one employed by poxviruses.

**Neutralization of IL-1β and IL-18**

Even after inflammasome activation and caspase-1-mediated cytokine processing have occurred successfully, viruses may still prevent effector functions of these cytokines. To this end, different members of the genus *Orthopoxviruses* encode decoy proteins binding IL-1β and IL-18. Vaccinia virus and CPV encode both a IL-1β-binding protein (IL-1β BP) and IL-18-binding proteins (IL-18 BPs), while EV and Yaba-like disease virus only secrete IL-1β BP and IL-18 BP, respectively (Alicam & Smith, 1992; Nazarian et al., 2008; Smith & Alicami, 2000; Smith et al., 2000). Thereby, downstream signalling of the cytokines IL-1β and IL-18 is prevented in effector cells. Decreased IFN-γ production and reduced natural killer and T-cell activity was observed in mice infected with wild-type vaccinia virus infection compared to vaccinia virus lacking IL-18 BP (Reading & Smith, 2003). This might explain the delayed clearance of wild-type virus compared to the mutant virus in mice.

In conclusion, there is growing evidence that viruses not only activate inflammasomes, but also have acquired mechanisms to counteract inflammasome assembly, activity or effector functions. Some viruses, like poxviruses, even modulate several of these events. This suggests that inflammasome activation and its downstream effects are important in the establishment of an antiviral immune response towards multiple viruses.

**Concluding remarks**

Since the discovery of the NLRP1 inflammasome almost a decade ago, knowledge on the molecular composition of inflammasomes and their ligands has expanded rapidly (Martinon et al., 2002). In addition to the ligands first identified, such as endogenous and bacterial danger molecules, viruses have now been accepted to activate (at least) a subset of inflammasomes, namely NLRP3, AIM2, IFI16 and RIG-1 inflammasomes. Both RNA and DNA viruses are sensed by NLRP3; but the exact stimulation and activation mechanisms of the NLRP inflammasomes remain unclear and need further clarification.

More recently, the ALR family members AIM2 and IFI16 have been added to the sensor molecules forming a functional inflammasome upon stimulation. AIM2 and IFI16 recognize dsDNA and, thereby, are important innate immune receptors directly sensing intracellular pathogens such as viruses and bacteria, but also cytosolic host DNA. Additionally, IFI16 may recognize nuclear viral DNA leading to inflammasome formation in the nucleus. This might contribute to our understanding of how viral, but not host DNA, is sensed in the nucleus. Moreover, indications are present that RIG-I can form an inflammasome upon VSV stimulation, although this is in contrast to another report. There are more studies needed to determine the activation mechanism and identify more ligands of the RIG-I inflammasome.

Beyond identification of different inflammasomes and their ligands, alternative effector functions of the inflammasomes are being recognized, for instance caspase-1-mediated pyroptosis (Lamkanfi, 2011). Pyroptosis might be triggered during stimulation of the NLRP3 and AIM2 inflammasome (Sauer et al., 2010). So far, pyroptosis has not been studied upon viral infection, even though it might be a relevant clearance mechanism for infected cells.

Whereas knowledge on activating ligands and subsequent effector functions is expanding substantially, regulation and shutdown mechanisms of inflammasome activation have not been explored in detail yet. Characterization of host proteins that only carry PYD (POP1 and POP2) or only CARD (COP, INCA and ICEBERG) revealed that
these proteins inhibit inflammasome assembly in vitro (Guarda et al., 2011). They are possibly acting as decoys in vivo, but their role and regulation remain elusive. In addition to host proteins, several DNA viruses, such as poxviruses and herpesviruses, encode evasion proteins inhibiting inflammasome assembly, caspase-1 activity, and downstream signalling of the cytokines IL-1β and IL-18 in effector cells (Kanneganti, 2010). Vaccinia virus combines multiple inflammasome inhibitors substantiating the importance of inflammasomes for vaccinia virus recognition and subsequent host immune activation eliminating the viral infection.

Besides by direct interaction of host and viral proteins, inflammasome activation might be regulated through cross-talk with other inflammatory signalling pathways. For example, incubation with type I IFNs prior to inflammasome stimulation resulted in the inhibition of IL-1β production (Guarda et al., 2011). This observation is in agreement with the impaired immune responses after viral infections (Guarda et al., 2011). Current knowledge on cross-talk between NLRs and other cytokine signalling pathways was reviewed recently (Barker et al., 2011).

So far, the mechanisms and ligands for activation of inflammasomes and its contribution to antiviral immunity are incompletely understood. Over the past decades, investigation of viral strategies to evade other immune response pathways has provided ample mechanistic insights into both viral infection and pathogenesis, as well as into the various cellular processes targeted. Therefore, a deeper understanding of immune evasion molecules evolved by viruses that perturb the function of inflammasomes will likely reveal novel concepts and may eventually identify targets in the treatment and prevention of infectious and inflammatory diseases.

**Acknowledgements**

We would like to thank Dr Sytse Piersma for helping with the figure design. This work was supported by the Netherlands Scientific Organization (NWO Vidi grant no. 917.76.330).

**References**


Recognition of RNA virus by RIG-I results in activation of the inflammasome and NF-kB. 


