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Commentary

Detection of Viral Infections by Innate Immunity

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ABSTRACT

Pattern recognition receptors (PRRs) and inflammasomes are a key part of the anti-viral innate immune system as they detect conserved viral pathogen-associated molecular patterns (PAMPs). A successful host response to viral infections critically depend on the initial activation of PRRs by viruses, mainly by viral DNA and RNA. The signalling pathways activated by PRRs leads to the expression of pro-inflammatory cytokines, to recruit immune cells, and type I and type III interferons which leads to the induction of interferon stimulated genes (ISG), powerful virus restriction factors that establish the "antiviral state". Inflammasomes contribute to anti-viral responses through the maturation of interleukin (IL)-1 and IL-18 and through triggering pyroptotic cell death. The activity of the innate immune system along with the adaptive immune response normally leads to successful virus elimination, although disproportionate innate responses contribute to viral pathology. In this review we will discuss recent insights into the influence of PRR activation and inflammasomes on viral infections and what this means for the mammalian host. We will also comment on how specific PRRs and inflammasomes may be relevant to how SARS-CoV-2, the virus responsible for the current COVID-19 pandemic, interacts with host innate immunity.

Abbreviations: AAV, Adeno-associated virus; AIM2, Absent in melanoma 2; ART, Antiretroviral therapy; ASC, Apoptosis-associated speck-like protein containing a CARD; CARD, Caspase activation and recruitment domain; CBV, Coxsackie B virus; cGAMP, Cyclic guanosine monophosphate-adenosine monophosphate; cGAS, Cyclic GMP-AMP synthase; cIAP, Cellular inhibitor of apoptosis protein; CLR, C type lectin receptors; COPD, Chronic obstructive pulmonary disease; COVID-19, Coronavirus disease 2019; CNS, Central nervous system; DAI, DNA-dependent activator of IFN-regulatory factors; DAMP, Damage-associated molecular patterns; DC, Dendritic cells; DC-SIGN, DC-specific intracellular adhesion molecule grabbing nonintegrin; DISC, Death induced signalling complex; dsRNA/DNA, Double stranded RNA/DNA; DV, Dengue virus; EBV, Epstein-Barr virus; EMCV, Encephalomyocarditis Virus; EV71, Enterovirus 71; FADD, FAS-associated death domain protein; FosL1, Fos-like 1; G-CSF, Granulocyte colony-stimulating factor; GSDMD, Gasdermin D; HBV, Hepatitis B virus; HCV, Hepatitis C virus; HIV, Human immunodeficiency virus; hnRNPA2B1, Heterogeneous nuclear ribonucleoprotein A2B1; HOIL-1, Heme-oxidized IRP2 ubiquitin ligase 1; HOIP, HOIL-1-interacting protein; HPV, Human Papilloma Virus; HSV, Herpes simplex virus; IAV, Influenza A virus; IFI16, IFN inducible protein 16; IFN, Interferon; IKK, Inhibitor of κB kinases; IL, Interleukin; IRF, Interferon regulatory factor; ISG, Interferon stimulated genes; KSHV, Kaposi's sarcoma-associated herpesvirus; LGP2, Laboratory of Genetics and Physiology 2; LUBAC, Linear ubiquitin chain assembly complex; MAP, Mitogen-activated protein; MAVS, Mitochondrial antiviral-signalling protein; MCMV, Murine cytomegalovirus; MCV, Molluscum contagiosum virus; MDA5, Melanoma differentiation associated gene 5; mDC, Myeloid dendritic cells; MDL-1, Myeloid DAP-12-associating lectin; MERS-CoV, Middle East respiratory syndrome coronavirus; MGL, Macrophage galactose-type lectin; MMTV, mouse mammary tumour virus; mtDNA, Mitochondrial DNA; mTORC2, Mammalian target of rapamycin (mTOR) complex 2; MVA, Modified vaccinia Ankara; mvRNA, Mini viral RNA; MyD88, Myeloid differentiation primary response 88; NDV, Newcastle disease virus; NEMO, NF-κB essential modifier; NF-κB, Nuclear factor kappa-light-chain-enhancer of activated B cells; NK, Natural killer; NLRP3, NLR Family Pyrin Domain Containing 3; PAMP, Pathogen-associated molecular patterns; PBMC, Peripheral blood mononuclear cell; pDC, Plasmacytoid dendritic cells; Pol III, RNA polymerase III; PPP6C, Protein phosphatase 6 catalytic subunit; PRR, Pattern recognition receptor; PRV, Pseudorabies virus; RIG-I, Retinoic acid-inducible gene I; RIP1, Receptor interacting protein kinase 1; RIPK, Receptor interacting protein kinase; RLR, RIG-I-Like Receptors; RNA5SP141, 5S ribosomal RNA pseudogene 141; ROS, Reactive oxygen species; RSV, Respiratory syncytial virus; RV, Rhinovirus; SARS-CoV-2, Severe acute respiratory syndrome coronavirus 2; SHARPIN, Shank-associated RH domain-interacting protein; SIV, Simian immunodeficiency virus; SOCS-1, Suppressor of cytokine signaling protein 1; ssRNA, Single stranded RNA; STING, Stimulator of interferon genes; SV, Sendai virus; TAK1, Transforming growth factor beta-activated kinase 1; TANK, TRAF family member associated NFκB activator; TBK1, TANK-binding kinase 1; TIR, Toll/interleukin-1 receptor; TLR, Toll like receptor; TNFα, Tumor necrosis factor α; TRAF, TNF receptor-associated factor; TRIF, TIR-domain containing adaptor inducing interferon (IFN)-β; TRIM, Tripartite motif; vRNP, Viral ribonucleoprotein; VSV, Vesicular stomatitis virus; VV, Vaccinia virus; VZV, Varicella-zoster virus; WHIP, Werner helicase-interacting protein 1; WNV, West Nile virus; ZBP1, Z-DNA-binding protein 1; ZV, Zika virus.

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1. Introduction

As humans we are under constant attack from invading microbes including viruses. How the human host detects and responds to invading viruses determines the outcome of these infections. Initial sensing of virus infection depends chiefly on the detection of viral nucleic acids, DNA or RNA, which are detected by the mammalian host by germline encoded Pattern recognition receptors (PRRs) [1]. PRRs and associated signalling pathways make up a large part of the innate immune system. Following engagement of these PRRs, type I and type III interferons (IFNs), chemokines and pro-inflammatory cytokines are produced to activate inflammation. Type I and type III interferons are powerful antiviral agents as they induce the expression of interferon stimulated genes (ISGs), some of which act as direct virus restriction factors, thus establishing the "antiviral state" in uninfected neighbouring cells. Hundreds of these ISGs are produced and they exert their antiviral activity by preventing viral entry, viral replication, and viral budding [2]. Proinflammatory cytokines production helps to shape the overall immune response by recruiting immune cells to the site of infection, and by activating adaptive immunity [3]. Therefore, the innate immune system powers the adaptive immune response, where specific antibodies neutralise viruses, and activated CD8+ cytotoxic T cells kill virus infected cells, ultimately leading to virus clearance. However, these viral sensing mechanisms can often trigger an overactive immune response that leads to tissue damage, such as the pathological over production of inflammatory mediators, known as the "cytokine storm". In this review we will explore the cellular mechanisms that exist to detect viral infections and examine how these mechanisms shape the overall anti-viral innate immune response. We will focus on newer developments within the field of virus sensing, especially those that have been made in the last 5 years. This field has gained even greater importance due to the current Coronavirus disease 2019 (COVID-19) pandemic, and a special focus will be placed on how the responsible virus, Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), interacts with the innate immune system.

2. Viral sensing PRRs of the innate immune system

A key component of the innate immune system is the network of PRRs and associated signalling pathways leading to IFN and inflammatory gene expressions. PRRs are germline encoded receptors which are conserved across evolution from plants, worms, *Drosophila,* and mammals. In contrast with immunoglobulin receptors of the adaptive immune system, generated by somatic gene rearrangements, PRRs are encoded within the germline of the host organism. These PRRs defend against infection by recognising conserved microbial structures. There are several classes of viral sensing PRRs which we will examine in this review, these include Toll-like receptors (TLRs), RIG-I-like receptors (RLRs), C type lectin receptors (CLRs), inflammasomes and DNA sensors. Although these receptors can detect a broad range of microbes, microbial products, and host derived damage-associated molecular patterns (DAMPs), we will limit our discussion to the role of these receptors in virus detection. Given the role of these antiviral defence mechanisms in protecting the host against viral infections it is not surprising then that viruses have evolved various strategies to evade these antiviral defence mechanisms to allow viral replication and establish successful infections. Therefore, our discussion will also include recent insights into strategies of viral immune evasion.

2.1. TLRs

TLRs are type I membrane spanning receptors structured with leucine rich repeats in the extracellular domain, for ligand binding, a transmembrane domain, and a Toll-interleukin-1 receptor resistance (TIR) domain in the cytoplasmic tails to activate intracellular signalling [4]. There are ten of these receptors in humans and 12 in mice [4]. Broadly speaking these receptors can be grouped into those that are expressed on the cell surface, and more associated with detection of bacterial products, and those that are expressed in endosomes, which are associated with the detection of nucleic acids. The endosomal TLRs include TLR3, TLR7, TLR8 and TLR9 [4]. TLR3 detects double stranded RNA, TLR7 and TLR8 both detect single stranded RNA (ssRNA), while TLR9 detects hypomethylated CpG DNA (Fig. 1) [4]. Since the presence of viral infections are mainly detected by nucleic acids, there is a key role for the nucleic acid sensing TLRs in virus detection and subsequent antiviral immune responses, in specific cell types where these TLRs are known to be expressed, especially plasmacytoid dendritic cells (pDCs) for TLR7 and TLR9 [4]. The restricted expression of TLRs contrasts with RLRs which are much more ubiquitously expressed. In addition to the nucleic acid sensing endosomal TLRs, surface TLRs such as TLR2 and TLR4 have also been linked with virus detection, by the recognition of virus coat proteins [5]. However, engagement of surface TLRs by viruses can be viewed as viral subversion as virus activation of TLR2 and TLR4 can favour the virus by inducing IL-10 production [5]. Unsurprisingly TLRs and their associated adaptor proteins are major targets of viral immune evasion by many different viruses such as poxviruses, hepatitis C virus (HCV) and herpesviruses [6,7]. We will examine new insights into the role of each of these TLRs in virus detection and the overall antiviral response (Fig. 1).

2.2. TLR3

TLR3 is located within endosomes and is a receptor for double stranded RNA (dsRNA). However, since many viruses have dsRNA within their genomes or generate dsRNA in their viral life cycles, TLR3 can sense the presence of ssRNA, dsRNA and DNA viruses [5]. Upon dsRNA binding, TLR3 recruits the TIR-domain containing adaptor inducing interferon (IFN)-β (TRIF), the sole adapter utilised by TLR3. TRIF recruits TNF receptor-associated factor 6 (TRAF6) to activate downstream nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and TRAF3 to activate downstream interferon regulatory factor (IRF) family members such as IRF3 and IRF7 [4]. TRAF6 associates with transforming growth factor beta-activated kinase 1 (TAK1) which recruits the Inhibitor of κB kinases (IKK) complex that included IKKα, IKKβ and NF-κB essential modifier (NEMO). This complex phosphorylates the NF-κB inhibitor IκBα leading to its ubiquitination and degradation. The NF-κB complex consisting of subunits p50 and p65 then translocates into the nucleus to induce gene expression. Once TRAF3 is engaged by TRIF, it recruits and activates the TRAF family member associated NF-κB activator (TANK) complex, consisting of NEMO, TANK-binding kinase 1 (TBK1) and IKKε, resulting in the phosphorylation of IRF3 and IRF7 [4]. These activated transcription factors translocate into the nucleus and together activate gene expression of chemokines, pro-inflammatory cytokines, type I and type III IFNs [4].

The NF-κB pathway is a major target for evasion by poxviruses such as vaccina virus (VV) and Molluscum contagiosum virus (MCV), where the MCV protein MC132 targets p65 for degradation [8] and MC005 binds to and inhibits the IKK subunit NEMO [9]. Similarly, IRF pathway members are also targets of viral evasion. C6 from VV binds to the TBK1 adaptor proteins TANK, SINTBAD and NAP1 to prevent IRF3 and IRF7 activation [10], while US3 from Herpes simplex virus (HSV) hyperphosphorylates and inhibits IRF3 to block IFNβ production [11] (Table 2).

