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Novel paradigms of innate immune sensing of viral infections

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ABSTRACT

According to the existing paradigm, cellular recognition of viral infection is mediated by molecular patterns within the virus particle or produced during virus replication. However, there are various physical cellular changes indicative of infection that could also trigger innate antiviral responses. The type-I interferon response is rapidly engaged to limit viral infection and a number of studies have shown that the interferon response, or components of it, are induced by general perturbations to cellular processes. Virus entry requires membrane and cytoskeletal perturbation, and both membrane fusion or actin depolymerising agents alone are able to activate antiviral genes. Viruses cause cellular stress and change the cellular environment, and oxidative stress or endoplasmic reticulum stress will amplify antiviral signaling. Many of these responses converge on interferon regulatory factor 3, suggesting that it plays a crucial role in determining the degree to which the cell responds. This review highlights novel paradigms of viral recognition and speculates that viral infection is sensed as a danger signal.

1. Introduction

There are multiple arms of innate immunity that organisms have at their disposal to combat pathogen infections. The primary weapon for combating viruses is the type I interferon (IFN) response. IFN is a cytokine produced in response to pathogenic stimuli that activates a set of IFN stimulated genes (ISGs) in both infected and surrounding cells. These ISGs act in a variety of ways, but collectively induce an antiviral state that targets nearly every stage of viral replication. However, to initiate an antiviral response, a cell must sense incoming virus and signal the induction of IFN, and viruses have evolved many ways to interfere with this process [1].

2. Viral recognition paradigms

2.1. The traditional paradigm of virus recognition

The existing paradigm of type I IFN induction requires recognition of pathogen associated molecular patterns (PAMPs) by pattern recognition receptors (PRRs). Double stranded RNA (dsRNA), a primary hallmark of viral infection, can signal a type I IFN response following detection by toll-like receptors (TLRs) in endosomes and RIG-I-like receptors (RLRs) in the cytoplasm [2,3]. Roles for TLRs and cytoplasmic DNA receptors (DNARs) in the recognition of viral glycoproteins and DNA have also recently been identified [4–6]. PRRs capable of detecting viral components signal through a variety of adaptors that converge on activation of the transcription factor IFN regulatory factor 3 (IRF3) [7]. IRF3 plays a central role in IFN induction through nearly every pathway and in most cell types, with the notable exception of plasmacytoid dendritic cells, which signal through IRF7 [8,9]. IRF3 together with activated NF-κB and ATF-2/c-Jun form a complex with nuclear proteins to induce transcription of IFNβ [10–12]. Low level IFNβ signaling is thought to be important for induction of IRF7 and subsequent activation of IFNα species [13]. Together, both species of type I IFN induce a range of ISGs specific for each cell and tissue type, culminating in an antiviral response. The intricacies of this response have been fairly well studied and there are many excellent reviews on the topic [2,3].

2.2. Non-traditional paradigms of virus recognition

Although the established pathways leading to IRF3 activation involve different sensing and signaling components, they are functionally similar in that they detect viral components via receptor–ligand interactions. However, it seems unlikely that this is the only form of viral detection. It is well known that viruses alter regular cellular processes such as endocytosis and cytoskeletal remodeling during entry. Viruses must also dramatically alter cellular conditions to mediate their replication and, consequently, cause cell stress. It is intriguing to speculate that perturbation of physical or homeostatic conditions within the cell could act as a danger

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signal for viral infection. Indeed, recent findings support this hypothesis.

Moreover, given the evolution of multiple viral sensing pathways, viruses have evolved strategies to avoid exposing their viral components to PRRs. Viruses are capable of sequestering their genomes and/or degrading dsRNA by-products of replication to prevent recognition [14,15]. On the other hand, physical changes to the cell are harder to conceal because of their more global nature. For example, cytoskeletal rearrangements and various signs of cell stress cannot be sequestered or hidden. Another issue with reliance on detection of viral dsRNA for host defense is that for many viruses, dsRNA does not accumulate until later in the viral replication cycle when the virus has had opportunity to subvert the antiviral response. If nucleic acid recognition was the only predominant mechanism of initiating an antiviral defense, viruses likely would have overcome the obstacle of innate immunity. Instead, cells have evolved to combat infection through the use of a variety of sensors and signaling pathways, so that only the larger and more complex viruses efficiently establish persistent infections.

3. Virus infection as a danger signal

3.1. Innate responses mediated by danger-associated molecular patterns

It has been established that cells can initiate inflammatory pathways following physical danger signals [16]. A broad group of molecules including asbestos, silica, uric acid crystals, ATP, and alum are considered danger-associated molecular patterns (DAMPs) and can initiate sterile inflammation. This danger-associated response signals through the NLRP3 inflammasome [17–20]. It is currently unclear how a wide range of stimuli is sensed by a single molecule. While a decrease in intracellular potassium was initially considered as a point of convergence for these stimuli [21], NLRP3 activation in the absence of potassium level alteration has been observed [22]. While the NLRP3 inflammasome is not considered a major component of the antiviral response and is not involved directly in IFN production, it has been linked with innate immune sensing of viruses [23]. Furthermore, adapters in the TLR and RLR pathways have been associated with NLRP3 inflammasome activation [24,25]. While mechanistic details of inflammasome activation remain elusive, it is now clear that cells can detect physiological perturbations independent of prototypic protein or nucleic acid ligand–receptor interactions and initiate innate immune responses.

