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Antiviral Innate Immunity: Editorial Overview

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Innate immune responses are the first line of defense against microbial infection, and they play a major role in restricting infection by viruses. These responses, which are well documented in organisms ranging from simple invertebrates to mammals, classically involve recognition of pathogen-associated molecular patterns on the invading infectious agent by host pattern recognition receptors (PRRs) such as retinoic-acid-inducible gene-I (RIG-I)-like receptors (RLRs), nucleotide-oligomerization-domain-like receptors, and Toll-like receptors (TLRs) [1]. The interaction between pathogen-associated molecular patterns and PRRs triggers a cascade of events that leads to the production of pro-inflammatory cytokines and three classes of interferons (IFNs): type I (IFN-α and IFN-β), type II (IFN-γ), and type III (IFN-λ1, IFN-λ2, and IFN-λ3). Key steps in the induction of IFNs and pro-inflammatory cytokines include activation and nuclear translocation of NF-κB, IFN-regulatory factor 3 (IRF3), and IFN-regulatory factor 7 (IRF7). Activation of IRF3 leads to the expression of a variety of innate immune genes that restrict infection, while production of IFNs by virus-infected cells ultimately triggers, by both paracrine and autocrine mechanisms, the expression of hundreds of IFN-stimulated genes (ISGs). ISGs function to suppress viral replication, regulate cellular metabolism toward an antiviral state, and modulate the ensuing adaptive immune response [1].

This special issue contains reviews that describe in detail a number of antiviral genes that are either IFN induced, directly acted upon by ISGs, or constitutively expressed. These include tetherin [2], viperin (virus inhibitory protein, endoplasmic reticulum associated, IFN inducible; also known as cig5 and RSAD2) [3], tripartite motif (TRIM) proteins [4], SAMHD1 [sterile alpha motif and histidine-aspartic domain (HD)-containing protein 1] [5], APOBEC (apolipoprotein B messenger-RNA-editing enzyme catalytic polypeptide) proteins [6], mitochondrial antiviral signaling (MAVS) protein [7], ISG15 (IFN-stimulated gene 15) [8], IFITM (IFN-induced transmembrane protein) [9], and defensins [10]. In many cases, viruses have evolved strategies to counteract the activity of these antiviral proteins. The mechanism by which specific virus families are targeted by the host innate immune response and the strategies used to avoid or escape the host's attempt to establish an antiviral state are

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reviewed here. Not only proteins but also microRNAs and small interfering RNAs contribute to the host defense [11]. Indeed, in insects, the RNAi pathway is the primary antiviral defense mechanism [12]. Several primary research articles that report interesting new data related to antiviral innate immunity are also included here [13–19].

Tetherin (originally known as CD317 or BST-2) was identified as an IFN-induced protein that restricts the release of human immunodeficiency virus (HIV)-1 virions from the plasma membrane [2]. Tetherin contains membrane-anchoring domains at both its N- and C-termini and a central coiled-coil domain. This unusual topology allows it to link viral and cellular membranes, thereby tethering particles to the cell surface. The antiviral activity of tetherin is not limited to HIV-1; this protein restricts the release of a number of other enveloped viruses. Tetherin also appears to sense retroviral particles and stimulate NF-κB signaling, leading to induction of IFN and pro-inflammatory cytokines. The importance of tetherin as a restriction factor is illustrated by the discovery that primate lentiviruses have evolved several distinct strategies for counteracting this protein, using independent viral genes Vpu (HIV-1), Nef [simian immunodeficiency virus (SIV)], or the envelope (Env) glycoprotein (HIV-2). Although the mechanisms by which these viral proteins counteract tetherin differ, in each case the final outcome is to reduce tetherin levels at the site of budding.