TLR3 is expressed on immune cells such as conventional dendritic cells (DCs), macrophages, natural killer (NK) cells and non-immune cells such as epithelia, endothelia, fibroblasts, astrocytes and hepatocytes [5]. Within the central nervous system (CNS) TLR3 is expressed in neurons, astrocytes, and microglia, suggesting a key antiviral role for TLR3 within the CNS [12]. Much of what we know about TLR3 has been learned from mice lacking TLR3. Mice deficient in TLR3 are susceptible to RNA viruses, such as West Nile Virus (WNV), Encephalomyocarditis Virus

(EMCV), Polio virus, Coxsackie B virus (CBV) and rotavirus, as well as DNA viruses, such as murine cytomegalovirus (MCMV) and HSV [5]. While the immune response elicited by TLR3 can contribute to virus elimination, TLR3 can also drive a damaging inflammatory response to some viruses. Hence there are several viral infections in mice where outcomes are improved in the absence of TLR3, including, Punta Toro virus, influenza A virus (IAV) and VV (Table 1) [5].

There is a well-established role for TLR3 in the defence against HSV induced encephalitis. Human studies have shown that HSV induced encephalitis is prevented by the TLR3-TRIF-UNC93 pathway, as individuals deficient in any of these pathway members are susceptible to this condition [12]. Furthermore, TLR3 has been implicated in the detection and restriction of varicella-zoster virus (VZV) in humans [13,14]. Therefore, TLR3 a dsRNA sensor, has a major role in host defence against neurotropic DNA viruses in humans.

It has been suggested that TLR3 might recognise dsRNA from virus infected cells that have undergone apoptosis, as endosomal TLR3 is located near phagosomes containing apoptotic cell debris. Fusion of the endosome with the phagosome allows viral dsRNA recognition by TLR3. This may represent a cross-priming method to activate cytotoxic T lymphocytes by DCs that have taken up these apoptotic bodies of virus infected cells but have not been infected by viruses [4]. TLR3 can also influence the humoral immune response by contributing to the optimal production of neutralising antibodies against Chikungunya virus [15]. These are examples where TLR3 can directly mobilise the anti-viral adaptive immune response [5]. Homeostatic roles for TLR3 have also been defined. Viruses resident in the gut reduce damaging inflammation by TLR3 and TLR7 mediated IFN production by pDCs [16]. In contrast lethal infection with avian IAV is associated with a potent TLR3 dependent inflammatory response [17].

As described above early work showed that TLR3 mediates a detrimental response to IAV infection, however a more recent report showed that TLR3 is required to initiate an adequate antiviral host response to the IAV strain A/Puerto Rico/8/1934 (H1N1) [18]. This might indicate that the TLR3 response to IAV infection is dependent on the IAV strain used [18]. Shank-associated RH domain-interacting protein (SHARPIN), heme-oxidized IRP2 ubiquitin ligase 1 (HOIL-1) and HOIL-1-interacting protein (HOIP) are members of the linear ubiquitin chain assembly complex (LUBAC) and are downstream signalling components of TLR3. SHARPIN is required for TLR3 mediated gene activation but also prevents excessive TLR3 dependent cell death following IAV infection. The absence of LUBAC components such as HOIP, allows the formation of a previously unrecognised TLR3 activated death induced signalling complex (DISC) consisting of LUBAC, receptor interacting protein kinase 1 (RIP1), FAS-associated death domain protein (FADD), cellular inhibitor of apoptosis protein-1 (cIAP1)/2, and caspase-8. The excessive TLR3

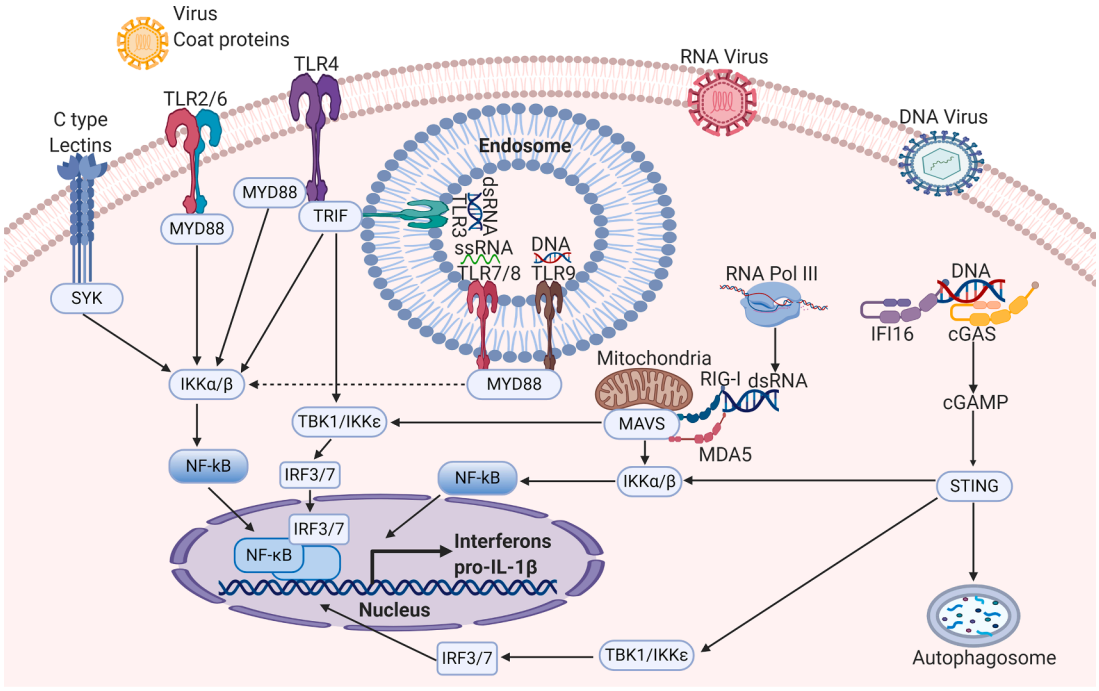

Fig. 1. The innate immune system senses viruses using different PRRs. Virus sensing mainly depends on the detection of viral nucleic acids both DNA and RNA. This is achieved by the nucleic acid sensing TLRs which are located on endosomes and cytosolic nucleic acid sensors, RLRs and DNA sensors. In endosomes TLR3 detects dsRNA, TLR7/8 detects ssRNA while TLR9 detects hypomethylated CpG DNA. TLR7/8 and TLR9 utilise the prototypical TIR adaptor MyD88 to activate the IKK and IKKε/TBK1 complexes which result in the activation of NF-κB and IRF family members. TLR3 by contrast utilises TRIF to activate downstream signalling. Viruses are also detected by viral coat proteins which sometimes activates surface TLRs such as the TLR heterodimer TLR2/6 and TLR4. However, engagement of these surface TLRs can be viewed as viral subversion if the outcome favours the virus, which occurs in instances where IL-10 is produced. Downstream signalling from the surface TLRs also requires MyD88 and TRIF in the case of TLR4. Innate immunity is also activated by viruses that engage C-Type lectin receptors, which via SYK leads to NF-κB activation, but are sometimes used by viruses to facilitate cell entry. In the cytosol viral dsRNA is sensed by the RLRs, RIG-I and MDA5. These receptors which have ATPase activity signal downstream via the RLR adaptor MAVS located at the mitochondria. MAVS also activates the IKK and IKKε/TBK1 complexes which result in the activation of NF-κB and IRFs. RNA polymerase III (Pol III) transcribes A-T rich DNA from both viral and host origins, and these transcripts act as ligands for RIG-I, placing Pol III upstream of the RLRs in the innate antiviral response. DNA viruses are sensed in the cytosol by the presence of the DNA sensors, the cGAS-STING pathway and IFI16. Viral DNA activates the enzymatic activity of cGAS to synthesise cGAMP using ATP and GTP as substrates. cGAMP binds to and activates STING and STING activates IKK and IKKε/TBK1 complexes, resulting in the activation of NF-κB and IRFs. STING also triggers autophagy which is another antiviral defence strategy. IFI16 binds to viral DNA and collaborates with cGAS to activate STING either in a manner dependent or independent on promoting cGAMP production. Once NF-κB and IRFs are activated they enter the nucleus and trigger the expression of pro-inflammatory cytokines, chemokines, type I and type III interferons. Production of these mediators promotes ISG expression and establishment of the "antiviral state", recruits immune cells to the infection site, and activates adaptive immunity to shape the overall antiviral immune response. Figure drawn with the aid of biorender.

Table 1
Interactions between TLRs and viruses and consequences for host and virus.

TLR (Ligand)	Virus (genome)	Outcome (Favours host or Virus)	Reference
TLR3 (dsRNA)	Hantaan virus (ss(−) RNA)	Favours host	[157]
	Punta Toro virus (ss(−) RNA)	Harmful to host	[158]
	Influenza (ss(−) RNA)	Harmful to host	[159]
	HCV (ss(+) RNA)	Favours host	[160]
	Dengue virus (ss(+) RNA)	Favours host	[161]
	EMCV (ss(+) RNA)	Favours host	[162]
	Coxsackievirus (ss(+) RNA)	Favours host	[163]
	Poliovirus (ss(+) RNA)	Favours host	[164]
	West Nile virus (ss(+) RNA)	Protective/harmful	[165,166]
	Rotavirus (dsRNA)	Favours host	[167]
	HSV-2 (dsDNA)	Favours host	[168]
	Vaccinia virus (dsDNA)	Harmful to host & favours virus Favours host	[169]
	MCMV (dsDNA)	Favours host	[170]
	VZV (dsDNA)	Favours host	[13]
	Chikungunya virus	Harmful to host	[15]
	Avian Influenza (H5N1) (ss(−) RNA)	Favours host	[17]
	HSV-1 (dsDNA)	Favours virus	[19]
	HIV (ss(+) RNA)		[23]
TLR7 (ssRNA)	West Nile virus (ss(+) RNA)	Favours host/ harmful to host	[171,172]
	HIV (ss(+) RNA)	Harmful to host	[173]
	Enterovirus 71 (ss(+) RNA)	Harmful to host	[33]
	VSV (ss(−) RNA)	Favours host	[35]
	Murine gammaherpesvirus 68 (dsDNA)	Favours host	[61]
TLR8 (ssRNA)	West Nile virus (ss(+) RNA)	Harmful to host	[25]
TLR9 (CpG DNA)	MCMV (dsDNA)	Favours host	[62,174]
	ECTV (dsDNA)	Favours host	[175]
	HSV (dsDNA)	Favours host	[44,176]
	Dengue virus (ss(+) RNA)	Favours host	[41]
	Murine gammaherpesvirus 68 (dsDNA)	Favours host	[61]
TLR2 (virus coat proteins)	RSV (ss(−) RNA)	Favours host	[177]
	HCV (ss(+) RNA)	Harmful to host	[178]
	HSV (dsDNA)	Favours host	[44,176]
	Pseudorabies virus (dsDNA)	Favours virus	[56]
TLR6 (virus coat proteins)	RSV (ss(−) RNA)	Favours host	[177]
	Dengue virus (ss(+) RNA)	Harmful to host	[52]
TLR4 (virus coat proteins)	RSV (ss(−) RNA)	Favours host	[177]
	VSV ss(−) RNA	Favours host	[179]
	Influenza (ss(−) RNA)	Harmful to host & favours virus	[180–182]

dependent cell death following IAV infection, that occurs in the SHARPIN KO mice, leads to increased susceptibility to IAV infection. Interestingly the absence of SHARPIN did not result in increased viral loads, and therefore the poorer outcome of these mice is explained by increased cell death. This shows that the balance of gene induction versus cell death following TLR3 activation, determines the host response to viral infections, and reminds us once again that cell death is a powerful driver of inflammation and immunopathology. Interestingly the TLR3 associated DISC, which can be activated using poly (I:C), contributes to the inflammatory phenotype characteristic of the SHARPIN KO, chronic proliferative dermatitis mice [18].

Further additional insights into TLR3 signalling pathways include reports that mammalian target of rapamycin (mTOR) complex 2 (mTORC2) can specify TLR3 subcellular localisation. In HSV infected neurons and astrocytes TLR3, located in perinuclear Rab7a+ lysosomes, forms a complex with mTORC2 and TRAF6, which activates chemokine gene expression. However, mTORC2 can relocate these TLR3 containing Rab7a+ lysosomes to the cell periphery. This allows TLR3 to form a complex with mTORC1 and TRAF3, which leads to the expression of type I IFNs. This shows that pathways activating mTORC2 could regulate TLR3 dependent responses. Interestingly an antibody agonist for TLR3 was shown to improve the antiviral response to HSV, suggesting that an augmented TLR3 response might provide a therapeutic strategy for the treatment of HSV encephalitis [19].