3.2. Membrane perturbation

To enter a cell, all viruses must cross the cell membrane either at the surface or within endosomal compartments. For enveloped viruses, this entails membrane fusion. Membrane fusion is energetically unfavourable because of the need to disrupt hydrophobic interactions within the phospholipid bilayer. Enveloped viruses apply force with fusion proteins to bring the membranes together and induce curvature and eventual fusion leading to incorporation of the envelope into the cellular membrane [26]. These alterations at the cellular membrane are characteristic of viral entry and could alert the cell to the presence of the virus.

Inactivated virus is a convenient tool for studying virus entry independent of complications associated with live virus infection. Replication competent viruses can be inactivated genetically or through heat or ultraviolet radiation treatment to render their genomes non-functional; therefore, particles are capable of entry but not gene expression or replication. A broad range of inactivated viruses are capable of inducing ISGs independently of TLRs or RLRs [27–30]. Furthermore, the response to virus particles is dependent on entry, suggesting that viral glycoprotein binding and recognition are not sufficient [28,31]. These observations suggest that virus particle entry in the absence of replication may not present a sufficient volume of PAMPs for TLR or RLR recognition. A strong possibility is that the physical act of viral entry is sufficient to alert a cell to impending danger. Recent studies using virus-like particles (VLPs) have supported this hypothesis.

Certain VLPs mimic enveloped viruses and are capable of membrane fusion but do not contain packaged virus genome or capsid. Light particles (or L-particles) are produced during natural infection by alphaherpesviruses and are composed of an envelope without capsid or genome [32]. They can be separated from replication competent virus by density gradient centrifugation or with mutant viruses incapable of capsid assembly [32,33]. Pre-viral replication enveloped particles (PREPs) are produced during viral replication when the viral polymerase is blocked [34]. PREPs contain viral capsid and tegument within a fusion competent envelope but do not contain genome. Both types of VLPs induce ISGs [35]. However, because these particles are produced in the context of viral replication, there is still the possibility of protein or nucleic acid contaminants inadvertently packaged within the envelope. Fusion-associated small transmembrane (FAST) proteins are non-structural, syncytia forming proteins expressed by reoviruses, a family of non-enveloped viruses [36]. Purified p14 FAST protein in complex with liposomes can induce ISGs in the absence of viral or cellular contaminants [37]. Finally, fusogenic liposomes capable of spontaneous fusion with cells recapitulate a similar response [35].

The mechanism of antiviral signaling following membrane fusion is largely unknown. Phospholipase C-γ (PLC-γ) and phosphatidylinositol-3-OH kinase (PI3K) pathways are associated with membrane signaling and inhibitors of PLC-γ and PI3K pathways interfere with the antiviral response to membrane perturbation [35,38]. However, the specific family members involved have yet to be identified as the response to UV-inactivated enveloped virus was shown to be independent of prototypic PI3K family members [38]. The ER resident stimulator of IFN genes (STING) also plays a role in signaling the response to membrane perturbation [35]. STING is associated with sensing cytosolic DNA and various enveloped viruses [6,39]. After stimulation, STING translocates from the ER to cytoplasmic punctuate structures where it co-localizes with Tank-binding kinase 1 (TBK1), which is normally responsible for phosphorylation of IRF3 and consequently regulation of IFN and ISGs [6]. In response to membrane fusion, STING was shown to translocate similarly and was essential for IFN and ISG induction [35].

The nature of the antiviral response to membrane perturbation is also controversial. Several studies in primary fibroblasts suggest that IRF3 mediates the direct activation of a subset of ISGs independent of IFN production [27,37,38,40]. In this context, IRF3 is essential for ISG induction, unlike the prototypic induction of IFN in response to dsRNA, which can occur in the absence of IRF3, even in fibroblasts [41]. Moreover, TBK1 is absolutely essential for the direct activation of IRF3 [29]. Other groups working predominantly with immune cells have found that IFN is produced following membrane perturbation and that TBK1 is involved, but not essential [35]. The apparent discrepancy could be a cell specific phenomenon, or it could relate to the extent of membrane perturbation. Studies in fibroblasts found that a low threshold of stimulation was sufficient to activate IRF3, but not NF-κB, suggesting that a critical level of virus particle entry is required to induce the production of IFN [29].

3.3. Cytoskeletal perturbation

Cytoskeletal perturbation has also been implicated in antiviral signaling. The cytoskeleton is involved in multiple aspects of virus