TRIM proteins are encoded by a large family of genes, many of which bear evidence of rapid positive selection suggestive of a role in combating pathogen invasion. Many TRIM proteins possess antiviral activity [4]. As their name implies, TRIM proteins bear three conserved motifs: a RING-type E3 ubiquitin ligase domain, one or two B-boxes, and a coiled-coil domain. The best-characterized antiviral TRIM protein is TRIM5α, which restricts retroviral infection. Although the precise mechanism of restriction is still being deciphered, it appears that TRIM5α recognizes the hexameric lattice of the incoming viral capsid and promotes its rapid uncoating and/or degradation. Retroviral capsid proteins have adapted to the TRIM5α expressed in their host; thus, for example, HIV-1 is potently inhibited by rhesus macaque but not human TRIM5α.

Viperin was first identified as a protein whose expression is induced by human cytomegalovirus (HCMV) infection and later shown to be induced by a number of other viruses (including Sendai virus, Sinbis virus, and lymphocytic choriomeningitis virus), IFN, and double-stranded nucleic acids [3]. Induction of viperin expression by some viral infections is mediated by activation of PRRs and downstream signaling through IRF3 and IRF7 to produce IFN-β. The mechanisms by which viperin interferes with viral replication are varied, complex, and poorly understood, as are the strategies developed by viruses to evade this protein’s activity. Effects on host cell lipid metabolism and lipid raft formation may be key to viperin’s antiviral activity.

Following up on the observation that the Vpx protein of HIV-2 and some strains of SIV greatly stimulate retroviral infection in myeloid cells, it was discovered that Vpx binds and induces the proteasomal degradation of SAMHD1 [5]. Depletion of SAMHD1 by RNA interference likewise reportedly stimulates retroviral infection. Mechanistic studies indicate that SAMHD1 blocks retroviral reverse transcription, an observation consistent with the finding that SAMHD1 is a deoxynucleoside triphosphate (dNTP) triphosphohydrolase that

reduces the pool of intracellular dNTPs by cleaving dNTPs to deoxynucleosides and triphosphate. SAMHD1, which, interestingly, is defective in patients with the autoimmune disorder Aicardi-Goutières syndrome, bears several functional domains including the SAM (sterile alpha motif) domain and the HD (histidine-aspartic domain), the latter of which is critical for triphosphohydrolase activity. The reduced intracellular concentrations of dNTPs that are a consequence of SAMHD1 expression are thought to be responsible for the impairment in reverse transcription induced by this protein. The antiviral activity of SAMHD1 extends to a number of retroviruses and also perhaps to non-retroviruses such as herpes simplex virus type 1 and vaccinia virus.

Enveloped viruses must fuse with target cell membranes (e.g., the plasma membrane or endosomal membrane) to gain access to the cytoplasm. The IFN-inducible proteins IFITM1, IFITM2, and IFITM3 exert antiviral activity against a broad range of enveloped viruses at this early step in the virus replication cycle, apparently by trapping incoming viral complexes at the membrane and inducing their lysosomal degradation [9]. The ability of IFITM proteins to inhibit membrane fusion may result from their localization to lipid rafts (where many enveloped viruses bind and enter), effects on membrane fluidity and dynamics, and perhaps effects on cellular lipid distribution.

In addition to serving as the “powerhouse” of the cell, mitochondria play a number of roles including serving to promote the host innate immune response to viral RNA. The signaling adaptor protein MAVS is the key player in this response [7]. Viral RNA is first sensed by the RLRs RIG-I and melanoma-differentiation-associated gene 5 (MDA-5). Both RIG-I and MDA-5 bind MAVS at the cytosolic surface of the mitochondrial membrane. These binding events result in the activation of key components of the antiviral response, including IRF3 and NF-κB, triggering the production of IFN and pro-inflammatory cytokines.