In vitro TLR3 senses and mediates antiviral responses to HCV, dengue virus (DV), and hantaan virus, while TLR3 agonists protects against a range of viruses including hepatitis B virus (HBV), IAV, human immunodeficiency virus (HIV) and coronaviruses [5]. These studies highlight the therapeutic potential of TLR3 ligands in enhancing antiviral immunity. Related to this, topical administration of aminoglycoside antibiotics such as neomycin enhanced host resistance to viral infections. This enhanced antiviral defence was due to TLR3, TRIF, IRF3/7 mediated ISG induction in XCR1+ DCs recruited to mucosal surfaces. This resulted in protection against both RNA and DNA viruses such as Zika virus (ZV), IAV and HSV infections, independent of the microbiota [20]. The raises the interesting possibility that aminoglycoside antibiotics might be useful as vaccine adjuvants or as antiviral treatments [21]. This study shows that activation of TLR3 clearly enhances antiviral immunity, however the specific RNA responsible for aminoglycoside activation of TLR3 remains unknown [20]. Other antibiotics possess similar antiviral properties. The macrolide antibiotic azithromycin has antiviral activity against a range of viruses such as Ebola virus, ZV, respiratory syncytial virus (RSV), influenza, enterovirus, and Rhinovirus (RV), and may be useful in the treatment of COVID-19, due to its ability to induce expression of type I and type III IFNs and the PRRs melanoma differentiation associated gene 5 (MDA5) and retinoic acid-inducible gene I (RIG-I) [22].

However, activating TLR3 can also have detrimental outcomes. Using a recently developed human microglia cell line, huglia, it was shown that stimulation of TLR3 can reactivate latent HIV in these cells, whereas stimulation of TLR3 does not have this effect in monocytes or T cells [23]. Interestingly reactivation of latent HIV in these microglial cells appears to depend on IRF3 activation, which can be blocked by bufalin, an IRF3 inhibitor. Although not all studies corroborate these findings the authors emphasise that studies need to be carefully designed to distinguish between the effect of TLR3 stimulation on pre-integrated HIV and post-integration reactivation of the HIV proviral DNA, which these authors examined [23]. In addition, HIV latency is maintained by chromatin restrictions, and treatment of these cells with a novel histone deacetylase inhibitor, HDACi4b, resulted in HIV reactivation [23]. Since reversal of HIV latency requires both the activation of transcription factors and de-repression of chromatin restrictions, it is likely that TLR3 stimulation impacts both in a manner that is cell specific. These findings also highlight the danger of therapeutic TLR3 stimulation which may lead to reactivation of latent HIV in affected individuals.

2.3. TLR7 & TLR8

TLR7 and TLR8 are encoded on the same genetic loci on the X chromosome (https://www.ncbi.nlm.nih.gov/omim) and share 43% amino acids identity (https://blast.ncbi.nlm.nih.gov/). In addition, TLR7 can escape X-linked inactivation leading to increased expression of TLR7 in females, which might explain the greater prevalence of lupus among females, since TLR7-stimulated IFN is a likely driver of lupus, and may also explain why women have a stronger antiviral immunity [24]. In humans endosomal TLR7 and TLR8 are functionally autologous receptors for viral ssRNA and following their activation engage the myeloid differentiation primary response 88 (MyD88) and IFR7 pathways leading to the production of IFNs and proinflammatory cytokines [5]. In humans TLR8 can detect ssRNA however murine TLR8 lacks this ability, possibility due to a 5 amino acids deletion in the receptor ectodomain [5]. Although TLR7 and TLR8 sense ssRNA, functional differences exist between the two as they have different expression profiles

and activate different cellular programs. TLR7 is expressed mainly on pDCs, while TLR8 is expressed mainly on myeloid cells such as monocytes/macrophages and myeloid DCs (mDCs) [5]. In pDCs TLR7 activation results in IFN production, whereas in myeloid cells TLR8 activation results in pro-inflammatory cytokine production [5]. TLR7/8 sense the presence of ssRNA viruses such as influenza, HIV, vesicular stomatitis virus (VSV), Sendai virus (SV), CBV, coronaviruses such as mouse hepatitis virus and SARS-CoV, in addition to a number of flaviviruses such as HCV, DV, and WNV [5]. While most of the antiviral roles of TLR7 can be attributed to IFN production, other IFN independent antiviral roles for TLR7 have also been described such as IL-23 dependent macrophage cell homing to defend against WNV and MyD88 dependent antibody production, required to clear infection with murine retroviruses [5].

There is considerable interest in how precisely the RNA sensors TLR7 and TLR8 located in cellular endosomes access and respond to viral RNA during viral infection. Three different pathways have been described. The first of these is the endogenous pathway, where endocytosis and protease degradation of virions allows endosomal TLR7/8 activation, utilised for IAV and VSV detection [5]. The second is autophagy, which delivers cytoplasmic viral RNA to endolysomes, utilised for SV, VSV and HIV detection [5]. Interestingly TLR8 in human macrophages defends against HIV by the induction of autophagy [5]. The final mechanism of endosomal TLR engagement involves cell to cell contacts, allowing the transfer of viral RNA containing exosomes. This mechanism operates for HCV, Venezuelan equine encephalitis virus and HIV detection [5]. Interestingly these mechanisms of endosomal TLR engagement do not require viral replication and resemble mechanisms known to activate TLR9 [5].

Several studies have indicated that TLR8 may have a negative role in innate viral sensing. Overexpression of murine TLR8 does not activate IRF3 or IFNα and TLR8 KO mice develop lupus like autoimmunity, due to enhanced TLR7 activity. In addition, TLR8 negatively regulates expression of TLR7 in multiple tissues [25] and TLR8 interacts with suppressor of cytokine signaling protein 1 (SOCS-1) to blunt the TLR7 antiviral program, consistent with the observation that TLR8 KO mice are resistant to infection with WNV [25]. These studies show that TLR8 inhibits the antiviral functions of TLR7, suggesting that TLR8 inhibition might be an effective treatment strategy in WNV infection to boost the anti-viral programme [25].

The expression patterns of TLR7 and TLR8 together with other innate receptors can also impact the outcome to viral infections. During HCV infections, HCV virions are taken up into macrophages in a manner dependent upon the C type lectin receptor DC-specific intracellular adhesion molecule grabbing nonintegrin (DC-SIGN). Following virus uptake, TLR7&8 expressed in macrophages leads to induction of pro-inflammatory cytokines such as tumor necrosis factor α (TNFα) and IL-6, but importantly not the antiviral type I IFNs. In contrast, monocytes, mDCs and pDCs, which are all capable of producing TLR7/8 dependent IFNs, do not express DC-SIGN and HCV virions are not taken up into these cells, which explain the lack of IFN response in HCV infected individuals [26]. Therefore, vaccine strategies against HCV should include methods to activate a TLR7/8 response in monocytes and DCs [26].

The advent of antiretroviral therapy (ART) has been transformative for individuals suffering with HIV. However, despite such advances, these treatments do not constitute a cure and cessations of treatments results in re-emergence of the virus. Therefore, considerable efforts have been directed at removing the latent viral reservoir in HIV infected patients, including efforts to manipulate innate immunity. Stimulation of TLR7 following vaccination with a combination of recombinant adenovirus serotype 26 prime and modified vaccinia Ankara (MVA) boost, expressing gag–pol–env, reduced simian immunodeficiency virus (SIV) viral loads in monkeys, indicating that vaccination followed by innate immune stimulation via TLR7 might lead to a functional cure of HIV infections [27]. In a follow-up study by the same investigators, a combination of a TLR7 agonist vesatolimod (GS-9620) and a broadly neutralising antibody to ENV, PGT121, delayed re-emergence of the virus upon discontinuation of ART [28]. Interestingly in a subset of monkeys that did not show rebound of the virus, depletion of CD8+ T cells did not permit a resurgence of the virus [28–30]. These reports clearly indicate the power of innate immune signalling and TLR7 activation to potentially remove the latent HIV reservoir.

In the search for a HIV cure strategy, the impact of TLR8 manipulation has also been explored. Stimulation of TLR8 resulted in cytokine secretion from CD4+ T cells, Th1 and Th17 cell differentiation, increased viral replication and a reversal of latency in patient derived T cells, resulting in viral dissemination within lymph nodes and low grade inflammation, characteristic of HIV infections [31]. The dramatic difference in outcome of TLR7 and TLR8 stimulation in HIV can be explained by the fact that TLR7 stimulation on pDCs triggers the production of antiviral type I IFNs, while activation of TLR8 on mDCs, and macrophages produces no such antiviral response [31]. A HIV cure strategy which has been proposed, involves the reversal of latency followed by intensive ART therapy. This “shock and kill” strategy might first involve stimulation of TLR8 [31]. Despite these intriguing studies, the only possibility of curing HIV infection ultimately lies with the elimination of the host cells that harbour integrated HIV proviral DNA. Another example of a viral infection improved by TLR7 stimulation is patients chronically infected with HBV, where TLR7 stimulation with GS-9620 did not alter viral levels but enhanced NK and T cell activity [32].

However, stimulation of TLR7 is not always beneficial during viral infections. The neurotropic human enterovirus 71 (EV71) induces TLR7 expression and drives TLR7 dependent neurodegeneration via IL-6 production and TLR7 dependent astrocyte apoptosis [33]. IAV infection is associated with increased risk of heart attack, however the underlying mechanism remained obscure. New advances revealed that TLR7 senses influenza virus on platelets which leads to the release of the complement component C3, DNA release from neutrophils, platelet adhesion, increasing thrombotic events [34]. Further, while TLR7 has been reported as the main VSV sensor and prevents VSV neuroinvasion, paradoxically TLR7 signalling supports infection of $CD169^{+}$ subcapsular sinus macrophages in the lymph node early in VSV infection [35]. This demonstrates the complex roles of PRRs in determining the outcome of viral infections, which depends on the anatomical site of expression.

In human blood monocytes TLR7 and TLR8 have differential activities in response to a variety of ssRNA viruses, such as CBV, EMCV, IAV, measles virus, SV, and VSV. TLR7 enhances Th17 polarizing cytokines via mitogen-activated protein (MAP) kinase activation and Fos-like 1 (FosL1), while TLR8 induced the expression of Th1 polarizing cytokines via NF-κB. Furthermore, TLR7 simulated calcium mobilisation inhibited the type I IFN response. While the mechanism is unknown, other reports confirm that calcium mobilisation inhibits type I IFN production [36]. Interestingly in this study, the human blood monocytes can mount an IFN response to viruses downstream of TLR8, which contrasts with other studies of myeloid cells described above. [36].

There are several viral infections that are ameliorated by TLR7/8 agonists, including the TLR7 agonist imiquimod, which reduces vaginal lesions associated with HSV-2 [37] and genital warts caused by human papilloma virus (HPV) [5]. In clinical trials an alternative TLR7 agonist, ANA-773, reduced serum HCV RNA levels in HCV patients. This compound triggers IFN production and activates NK cells [5]. Finally, stimulation with the TLR7/8 agonist R848 blocks replication of ZV due to the induction of the ISG viperin, suggesting that TLR7/8 stimulation might be useful in the treatment of this infection [38]. It will be interesting to determine if imiquimod or other TLR7 agonists show efficacy against SARS-CoV-2, especially since TLR7 can sense the closely related SARS-CoV virus in pDCs [5].

2.4. TLR9

The presence of DNA viruses within cells is detected by TLR9 located in endosomes. TLR9 detects hypomethylated CpG DNA, indicative of DNA viruses such as HSV, MCMV, HCMV, pox viruses and HIV, with TLR9 providing protective immunity to most of these virus infections [39,40]. More recent investigations into TLR9 reveal new connections to other innate signalling pathways in response to several viral infections.

DV is one of the most common mosquito born viruses with 390 million annual infections worldwide, mainly in developing countries. In addition to the detection of DNA viruses, it was recently shown that TLR9 also has a role in the detection of RNA viruses such as DV. This is due to the release of mitochondrial DNA (mtDNA) from infected human DCs [41]. The release of mtDNA from DV infected cells activates TLR9 and cyclic GMP-AMP synthase (cGAS) (see Section 6.2), where both DNA sensors have an equal role in the induction of type I IFNs. Since treatment with MCC950, a specific inhibitor of the NLR Family Pyrin Domain Containing 3 (NLRP3) inflammasome (see Section 5.1), prevented mitochondrial reactive oxygen species (ROS) production and mtDNA release, this places the NLRP3 inflammasome as a proximal sensor of DV infection resulting in mitochondrial ROS production, mtDNA release and activation of the DNA sensors cGAS and TLR9. Surprisingly, there was no alteration in mitochondrial membrane potential or indeed cell viability in DV infected human DCs despite the release of mtDNA, demonstrating that mtDNA release is a mechanism to activate innate immunity in live virus infected cells. Furthermore, both oxidised and non-oxidised forms of mtDNA are released following DV infection, where the oxidised mtDNA is a more potent activator of TLR9 [41]. Hence DV infection elicits the activation of three different classes of innate immune receptors, TLRs, DNA sensors, and the NLRP3 inflammasome resulting in type I IFN production. One question that remains outstanding and not addressed in the study, is how mtDNA released into the cytosol activates TLR9 present in endosomes.

Adeno-associated virus (AAV) are commonly used as vectors in gene therapy, however these viruses are often associated with unwanted CD8+ T cell activation. To explore this mechanism further it was shown that crosspriming of CD8+ T cells in response to AAV introduction was dependent upon TLR9 sensing of the viral genome in pDCs. This process was also dependent upon type I IFN and inhibition of either TLR9 or IFN prevented this undesired activation of CD8+ T cells [42].

There is a long-established role for TLR2 in partnering with TLR9 in sensing HSV [43]. More recent findings show that TLR2 and TLR9 defend against HSV by the recruitment of monocytes and NK cells into the vaginal mucosa, preventing viral dissemination into the CNS [44]. However, activation of TLR2 and TLR9 is not always associated with beneficial responses to HSV infections. Suppression of TLR2/TLR9 signalling with chlorogenic acid improves inflammation associated with HSV [45] and vitamin D was shown to downregulate TLR2 and reduced viral titres of HSV in a Hela cell culture infection model [46]. In response to enteric HSV infection, TLR2, in infected neurons, drives neuromuscular dysfunction by CCL2 dependent recruitment of $CD11b^{+}$ macrophages, indicating that TLR2 inhibitors might alleviate neuromuscular dysfunction during enteric HSV infection [47]. These studies show that TLR2 and TLR9 can exert both protective and damaging responses to HSV infections. Immunopathology due to CBV infection also involves TLR9. Myocarditis caused by CBV infection triggers activation of the TLR9-IRF5 pathway and production of IL-6 and TNFα, which is associated with the severity of cardiac tissue damage [48]. Stimulation of PRRs, including TLRs, may contribute to improved vaccine design. During infection with HCV, activation of TLR7 and TLR9 augment the antiviral activity of pDCs, increasing antigen presentation and expression of lymph node homing markers. This shows that TLR7 and TLR9 agonists might be useful in the design of a successful HCV vaccine [49]. Given current efforts to develop a SARS-CoV-2 vaccine, it will be interesting to see if TLR or other PRR ligands improve responses to these vaccines.

2.5. TLR4, TLR2&TLR6

Although viruses are mainly detected by nucleic acid sensors including the nucleic acid sensing TLRs, surface expressed TLRs also have a role in cellular responses to virus coat proteins [5]. This can be viewed as viral detection, or viral subversion, depending if the outcome favours the virus or the host. An example of such viral subversion is the activation of TLR4 by mouse mammary tumour virus (MMTV) which results in the production of IL-10 to drive virus replication and also upregulates the expression of the MMTV entry receptor CD71 [50,51].

In addition to the innate mechanisms to sense DV described above, DV is also sensed by TLR2 and TLR6 which detect the virus via NS1. Interestingly survival is enhanced in TLR6 KO mice, suggesting that the NS1-TLR6 axis drives a pathological response associated with DV infection [52]. Other studies indicate that DV activates platelets via CLEC2 resulting in the generation of DV containing microvesicles and exosomes derived from platelets which are sensed by CLEC5A and TLR2 on macrophages and neutrophils, resulting in the production of pro-inflammatory cytokines and neutrophil extracellular traps formation. Inhibition of CLEC5A and TLR2 reduced DV associated inflammation and enhanced survival rates [53].

DV has other mechanisms to drive pathology within the human host. DV secreted NS1 drives inflammatory cytokine production which disrupts the endothelial cell monolayer causing vascular leakage resulting in haemorrhagic fever and shock. Therefore, the cellular pathways activated by NS1 are of interest from a therapeutic viewpoint. However, the TLR responsible for the detection of NS1 is controversial. Recently it was reported that TLR4 is the sensor for NS1 of DV and not the TLR2/6 heterodimer as previously reported [54]. This may be explained since the commercially available NS1 used in other studies does not fold correctly and is contaminated with TLR2/TLR6 ligands, explaining earlier reports of TLR2/6 as the DV NS1 sensor. Thus, TLR4 inhibition might be a useful strategy in the treatment of DV infection [54,55]. These studies reveal important new insights into sensing and immunopathogenesis of this infection.

The alphaherpesvirus pseudorabies virus (PRV) triggers severe and lethal neuropathy in mice. This infection starts in the epithelia and leads to invasion of the peripheral nervous system. To uncover the underlying mechanism a mouse footpad model of infection was used, and it was found that animals lacking TLR2 were asymptomatic, indicating that TLR2 may be a receptor for PRV on dorsal root ganglion neurons to facilitate virus dissemination [56]. These observations are reminiscent of early work on HSV, which showed that TLR2 mediates lethal inflammation in response to HSV [56,57]. Activation of TLR2 by PRV drives production of both IL-6 and granulocyte colony-stimulating factor (G-CSF) leading to a pathological neuroinflammatory response. Indeed, immunopathogenesis by many viral infections is driven by production of IL-6 including SARS-CoV-2. The proposal that TLR2 is a sensor for PRV by binding to the gB protein is supported by earlier studies on HSV [56]. Finally, PRV is proposed to activate TLR2 to downregulate type I IFN production by TLR3 and TLR9 expressed on DCs [56]. Other studies have also shown that TLR2 can modulate the IFN response. TLR2 was found to reduce the IFN response following RV infection by inhibiting STAT1 and STAT2 phosphorylation, which was associated with TLR2 dependent SIRT-1 expression. Interestingly this pathway is dysregulated in chronic obstructive pulmonary disease (COPD) patients resulting in heightened IFN production [58]. Expression of TLR2 also impacts treatment outcomes in individuals treated for HBV infection. Here, higher expression of TLR2 in peripheral blood mononuclear cell (PBMCs) and lower levels of serum soluble TLR2 are associated with a complete response to telbivudine, an antiviral nucleoside analogue used in the treatment of HBV infections [59].

Other recent insights into TLR sensing of viral infections include reports that Norovirus, the causative agent of major gastroenteritis outbreaks, are sensed by TLR2 and TLR5 [60]. Both TLR7 and TLR9 cooperate to detect murine gammaherpesvirus 68 infection in pDCs

leading to the production of IFNα [61]. Immune defence against MCMV is dependent on TLR9/MyD88 expression on conventional CD11c+ DCs, which leads to increased MCMV clearance by enhanced expression of CD69 on NK cells and IFNγ production [62]. Finally, DHX9, once thought to be a DNA sensor, has a key role in IL-6 production following TLR2 and TLR8 stimulation, which is blocked by the poxviral protein E3 by directly interacting with DHX9 [63].

3. RLRs

RLRs are a family of cytoplasmic RNA receptors that are key players in the detection of viral RNA genomes and RNA replicative intermediates (Fig. 1). This family of receptors includes RIG-I, MDA5 and laboratory of Genetics and Physiology 2 (LGP2). Structurally both RIG-I and MDA5 consists of two caspase activation and recruitment domain (CARD) domains, a helicase domain, and a C-terminal domain. LGP2 differs from the other two as it lacks the CARD domains. RIG-I and MDA5 signal via the mitochondrial anchored adaptor mitochondrial antiviral-signaling protein (MAVS) that signals via TBK1 and IKKε to activate NF-κB and IRF family members, resulting in the expression of type I IFNs and pro-inflammatory cytokines [64]. Great progress has been made in the last decade to define the exact viral RNA ligand responsible for RIG-I activation. RIG-I is activated by uncapped 5′-triphosphate RNA, with blunt ends being the most potent activator, uncapped 5′- diphosphate RNA and RNA with a 5′ - terminal nucleotide unmethylated at its 2′- O position [64]. Less is known about the exact RNA ligand for MDA5, which is activated by an accumulation of dsRNA in infected cells, but MDA5 can bind one strand of viral dsRNA [64]. Several studies have shown that LGP2 can have positive or negative effects on RIG-I and MDA5 signalling [64]. LGP2 inhibits RIG-I by sequestering viral RNA, blocks RIG-I binding to MAVS and prevents RIG-I ubiquitination by tripartite motif (TRIM)25 [64]. Alternatively, LGP2 enhances the MDA5 response by promoting MDA5 oligomerisation on dsRNA [64]. Surprisingly LGP2 inhibits the antiviral RNA interference pathway in mammals, by blocking DICER processing of long dsRNA [64]. Intriguingly this antiviral RNA interference system is active in mammalian cells in situations where the IFN system cannot operate [64]. The RLRs are capable of recognising viruses across all seven viral genome groups according to the Baltimore classification, with RIG-I and MDA5 both equally capable of virus recognition, with just occasional exceptions where either RIG-I or MDA5 individually mediate virus detection [64]. Here we will describe the recent advances in viral sensing by RLRs.

3.1. RIG-I & RNA polymerase III

The outcome of influenza virus infection in a host depends on several factors such as the host immune response, viral and bacterial co-infection [65]. The dysregulated innate immune response responsible for the “cytokine storm” characteristic of the 1918 H1N1 pandemic or the highly pathogenic avian influenza virus H5N1 remains to be fully understood. To further explore influenza virus sensing mechanism, an infection model of ferret lungs was used and it was found that short aberrant RNAs, known as mini viral RNAs (mvRNAs), generated by the viral RNA polymerase during replication of the viral RNA genome, are sensed by RIG-I resulting in IFNβ production. The authors show that these mvRNAs produce a higher level of IFN compared with other RNA species such as full-length segment 4 or 5 from the virus. In addition the investigators show that mvRNAs longer than 47 and shorter than 125 nt are capable of RIG-I activation, and these mvRNAs must be triphosphorylated (a key feature of other RIG-I agonists) in order to trigger the ATPase activity of RIG-I, essential for receptor activation after RNA binding. mvRNA production was associated with increased cytokine production and cell death, indicating that these mvRNAs are an important driver of influenza virus induced virulence. The authors propose that mvRNAs are the main RIG-I agonist during influenza infection [65]. Given the ongoing search for more effective vaccine adjuvants mvRNAs might serve as interesting candidates. Other possible vaccine adjuvants or antiviral treatments may include a small RNA hairpin based on an RNA scaffold 3p10L of 25 nucleotides in length, which was reported to be a very potent activator of RIG-I and IFN production. Treatment of cells with this RIG-I agonist protected cells from infection with DV [66].

During DV infection RNAs that bind RIG-I are immunostimulatory, whereas those that bind MDA5 fail to activate an immune response. To understand this phenomenon next generation sequencing was performed which revealed that RIG-I, but not MDA5, binds the 5′ region of the DV genome. *In vitro* production of the DV genome fragments confirmed that the 5′ triphosphorylated end of the DV genome is the actual RIG-I ligand. In addition, RIG-I also detects the 5′ region of the ZV genome, suggesting a common mechanism of flavivirus detection by RIG-I [67]. Although RIG-I is a sensor of RNA viruses it is known that RIG-I can respond to infections with DNA viruses. RNA polymerase III (Pol III) has previously been associated with herpesviruses sensing by the transcription of A-T rich DNA into RNA ligands detected by RIG-I [68], therefore, Pol III and RIG-I collaborate to form a unique antiviral defence system against herpesviruses. To identify physiological RNA species generated during infection with herpesviruses that are sensed by RIG-I, RNAseq was performed in HSV infected cells. It was found that the host 5S ribosomal RNA pseudogene 141 (RNA5SP141) was bound to RIG-I during infection with HSV. Interestingly mammalian 5S ribosomal RNA pseudogenes, including RNA5SP14, are transcribed by Pol III, clearly highlighting the antiviral role for Pol III [68]. HSV infection induced the relocalisation of RNA5SP141 from the nucleus to the cytoplasm. Virus induced inhibition of protein synthesis reduced the levels of RNA5SP141 protein binding partners, allowing RIG-I binding to RNA5SP141 resulting in IFN production. Silencing of RNA5SP141 reduced the antiviral response to both DNA and RNA viruses, HSV, Epstein-Barr virus (EBV) and IAV. This study highlights the antiviral role of an endogenous RIG-I ligand, mobilised upon translational shutdown during viral infections, and is a good example of “altered self” activating innate immunity [68,69]. Furthermore, this modality of antiviral defence shows how cytoplasmic RNA sensors are deployed to defend against nuclear DNA viruses [68]. Further confirmation that Pol III sensing may be important in generating RIG-I ligands from AT-rich viral DNA has recently been provided, since humans with variations in Pol III components were more susceptible to VZV infections [70,71].

Recent RLR studies have also shed light on why exposure to cooler temperatures increase susceptibility to common cold viruses [72]. RV that infects the nasal cavity and causes the common cold in humans exhibits increased replication at 33–35 °C compared with core body temperature of 37 °C. To gain a greater insight into these observations, antiviral transcriptional responses were compared at 33 °C and 37 °C. Following RV infection, the expression of type I, type III IFN genes and ISGs was much stronger at 37 °C compared with 33 °C. The ATPase activity of recombinant RIG-I and MDA5 in the presence of poly (I:C) were also analysed, which is optimal at 37 °C and reduced at 33 °C, providing one rationale as to why innate antiviral responses are optimal at 37 °C and reduced at lower temperatures.

There are also fresh mechanistic insights into the activation and regulation of RLR signalling. During viral infections the E3 ubiquitin ligase FBXW7 translocates from the nucleus to the cytoplasm to stabilise RIG-I, by shutting off the SHP2 and the E3 ligase cCbl mediated destruction of RIG-I. Consistent with this FBXW7 deficient macrophages showed lower RIG-I protein levels and reduced type I IFN production. Mice lacking FBXW7 displayed reduced defence against VSV and IAV infections. Interestingly PBMCs from children infected with RSV show reduced expression of FBXW7, a means perhaps of virus innate immune evasion [73]. RLR signalling is also enhanced by the zinc-finger protein ZCCHC3. The functional importance of this was highlighted where mice lacking ZCCHC3 were more prone to infection with RNA viruses such as VSV and EMCV. Functionally ZCCHC3 enhances RLR function in 2 ways,

firstly it binds to dsRNA and enhances the binding of RIG-I and MDA5 to dsRNA. Secondly ZCCHC3 recruits the E3 ubiquitin ligase TRIM25 to promote the K63-linked polyubiquitination of RIG-I and MDA5 to activate downstream signalling pathways [74]. Further mechanistic insights into MAVS dependent RIG-I signalling have also been revealed. Using RNAi and yeast two-hybrid screens it was shown that a mitochondrial complex consisting of TRIM14, Werner helicase-interacting protein 1 (WHIP) and protein phosphatase 6 catalytic subunit (PPP6C) form a signalling platform with MAVS and RIG-I. Following viral infection, TRIM14 anchors the complex to MAVS, WHIP binds to RIG-I via ubiquitin chains at lysine 164, the WHIP ATPase domain stabilises the RIG-I-dsRNA interaction and the phosphatase PPP6C dephosphorylates RIG-I. Together this molecular arrangement triggers activation of RIG-I dependent signalling [75]. NLRP12 acts as a negative regulator of RIG-I signalling in response to RNA viruses, by regulating the association of RIG-I with its adaptor protein MAVS. In addition, NLRP12 regulates the ubiquitination pattern of RIG-I, where it inhibits TRIM25 mediated lysine 63-linked ubiquitination, but increases lysine 48 linked ubiquitination by RNF125, resulting in RIG-I degradation. Mice lacking NLRP12 in the myeloid compartment have increased IFN and TNF responses and display greater resistance to infection with VSV [76].

The RIG-I pathway which is a pivotal antiviral defence mechanism is a frequent target of evasion strategies by many viruses, including coronaviruses. The SARS-CoV and Middle East respiratory syndrome coronavirus (MERS)-CoV nucleocapsid protein (N protein) acts as an inhibitor of type I IFN production by blocking the interaction between TRIM25 and RIG-I and thus inhibiting the ubiquitination and activation of RIG-I by TRIM25 [77,78]. Thus, coronavirus N protein is a viral immune evasion factor. These findings may have relevance for the current COVID-19 pandemic, where SARS-CoV-2 potently blunts interferon production, possibly by utilising the virus N protein [79].

A recent report has challenged the accepted view that MAVS dependent RIG-I signalling occurs at the mitochondria. Using high resolution biochemical fractionation and electron microscopy it was found that components of the RLR pathway are in microsomes and not the mitochondrial fraction in resting cells. LGP2 was shown to interact with MAVS in microsomes and inhibit the RIG-I/MAVS interaction. Upon dsRNA treatment or infection with RNA viruses, LGP2 disassociates from MAVS and assumes a mitochondrial location which temporally correlates with IRF3 activation. Activation of IRF3 occurs on endoplasmic reticulum (ER)-derived membranes and not at the mitochondria as generally understood. This study shows that activation of the RLR pathway is mediated from ER derived membranes [80].

3.2. MDA5

The importance of MDA5 as a defence mechanism against viruses in humans was recently highlighted in a child suffering with life threatening recurrent respiratory tract infections with viruses such as RV, IAV, and RSV. It was found that there was a homozygous missense mutation in IFIH1, the gene encoding MDA5 [81]. This mutant did not recognise poly(I:C) and failed to activate a reporter gene the linked to the promoters of IFNβ1, ISRE or NF-κB [81]. In multiple cell models MDA5 inhibited the replication of enteric viruses such as HEV, human norovirus and rotavirus. Interestingly this antiviral defence mechanism is mediated by the activation of a non-canonical IFN pathway that is partly dependent upon the ability of MDA5 to induce the expression and phosphorylation of STAT1 [82]. MDA5 also confers antiviral defence in liver hepatocytes in response to infection with hepatitis D virus, resulting in the production of IFN-β and IFN-λ [83]. An interesting alternative role for MDA5 has also been reported, where MDA5 is crucial to contain infection with a mouse coronavirus and infection associated proinflammatory responses, suggestive of an immune-regulatory role for MDA5 [84].

There are also new insights into the mechanism of MDA5 activation. MDA5 also interacts with the E3 ligase TRIM65 and catalyses K63-linked ubiquitination of MDA5 at lysine 743, resulting in MDA5 oligomerization and activation. Mice deficient for TRIM65 are more susceptible to infection with EMCV and fail to produce type I IFN. These observations indicate that MDA5 is an important sensor for EMCV and MDA5 requires TRIM65 for activation [85]. Further insights into MDA5 activation include observations that an RNA helicase DHX29 was identified as a co-sensor of RNA with MDA5 for antiviral defence against EMCV. Interestingly DHX29 specifically enhances MDA5-dsRNA binding, but not RIG-I-dsRNA binding [86]. The cellular abundance of RIG-I and MDA5 are regulated by sumoylation. RIG-I and MDA5 sumoylation by the SUMO E3 ligase TRIM38 reduces the K48-linked polyubiquitination and degradation of both proteins [87].

The RLR pathway is subject to viral evasion by many different viruses that utilise different evasion strategies to switch off these antiviral defence mechanisms [88]. A further evasion strategy that has been recently reported is from ZV, where 14-3-3 protein family members that translocate RIG-I and MDA5 to the mitochondria upon viral infection are targeted for inhibition by the viral protein NS3 [89] (Table 2).

4. C-type lectin-like receptors

CLRs are mainly expressed by myeloid cells such as monocytes, macrophages and DCs. This large family are divided into 17 groups and consist of transmembrane and soluble receptors which recognise carbohydrates *via* their carbohydrate recognition domain (CRD). These PRRs recognise pathogen-associated molecular patterns (PAMPs) present on bacteria, fungi, parasites, and viruses (Fig. 1). CLRs take-up pathogens into myeloid cells by endocytosis, and leads to increased expression of inflammatory cytokines, chemokines, activation of inflammasomes and the presentation of processed antigens to T cells. Together with other PRR systems, these responses help shape the overall immune response [90]. Many viruses have evolved to encode glycosylated proteins to permit CLR binding and facilitate cell entry. Furthermore glycosylation of viral proteins is also important for their stability and antigenicity [90]. For example the glycoprotein of IAV binds to several CLRs such as langerin, mannose receptor, myeloid DAP-12-associating lectin (MDL-1 also known as CLEC5A) and macrophage galactose-type lectin (MGL) [91–93] as a means of cell entry. Interestingly the S protein of SARS-CoV, which is a glycoprotein, is recognized by both DC-SIGN and L-SIGN receptors which facilitates viral transmission in susceptible cells such as DCs [94].

5. Viruses and inflammasomes

Inflammasomes are cytosolic multiprotein complexes formed during cellular stress or infection that activates the inflammatory caspases, leading to the production of mature IL-1β and IL-18, and to pyroptotic cell death [95]. These multiprotein platforms generally consist of an NLR sensor, the adaptor apoptosis-associated speck-like protein containing a CARD (ASC) and caspase 1, where activation triggers the protease activity of caspase 1, that also results in the cleavage of gasdermin D (GSDMD) [95]. The cleaved N terminal domains of GSDMD form pores in the plasma membrane to permit IL-1β secretion and also mediates pyroptotic cell death [95]. Five canonical inflammasomes exits, NLRP3, NLRP1, NLRC4, PYRIN and the absent in melanoma 2 (AIM2) inflammasome. An additional or non-canonical inflammasome known as caspase 11 (caspase 4/5 in humans) also exists and detects the presence of cytosolic LPS [95]. Activation of inflammasomes is a two-step process. The first “priming“ step or signal 1 is required to induce the transcription of pro-IL-1β and NLRP3, usually involving TLR stimulation and requires NF-κB activation. The second step, or signal 2, directly activates the sensory protein and is different for all five inflammasomes. NLRC4 is activated by bacterial flagellin, while AIM2 is activated by cytosolic DNA. In contrast, activation of the NLRP3 inflammasome is associated with a diverse range of PAMPs, DAMPs and particulate matter, many of which activate the NLRP3 inflammasome by

Table 2
Summary of PRRs, their agonists, representative viral inhibitors, and their mechanism of immune evasion.

PRR	Agonist	Agonist origin (Virus/ host)	Viral inhibitors	Mechanism of evasion	References
TLR3	dsRNA	Viral/host	HSV US3	Inhibits TLR3 expression Inhibits TRIF	[7]
			VV A46R	Degrades TRIF	[6]
			HCV NS3–4A		[6]
TLR7	ssRNA	Viral/host	HSV ICP0	Inhibits MyD88	[7]
TLR8	ssRNA	Viral/host	HSV ICP0	Inhibits MyD88	[7]
TLR9	Hypomethylated CpG DNA	Viral/host	HSV ICP0	Inhibits MyD88	[7]
			VV A46R	Inhibits MyD88	[6]
			HCV NS5A	Inhibits MyD88	[6]
TLR2	Virus coat proteins	Viral	VV A46R	Inhibits MyD88	[6]
			VV A46R	Inhibits Mal	[6]
			HSV ICP0	Inhibits MyD88	[7]
			HSV ICP0	Inhibits Mal	[7]
TLR6	Virus coat proteins	Viral	VV A46R	Inhibits MyD88	[6]
			HSV ICP0	Inhibits MyD88	[7]
TLR4	Virus coat proteins	Viral	VV A46R	Inhibits MyD88	[6]
			VV A46R	Inhibits Mal	[6]
			VV A46R	Inhibits TRIF	[6]
			VV A46R	Inhibits TRAM	[6]
			HSV ICP0	Inhibits MyD88	[7]
			HSV ICP0	Inhibits Mal	[7]
RIG-I	dsRNA	Viral/host	ZV NS3	Inhibits 14-3-3ε	[89]
MDA5	dsRNA	Viral/host	ZV NS3	Inhibits 14-3-3η	[89]
Langerin	Glycosylated proteins	Viral	IAV Hemagglutinin glycoprotein	Langerin used as IAV cellular entry receptor	[91]
MR	Glycosylated proteins	Viral	IAV glycoproteins	MR used as IAV cellular entry receptor	[92]
MDL-1 (CLEC5-A)	Glycosylated proteins	Viral	IAV glycoproteins	MDL-1 used as IAV cellular entry receptor	[93]
MGL	Glycosylated proteins	Viral	IAV glycoproteins	MGL used as IAV cellular entry receptor	[92]
DC-SIGN	Glycosylated proteins	Viral	S protein SARS-CoV-2	DC-SIGN used as cellular entry receptor for SARS-CoV-2	[94]
L-SIGN	Glycosylated proteins	Viral	S protein SARS-CoV-2	L-SIGN used as cellular entry receptor for SARS-CoV-2	[94]
NLRP3	dsRNA, ATP	Viral dsRNA Host ATP	IAV NS1	Inhibits NLRP3	[101]
AIM2	dsDNA	Viral & host	HCMV pUL83	Inhibits AIM2	[128]
			HSV VP22	Inhibits AIM2	[129]
IFI16	dsDNA	Viral & host	HSV ICP0	Degrades IFI16	[146]
			HCMV pUL83	Inhibits IFI16	[147]
			HPV E7	Degrades IFI16	[148]
cGAS	dsDNA	Viral & host	HIV capsid	Inhibits IFI16	[88]
			KSHV ORF52	Inhibits IFI16	[88]

a common mechanism of K+ efflux [95].

Activation of inflammasomes is consistent with the known role of IL-1 in the response to viral infections. In human epithelial cells infected with RV, blockade of IL-1R1 prevented up-regulation of pro-inflammatory cytokines such as IL-6 and IL-8 and neutrophil chemoattractants such as CXCL2 but had no effect on T-cell chemoattractants, IFN production, ISGs expression or virus replication. This indicates that IL-1 has a role in the recruitment and activation of immune cells but has no direct anti-viral activity [96]. In agreement with this conclusion, the infection of IL-1R1$^{-/-}$ mice with IAV displayed reduced lung inflammatory pathology, but enhanced mortality induced by the virus. IL-1 was required to induce effective viral clearance by the recruitment of neutrophils and CD4^{+} T cells and the enhancement of the antiviral IgM antibody response by B cells [97].

Il-18, which is another product of inflammasome activation, also has a role in the response to virus infections. Since IL-18 is a potent inducer of IFN-γ and activates CD8+ T cells, suggests a key role for IL-18 in viral clearance and as expected, IL-18 is protective against several viruses such as Ectromelia virus, HSV and rotavirus [98]. However, the relationship between IL-18 and respiratory viruses differs to that of IL-1. Deletion of IL-18 enhances viral clearance of IAV from infected lungs where there is reduced viral loads in the IL-18 KO mice due to increased CD4+ T-cell activation [99].

In general activation of inflammasomes in response to viral infections can either favour the host or the virus. Inflammasome mediated pyroptosis can expel viruses from their replicative cellular niche and allow viral elimination by other arms of the immune system. By contrast there are examples of viral infections where inflammasome activation drives immunopathology and is therefore detrimental to the host. Since the NLRP3 and AIM2 inflammasomes are mainly activated during viral infections, we will limit our discussion to these and we will also cover the recently described role of MxA as it has recently been reported to form a unique inflammasome and explore their activity during viral infections (Fig. 2).

5.1. The NLRP3 inflammasome

The NLRP3 inflammasome has a protective role in immunity against IAV infection since mice lacking NLRP3, ASC or caspase 1 were shown to be hypersusceptible to IAV infection [100]. The importance of the NLRP3 inflammasomes in providing antiviral defence against IAV is highlighted by the fact that the NS1 protein from IAV binds to and inhibits NLRP3 [101]. Viral RNAs from IAV activates NLRP3 and the NLRP3 inflammasome is responsible for the subsequent adaptive immune response and initiates the healing process following infection with IAV [100]. Other sensors that are also important during infection with IAV are RIG-I, which detects viral dsRNA and the AIM2 inflammasome, which detects host DNA released from damaged tissues, but this drives lung injury and mortality [100]. There are 3 IAV associated PAMPs that have been reported to act as activators of the NLRP3 inflammasome,

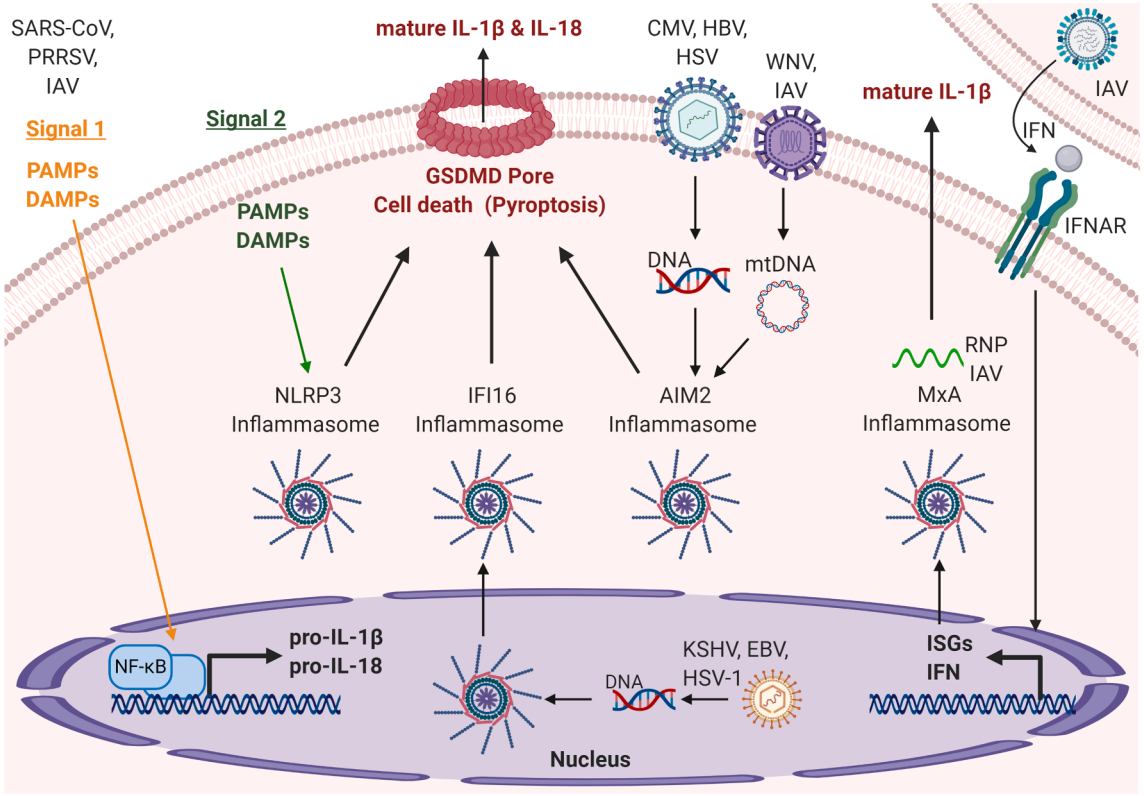

Fig. 2. Shown are inflammasomes implicated in mediating virus-induced pyroptosis, and/or generation of mature IL-1β and IL-18. Many of the PRR pathways described in Fig. 1 would be capable of increasing pro-IL-1β transcription (signal 1), which is a necessary step prior to direct inflammasome activation by viruses (signal 2) leading to processing of pro-IL-1β to mature IL-1β via caspase 1 activation. NLRP3 is activated by a wide range of pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs). In contrast, AIM2 and IFI16 can directly detect self or foreign double stranded DNA (dsDNA). The presence of viral dsDNA in the nucleus triggers the activation of the IFI16 inflammasome which then translocate into the cytoplasm. As for AIM2, self-DNA or viral dsDNA leads to the activation of the AIM2 inflammasome. Interestingly, RNA virus infection can also activate the AIM2 inflammasome, since viral infection causes mitochondrial damages resulting in the release of mitochondrial DNA (mtDNA) in the cytoplasm that is recognised by AIM2. As well as maturation of IL-1β and IL-18, the assembly and activation of these inflammasomes leads to caspase 1 dependent cleavage of gasdermin D, triggering pyroptotic cell death. Recently an IFN-dependent inflammasome has been described during IAV infection. The secretion of interferon (IFN) from IAV-infected cells triggers the up-regulation of ISGs in neighbouring cells leading to the production of MxA protein. In the cytoplasm, MxA binds to the ribonucleoprotein (RNP) of IAV leading to the activation of a MxA inflammasome and the secretion of mature IL-1β, in a process that is still poorly understood. Figure drawn with the aid of biorender.

viral RNA, the M2 channel protein and aggregated PB1-F2 [100].

While the in vivo investigations described above show that the NLRP3 inflammasome is protective in IAV infection, treatment of mice with MCC950, the specific NLRP3 inhibitor, revealed both beneficial and detrimental outcomes of NLRP3 inflammasome activation, depending on the timing of treatment. In mice treated 1 day after infection with IAV, the mice were more prone to infection, whereas treatment of mice 3 days post infection was protective, where lung inflammation was reduced. Treatment using anakinra also protected juvenile mice from a lethal challenge with IAV [100]. These data indicate that inhibition of the NLRP3 inflammasome might be beneficial during severe IAV infections. Furthermore, studies again using MCC950 indicated that NLRP3 inhibition might also prevent influenza associated secondary bacterial infection [100]. In summary the NLRP3 inflammasome can be both protective and harmful in the immune response to influenza infection.

The NLRP3 inflammasome is activated in response to infection with a range of RNA viruses. However, NLRP3 itself nor the other inflammasome components can bind RNA. There is growing evidence suggesting that members of the DExD/H-box family of helicases act as sensors of viral RNA to mediate activation of the NLRP3 inflammasome. In a further example of such a sensing and activating mechanism, it was shown that DDX19A is a novel component of the NLRP3 inflammasome following infection with porcine reproductive and respiration syndrome virus (PRRSV). During infection with this virus, DDX19A binds to the viral RNA to activate the NLRP3 inflammasome resulting in IL-1β production [102]. Indeed, other inflammasomes are also activated by DExD/H-box family of helicases. In epithelial cells of the intestine rotavirus is restricted by Nlrp9b via the RNA helicase Dhx9 recognition of short double-stranded RNA to form an inflammasome with ASC and caspase 1. This results in the maturation of IL-18 and GSDMD mediated pyroptosis. Deletion of Nlrp9b or other inflammasome components resulted in enhanced replication of rotavirus and rotavirus associated disease [103]. So, in the context of rotavirus infection at least, pyroptotic cell death does remove the replicative viral niche without damaging consequences for the host.

A second mechanism to activate the NLRP3 inflammasome during viral infections involves an interesting convergence of the antiviral RNAse L pathway, a DExD/H-box helicase, DHX33, leading to NLRP3 activation. During viral infections with IAV cleavage of viral and cellular RNAs by the antiviral RNAse L pathway is required to activate the NLRP3 inflammasome. The RNA cleavage products generated by RNAaseL containing 2′,3′-cyclic phosphate termini, are recognised by the DExD/H-box helicase, DHX33 leading to complex formation with the mitochondrial adaptor MAVS resulting in optimal activation of the NLRP3 inflammasome and IL-1β production [104].

A third mechanism to activate the NLRP3 inflammasome during viral infections requires NLRP3 to partner with another innate immune sensory protein. Z-DNA-binding protein 1 (ZBP1)/DNA-dependent activator of IFN-regulatory factors (DAI) was initially reported as a sensor of

DNA however this study could not be independently verified, and it was later discovered that ZBP1/DAI is an RNA sensor [105]. For example, ZBP1/DAI detects the presence of replicative intermediates of viral RNA via its Z-binding domains and leads to the activation of necroptosis resulting in restriction of the DNA virus MCMV [105]. The Z-DNA binding protein ZBP1/DAI also senses the presence of IAV by the detection of viral proteins NP and PB1 and leads to the activation of the NLRP3 inflammasome. By contrast ZBP1/DAI has no role in NLRP3 activation following infection with a different RNA virus, VSV. ZBP1/DAI mediates three different forms of cell death after IAV infection, pyroptosis and receptor interacting protein kinase (RIPK)3 dependent apoptosis and necroptosis. Mice lacking ZBP1/DAI, while displaying higher viral titres, were protected from IAV induced lethality, due to reduced inflammation and epithelial damage. This shows that while cell death removes the cellular niche required for viral replication, the ZBP1/DAI dependent IAV cell death is detrimental to the host [106]. A subsequent study showed that following IAV infection which is sensed by RIG-I, activates the MAVS-IFN pathway to upregulate ZBP1/DAI to drive cell death [107]. The search for the ZBP1/DAI activating ligands during IAV infection showed that the RNA genome of IAV together with NP and PB1 are present in ZBP1/DAI immunoprecipitates, however none of these individually could activate ZBP1/DAI dependent cell death. Rather assembly of a viral ribonucleoprotein (vRNP) polymer consisting of IAV RNA, NP, and PB1 are required to activate ZBP1/DAI dependent cell death. Thus, the actual ZBP1/DAI ligand during live IAV infection appears to be a complex vRNP polymer [107]. As expected from other innate immune sensors, the ZBP1/DAI sensor is subject to regulatory control and two independent studies reported that ZBP1/DAI induced necroptosis is inhibited by RIPK1 via its RHIM domain, which is independent of its kinase activity [108,109]. Interestingly ZBP1/DAI is a target of viral evasion by the E3 protein from VV, where E3 prevents ZBP1/DAI mediated necroptosis [110].

Several studies report that activation of the NLRP3 inflammasome following viral infections requires an association with the mitochondrial adapter MAVS and related to this, infection of human monocyte-derived macrophages with the highly pathogenic influenza A (H7N9) virus blocks inflammasome activation by inhibiting the interaction between NLRP3 and MAVS by the viral virulence factor PB1-F2 [111]. Interestingly macrophage phagocytosis of PB1-F2 protein aggregates results in hyperactivation of the NLRP3 inflammasome and over-production of IL-1β and IL-18 which contributes to the cytokine storm seen in highly pathogenic H7N9 infections but not in the WSN laboratory strain of human IAV [111]. Therefore, clinically relevant virus strains are needed in infection models to faithfully represent the actual disease process.

The NLRP3 inflammasome has been associated with virus induced inflammation and age-related inflammation. In bats it has been shown that there is reduced activation of the NLRP3 inflammasome in immune cells compared to that of mouse and human in response to a number of zoonotic viruses such as IAV, Melaka virus and MERS-CoV [112]. This reduction in NLRP3 activation was due to suppressed transcriptional priming, a novel splice variant and an altered leucine-rich repeats (LRR) domain of bat NLRP3. Interestingly this reduced activation of NLRP3 did not impact viral loads. This study supports the notion that there is enhanced innate immune tolerance and not enhanced antiviral defence in bats and is consistent with bats acting as a unique asymptomatic viral reservoir [112]. This study has obvious relevance towards our understanding of the emergence of the current SARS-CoV-2 pandemic, as the SARS-CoV-2 virus originated in bats, where the SARS-CoV-2 lineage has been circulating unnoticed in bats for many decades [113].

The coronaviruses SARS-CoV, MERS-CoV and SARS-CoV-2 can all trigger the activation of a cytokine storm, however the underlying mechanisms are unknown. Several studies have examined the effect of inflammasome activation by different coronaviruses. The SARS-CoV protein ORF3a activates both signals 1 and 2 to activate the NLRP3 inflammasome. Firstly, ORF3a activates NF-κB by switching on the TRAF3 dependent ubiquitination of p105 resulting in its processing to p50 to promote pro-IL-1β gene expression. Secondly ORF3a activates TRAF3 dependent K63 linked ubiquitination of ASC, leading to ASC speck formation. Therefore SARS-CoV ORF3a activates both NLRP3 inflammasome signals by targeting a single protein, TRAF3 [114]. Interestingly infection of human PBMCs with MERS-CoV activates the NLRP3 inflammasome, resulting in ASC speck formation and IL-1β production, both of which are inhibited by MCC950 [112], and this has also recently been shown for in vitro infection of human monocytes with SARS-CoV-2 [115]. Like SARS-CoV, this could be due to ORF3a in SARS-CoV-2. It has been reported that in moderate to severe COVID-19 patients, the NLRP3 inflammasome was activated in PBMCs and was also evident in tissues of post-mortem patients upon autopsy. Markers of inflammasome activation including the p20 form of cleaved caspase −1 and IL-18 in the sera correlated with markers of COVID-19 severity such as IL-6 and lactate dehydrogenase (LDH). Higher levels of these inflammasome markers were associated with disease severity and worse clinical outcome. These observations clearly indicate that the NLRP3 inflammasome is a key driver of COVID-19 pathophysiology and a possible therapeutic target in this disease [115]. Furthermore inflammasome activation and pyroptosis of macrophages releases membrane bound tissue factor, a crucial step in clot formation [116]. Therefore, it is conceivable that NLRP3 inhibition might block excessive inflammation and prevent clot formation, a major cause of mortality in COVID-19 patients [117].

It is increasingly clear that there are age dependent alterations in innate immunity, and inflammasomes may have a role in such alterations. It was shown that in IAV infected monocytes from older individuals, the IFN response is impaired but inflammasomes and proinflammatory response remains intact, key features of the aging human innate immune system [118]. Surprisingly, aged mice do not share these human characteristics, one of many mouse-human differences in innate immunity. Resistance to IAV infection is dependent upon IFN production generated by the TLR and RIG-I pathways [118]. In TLR7 and MAVS DKO mice IAV induced lethality was shown to be independent of viral loads, but dependent on bacterial burden, caspase 1/11 activity and neutrophil dependent tissue damage. These data show that during IAV infection a failure to produce IFN leads to increased viral loads which damage epithelial that is conducive to bacterial blooms. This leads to the recruitment and activation of neutrophils, producing lethal immunopathology. Surprisingly, it was also found that caspase 1 and caspase 11 were required for neutrophil netosis [118]. These findings explain why reduced IFN production in older individuals leads to secondary bacterial infection during influenza infection and suggest strategies to inhibit neutrophil associated inflammation might be beneficial in these groups. These observations may also help explain the increased susceptibility of these older groups to the current SARS-CoV-2 pandemic.

There are numerous examples of viral infections leading to inflammasome activation and the development of immunopathological responses. Such examples include norovirus, which is a major global cause of food-borne gastroenteritis. Norovirus activates the NLRP3 inflammasome and leads to the production of IL-1β and GSDMD dependent pyroptosis. However, norovirus activation of the NLRP3 inflammasome drives an immunopathological response as mice lacking either NLRP3 or GSDMD displayed reduced levels of the faecal inflammatory marker lipocalin-2 and delayed lethality after gastrointestinal norovirus infection [119]. The NLRP3 inflammasome also mediates pathology in response to Mayaro virus, an arbovirus that is emerging as a major public health concern in Latin America [120]. Mayaro virus activates the NLRP3 inflammasome by inducing the production of ROS and activating potassium efflux [120]. The NLRP3 inflammasome is protective against EV71, the main causative agent of hand foot and mouth disease. EV71 can antagonise the NLRP3 inflammasome by cleaving NLRP3 by the proteases 2A and 3C [121]. However a further study showed that pyroptosis drives pathology associated with EV71 infection [122].

It is crucial that the NLRP3 inflammasome and IL-1β production are

subject to tight regulation to prevent a damaging hyperinflammatory response, and it has been recognised that IFN acts as a potent negative regulator of expression and activity of the NLRP3 inflammasome [100]. Therefore, during viral infections, IFNs have a dual role both as powerful antiviral agents, and as homeostatic immune regulators. Inflammasome activation can also have a regulatory role in innate antiviral defence, where caspase 1 cleaves cGAS (see SECTION 6.2) to prevent cGAS-STING mediated IFN production during infection with DNA viruses but, interestingly not RNA viruses [123]. These findings reveal a regulatory circuit where type I IFNs inhibit inflammasomes and activated inflammasomes also inhibit type I IFN production. This study also indicates that inflammasome inhibition might be a strategy to augment antiviral defence against DNA virus infections such as HSV. Finally, Newcastle disease virus (NDV) activates the NLRP3 inflammasome, which may explain its oncolytic properties, since NDV activates pyroptosis [124]. This is because it was recently reported that pyroptosis of just 15% of tumour cells is sufficient to clear the entire tumour mass. This is due to lymphocyte recruitment and activation of anti-tumour immunity, driven by immunogenic pyroptosis [125].

5.2. AIM2

AIM2 is a member of the PYHIN family of proteins, some of which can act as sensors of dsDNA. AIM2 consists of a single PYRIN domain and a single HIN domain, with clear orthologues present in mammals such as mouse and human. AIM2 together with ASC and caspase 1 forms the AIM2 inflammasome, activation of which, like NLRP3, results in the maturation of pro-IL-1β, pro-IL-18 and GSDMD dependent pyroptosis [126]. The AIM2 inflammasome is activated by a wide range of DNA viruses such as CMV, VV, HPV, HBV, EBV, HSV, and enterovirus [126]. Interestingly this DNA sensing inflammasome is also activated by infection with RNA viruses such as Chikungunya virus, WNV, IAV and ZV [126]. Activation of the AIM2 inflammasome in response to RNA viruses is likely explained by the release of host derived nuclear or mtDNA as DAMPs during RNA virus infection into the cytosol where AIM2 is located, which occurs in the case of IAV infection [126]. In mice lacking AIM2 there is reduced lung injury and enhanced survival in response to IAV infection, but interestingly no alteration in lung viral titres. This suggests that any AIM2 dependent pyroptosis does not effectively remove the replicative niche associated with IAV infection in the lung and AIM2 drives a damaging inflammatory response [127]. In addition the adaptive immune response was also unaffected by the absence of AIM2 [127]. In experiments using mouse and human macrophages it appears that AIM2 mediates the proinflammatory response to IAV infection. The authors therefore suggest that therapeutic targeting of AIM2 might be beneficial during influenza infection as it would leave the antiviral immune response intact [127].

Further recent insights into AIM2 include the observation that during infection with HCMV, the tegument protein pUL83 blocks activation of the AIM2 inflammasome by directly binding to AIM2 [128]. The tegument protein VP22 from HSV also targets AIM2 by a direct interaction to block AIM2 oligomerisation and AIM2 inflammasome activation to allow in vivo replication of HSV [129]. Finally, immune complexed human adenoviruses taken up into monocyte-derived dendritic cells engages the AIM2 inflammasome and leads to IL-1β production and GSDMD dependent pyroptosis [130].

5.3. MxA inflammasome

Apart from NLRP3 and AIM2, a third novel inflammasome has been recently linked to viral infections that involves the MxA protein. *Mx* genes, which are found in almost all vertebrates, are ISGs that are upregulated after IFN production and have antiviral activities, especially against RNA viruses [131]. In human two Mx proteins have been described, MxA, which is localised in the cytoplasm while MxB resides in the nucleus [131]. MxA targets different steps of the life cycle across many virus families, ssRNA, dsRNA and dsDNA viruses [131]. Following IAV infection, MxA is thought to recognises the IAV nucleoprotein, which then interacts with ASC and leads to caspase 1 activation and IL-1β production [132]. This MxA-dependent IL-1β response is not found in myeloid cells and is a specialised inflammasome unique to respiratory epithelial cells [132]. While pyroptosis was not examined in this study, the MxA inflammasome may explain how virus infection of epithelial cells, which do not express NLRP3, elicit IL-1 production [132].

6. DNA sensors

The immunostimulatory properties of DNA have been known about since the early 1960s [133]. However, it is only in the last decade that the immune sensing mechanism responsible for the detection of DNA have been described. This response can be triggered by self-DNA from the nucleus or, relevant to our discussion, the presence of viral DNA genomes in the cytoplasm. In fact, the observation that both self-DNA and viral DNA can activate innate immunity helped to resolve early confusion whether such DNA recognition in the cytoplasm was sequence dependent or not. It is now established that free DNA is sensed in the cytoplasm in a sequence independent manner [134]. The long list of putative innate DNA sensors that were initially reported ten years ago have undergone somewhat of a revision. It now appears that the main DNA sensors for IFN production are IFN inducible protein 16 (IFI16) and cGAS [134], with cGAS playing a major role [135]. Here we will describe the roles of the PYHIN protein IFI16 and the cGAS-STING pathway in response to DNA virus infections.

6.1. IFI16

Human IFI16 has emerged as a sensor of DNA viruses both in the cytoplasm and nucleus. Structurally IFI16 is composed of an N terminal Pyrin domain, which mediates protein–protein interactions and two C terminal HIN domains which are sequence independent DNA binding motifs [134]. In human macrophages IFI16 detects HIV proviral DNA leading to the activation of cGAS, stimulator of interferon genes (STING) and type I IFN production [136,137]. In keratinocytes IFI16 has a similar function where IFI16 senses DNA and DNA viruses, and co-operates with cGAS to activate STING resulting in IFN production [138]. Interestingly while IFI16 increases cyclic guanosine monophosphate-adenosine monophosphate (cGAMP) production by cGAS in macrophages, IFI16 does not have this function in keratinocytes, indicating cell specific roles for IFI16 in cooperating with the cGAS pathway [138].

In CD4+ T cells, HIV proviral DNA detection by IFI16 has a different outcome. Here IFI16 forms an inflammasome with ASC to activate caspase 1 dependent pyroptotic cell death, resulting in abortive infection of these cells, thus promoting clinical progression of AIDS [133]. Supporting these conclusions, silencing of IFI16 or ASC by shRNA or caspase 1 inhibition rescued CD4+ T cells from death. Importantly silencing of NLRP3 did not rescue these cells. This discovery may explain how CD4+ T cells die during HIV infection. While the contribution of gasdermin family members to pyroptosis of CD4+ T cells during infection with HIV remains to be reported, a clear role would be predicted. Two additional important observation were also made. Firstly, HIV induced pyroptosis of CD4+ T cells occurs in tissue resident CD4+ T cells and not in CD4+ T cells from the circulation. This is explained since CD4+ T cells in the circulation are in a deep resting state, support the generation of fewer incomplete reverse transcripts of HIV and have reduced expression of IFI16. Indeed, we are reminded that while viral loads are commonly measured in the blood, HIV replication primarily occurs in lymphoid tissues [133]. Secondly, cell to cell transmission of HIV is required to induce pyroptosis in HIV infected CD4+ T cells, something that does not occur with cell free virions [133]. Interestingly cGAS is not expressed in lymphoid cells, but strongly expressed in myeloid cells, suggesting that IFI16 might be the cytoplasmic DNA sensor in lymphoid cells, whereas cGAS might be the major cytoplasmic DNA sensor in myeloid cells

[133]. It has been proposed that IFI16 dependent pyroptosis of abortively infected CD4+ T cells, might release DAMPs such as ATP resulting in a subsequent torrent of NLRP3 dependent pyroptosis in non-infected primed tissue resident CD4+ T cells [133]. In this scenario therapeutic blockade of NLRP3 might prevent CD4+ T cell loss and limit the chronic inflammation associated with HIV infected individuals. Inhibitors of the IFI16 inflammasome or caspase 1, by VX-765, which is well tolerated in humans, may also rescue CD4+ T cells during HIV infections and may also block the chronic inflammation characteristic of this infection. Indeed, why infected CD4+ T cells die when infected with HIV, while other cells such as microglia can be infected for years, likely depends on formation of this specialised IFI16 inflammasome [133].

In addition to the activation of innate signalling pathways as we have seen above, IFI16 has other more direct antiviral activities, especially to restrict herpesviruses. The formation of IFI16 oligomers via surface alpha helices in the pyrin domain is required for IFI16 to associate with HSV genomes and prevent viral replication [139]. Mechanistically this is due to IFI16 oligomers forming associations with ND10 body components, UBTF, and PAF1C. PML and DAXX are ND10 body components and are viral restriction factors. DAXX promotes formation of transcriptionally inactive chromatin on the HCMV genome by histone deacetylase, while PML knockdown enhances HSV and HCMV replication. Like IFI16, UBTF also restricts HSV replication, while PAF1C restricts HSV possibly due to its ability to mediate H3K9 trimethylation [139]. Interestingly in patients with the systemic autoimmune disease Sjögren's syndrome, IFI16 forms oligomers spontaneously, suggesting activation by host DNA is due to a breakdown of self-tolerance [140].

IFI16 also restricts replication of herpes viruses by promoting heterochromatin formation on viral genes. Mechanistically IFI16 promotes histone H3K9 trimethylation but reduces histone H3K4 trimethylation on HSV DNA [141]. These studies show that IFI16 restricts expression of under-chromatinised DNA [141]. These data indicate that IFI16 may have the capacity to recruit histone modifiers to viral genomes [141]. This in fact turns out to be the case, as it was recently reported that IFI16 recruits the H3K9 methyltransferases SUV39H1 and GLP to epigenetically silence herpesviruses [142].

An important question often raised in the field of innate DNA sensing is how can the host "sees" the viral DNA genomes within the nucleus and distinguish it from host nuclear DNA. In response to the presence of Kaposi's sarcoma-associated herpesvirus (KSHV), EBV and HSV genomes in the nuclei of infected cells, BRCA1 forms a complex with IFI16 and promotes the formation of an IFI16 inflammasome complex consisting of ASC and caspase 1. This BRCA1 triggered IFI16 inflammasome then translocates into the cytoplasm and cleaves IL-1β into its mature form [143]. In addition, once in the cytoplasm, IFI16 also activates the production of type I IFNs via the cytoplasmic STING-TBK1-IRF3 pathway [143]. The presence of BRCA1 was required for formation of the IFI16 inflammasome, IL-1β processing and IFN-β production during infection with KSHV and HSV. Therefore, BRCA1 together with IFI16 plays an important role in sensing the presence of viral DNA genomes within the nucleus which can then mobilise cytoplasmic antiviral defence mechanisms [143]. The surprising finding was then made that histone H2B is required for KSHV and HSV genome recognition by IFI16 in the nucleus. H2B forms a complex with IFI16 and BRCA1 under normal physiological conditions and infection with KSHV, HSV and EBV triggers the cytoplasmic relocation of H2B-IFI16, H2B-BRCA1 and IFI16-ASC complexes [144]. However, infection with the cytoplasmic dsDNA virus vaccinia did not have this cytoplasmic relocating effect but required a nuclear virus. In the cytoplasm the H2B-IFI16-BRCA1 complex interacted with cGAS and STING resulting in TBK1 and IRF3 phosphorylation and IFNβ production [144]. Hence, BRCA1 interacts with IFI16 both to form an inflammasome and mediate IL-1 production and activates cGAS leading to IFN production.

As a sensor of retroviral DNA intermediates, IFI16 has a major role in restricting retroviruses such as HIV. However, IFI16 also restricts HIV independent of its DNA sensing capabilities, where IFI16 binds to Sp1 to prevent Sp1-dependent expression of HIV genes [145]. To summarise IFI16 has four potentially distinct mechanisms to restrict virus infections, (1) activation of the STING-IFN pathway, (2) formation of inflammasome complexes, (3) epigenetic silencing of viral promoters, (4) limiting access to host factors required for viral replication such as Sp1. This explains why IFI16 is such a frequent target of immune evasion by many different viruses [88]. ICP0 from HSV targets IFI16 for degradation [146], while the tegument protein pUL83 from HCMV binds IFI16 and blocks IFI16 oligomerisation, thus preventing the activation of the STING-TBK1-IRF3 pathway [147]. Finally it was recently reported that the E7 protein from HPV recruits the E3 ligase TRIM21 to ubiquitinate and degrade IFI16 to inhibit IL-1 production and pyroptosis, both in Hela cells and HaCaT keratinocytes [148].

6.2. The STING-cGAS pathway

The discovery of the cGAS-cGAMP-STING pathway in 2013 represented a major advance in our understanding of how the innate immune system detects the presence of cytoplasmic DNA and DNA viruses. The presence of cytoplasmic DNA results in the activation of the enzymatic activity of cGAS and the synthesis of cGAMP from the substrates ATP and GTP. cGAMP then directly binds to and activates STING, via a conformational change of a preformed STING dimer, resulting in STING mobilisation from the ER to the Golgi. Here STING recruits and activates the kinases TBK1 and IKKβ resulting in the activation of IRF3 and NF-κB, which culminates in the production of pro-inflammatory cytokines and type I IFNs [149].

From the use of cGAS or STING knockout mice it is clear that mammalian host defence against DNA viruses such as herpesviruses, adenoviruses, murine gammaherpesvirus 68, VV, cytomegalovirus and papillomavirus are critically dependent on the cGAS–STING pathway [149]. In addition to DNA viruses, retroviruses such as HIV, SIV and murine leukaemia virus also activate cGAS [149], due to the formation of proviral DNA by the activity of reverse transcriptase enzymes. Some RNA viruses such as DV, VSV and WNV also activate the cGAS pathway [149]. DV activates the cGAS pathway by the release of mtDNA, as we have seen above, and VSV infection activates STING to block protein translation [149] and STING is required to control infection with WNV [150]. Finally, viral membrane fusion can also activate STING, independent of its DNA sensing capability [149].

The current understanding that cGAS is a cytoplasmic protein in search of DNA within the cytoplasm has been challenged by the recent finding that cGAS is anchored to the plasma membrane by an N-terminal phosphoinositide-binding domain located in cGAS. This subcellular localisation of cGAS is required to efficiently sense MVA and helps prevent cGAS from reacting to nuclear DNA and is a mechanism to help distinguish self from non self [149].

In addition to the induction of the IFN response the cGAS-STING pathway also confers antiviral protection by the induction of autophagy. Following the binding of cGAMP to STING, STING translocates to the endoplasmic reticulum-Golgi intermediate compartment and via WIPI2 and ATG5, triggers LC3 lipidation, a key step in autophagosome formation. This STING induced autophagy mediates the clearance of both cytoplasmic DNA and DNA viruses such as HSV. Interestingly STING induced autophagy is evolutionary conserved and is present in the sea anemone Nematostella vectensis, while the STING IFN response is absent in this species, suggesting that autophagy activation is the primordial function of STING [149].

STING KO mice display increased viral loads, dissemination within the CNS and decreased survival in response to challenge with WNV. The CNS of STING KO mice showed increased lesions, mononuclear cellular infiltration, neuronal death, and sustained pathology after clearance of the virus. T cell responses were compromised in STING KO mice both in the spleen and brain which was associated with the sustained CNS pathology observed in these mice. These data are indicative of novel roles for STING in immune homeostasis, immune programming, and

neuropathological defence during WNV infection. Interestingly STING signalling was not required to control WNV and WNV does not activate canonical STING activation, thus raising important questions as to how STING is activated by RNA viruses [150].

New players that detect and respond to DNA viruses have recently been reported. The heterogeneous nuclear ribonucleoprotein A2B1 (hnRNPA2B1) binds viral DNA in the nucleus, translocates into the cytoplasm where it engages TBK1 to activate IRF3, resulting in the production of type I IFNs [151]. hnRNPA2B1 also promotes the nucleocytoplasmic trafficking of cGAS, IFI16, and STING mRNAs, to enhance cytoplasmic DNA sensing and boost antiviral defence mechanisms [151].

There are several ways in which DNA and RNA viruses evade cGAS-STING defence mechanisms [152,153]. During HIV infection the 3′–5′ DNase TREX1 degrades cytosolic HIV DNA, the HIV capsid recruits cyclophilin A, to prevent cGAS activation, while ORF52 of KSHV also prevents activation of cGAS by viral DNA [88]. STING itself is also subject to similar viral evasion strategies. The HBV polymerase blocks lysine-63 linked ubiquitination and STING activation, the papain-like proteases from human coronaviruses including SARS-CoV also block STING lysine −63 linked ubiquitination and the viral oncoproteins E7 from HPV-18 and E1A from human adenovirus 5 bind to and inhibit STING activation. In addition the DV protease NS2B–NS3 cleaves and inactivates STING [88]. New virulence evasion factors have also been discovered in DNA viruses to neutralise host defence mechanisms. Nucleases present in poxvirus known as poxins which are 2′,3′-cGAMP-degrading enzymes, to prevent STING activation, provides further insights into the ongoing evolutionary arms race between viruses and their mammalian host [154].

Finally, as described above, due to the current SARS-CoV-2 pandemic there is huge interest in the innate immune system of bats. And like bat NLRP3 described above STING in bats has also been examined, and just as the NLRP3 inflammasome is subdued in bats so too is the IFN response. This is due to a substitution at the highly conserved serine residue S358 in STING. The introduction of this serine restores STING activity, IFN production and virus restriction [155]. It is also intriguing that the entire PYHIN locus, which includes IFI16 and AIM2, is absent in all bat genomes sequenced so far, suggestive of subdued DNA sensing [155]. These observations serve to reinforce the theme that in bats their propensity to act as unique viral revivors is due to increased innate immune tolerance, because of blunted innate sensing and responses and not due to increased innate immune defence.

7. Concluding comments and future directions

Huge progress has been made in our understanding of how the body detects virus infections. In the coming years, the challenge for immunologist will be to translate this knowledge into finding ways to activate PRRs as novel antivirals to treat virus infections and as effective vaccine adjuvants. Conversely blockade of PRRs might alleviate the damaging inflammation associated with viral infections such as SARS-CoV-2. The ability to manipulate PRRs with more refined ligands and more specific inhibitors will impacts other infectious, autoinflammatory and autoimmune conditions. For example, finding innovative ways to block low grade inflammation would be beneficial in several conditions such as HIV infection and cancer, which will also reduce aging or inflammaging associated with these conditions.

While much of our understanding of how viral sensing occurs is from gene KO studies in mice, in the future greater emphasis will need to be placed on understanding the human system and there is a pressing need for the development of human models that more faithfully represent the actual disease process of clinically relevant virus strains. In this regard human organoid models of disease, which now exist may be further utilised to understand PRR biology during viral infections [156]. It is clear from the discussion above that the impact of innate antiviral immune response differs for different virus infections, where individual PRRs and inflammasomes favour either the host or the virus, and sometimes in a spatiotemporal fashion. Therefore, any manipulation of innate immune signalling pathways as antiviral treatments will have to be virus specific and minimise the possibility of triggering a hyper-inflammatory or autoimmune response. More accurate insights into the course of viral infections will permit the identification of participating cells and molecules, which will result in the development of better therapies for viral infections.

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