The APOBEC family of proteins are cytidine deaminases that act on single-stranded DNA or RNA substrates by converting cytidines to uridines (C-to-U) or deoxycytidines to deoxyuridines (dC-to-dU) [6,20]. More than a decade ago, it was observed that one human APOBEC family member, APOBEC3G, potently restricts HIV-1 infection in the absence but not in the presence of the accessory protein Vif. Vif was subsequently shown to induce the degradation of APOBEC3G by connecting it to the proteasomal degradation machinery. In the absence of Vif, APOBEC3G is incorporated into HIV-1 particles and induces C-to-U deamination during reverse transcription in the next round of infection, resulting in G-to-A hypermutation in the viral DNA. Other APOBEC family members, most notably APOBEC3F, also possess anti-retroviral activity. The APOBEC proteins broadly inhibit replication of a wide range of retroviruses as well as retrotransposons and other viruses (e.g., hepatitis B virus).

ISG15 is a ubiquitin-related protein that, like ubiquitin itself, is conjugated to hundreds of target proteins and also exists as a free protein both intracellularly and as a secreted factor [8]. ISG15 conjugation to target proteins (referred to as ISGylation by analogy to ubiquitylation) is carried out by a small number of E1, E2, and E3 enzymes, which, like ISG15 itself, are induced by IFN. There is no clear evidence that ISGylation leads to proteasomal degradation of the conjugated target protein, in contrast to a large subset of

ubiquitylated proteins. The replication of many viruses, including influenza virus, retroviruses, paramyxoviruses, human papilloma virus, vaccinia virus, vesicular stomatitis virus, and flaviviruses is inhibited by ISG15. The protective effect of ISG15 is illustrated by increased lethality following viral infection of ISG15 knock-out mice relative to $ISG15^{+/+}$ mice. In some cases, it appears that viral proteins are the targets of ISGylation; in other instances, ISG15 expression may disrupt cellular machinery used by the virus to replicate. In yet other systems, depletion of ISG15-conjugating enzymes has little effect on ISG15 antiviral activity, suggesting that ISG15 may possess antiviral properties in its unconjugated form. As one might expect, viruses have developed strategies to counteract the antiviral activity of ISG15. For example, some encode proteins that either prevent ISGylation or catalyze the deconjugation of ISG15 from modified proteins.

The defensins are antimicrobial peptides that constitute a primitive defense mechanism against bacterial and viral infection [10]. These small peptides, whose expression is often induced by viral infection, typically block various aspects of virus entry and are active against both enveloped and nonenveloped viruses. Mechanisms of action vary and are reported to include direct interactions with viral membranes, down-regulation of viral receptors, and blocking of viral membrane fusion.

Above, we briefly discuss examples of individual host restriction factors against which viruses have evolved specific countermeasures. A number of viruses have developed much broader strategies to disable or manipulate the host innate immune response. For example, several viruses (e.g., hepatitis C virus [21]) antagonize TLRs by targeting the TLR adaptor TIR-domain-containing adapter-inducing IFN-β (TRIF). A favored approach to targeting TRIF appears to be direct cleavage mediated by viral proteases. Inactivation of TRIF blocks both NF-κB- and IRF3-dependent signaling [22]. In addition to its ability to counteract specific host restriction factors (see above), HIV-1 counteracts IRF3 [20]. Dengue virus uses its nonstructural proteins and an RNA to target several aspects of the innate immune response [23]. The viral RNA sfRNA, encoded by the 3′ untranslated region of the dengue genome, and nonstructural protein NS4B both reportedly interfere with dicer, a key enzyme in the RNAi pathway. The dengue virus NS2B/3 protease cleaves a critical factor downstream of RIG-I and MDA-5, thereby interfering with IFN production. Dengue virus also encodes nonstructural proteins that inhibit STAT1 phosphorylation and degrade STAT2, thereby disrupting IFN signaling [23]. In the case of hepatitis C virus, the viral protease NS3/4A targets and cleaves MAVS, blocking downstream signaling and blunting the production of IFN and pro-inflammatory cytokines [21]. As detailed in the Parks review [24], paramyxoviruses have evolved several distinct strategies for blocking IFN induction. These include limiting RNA synthesis to avoid RNA sensors RIG-I and MDA-5 and direct inhibition of MDA-5 by the viral V protein. The latter protein also serves as a decoy for phosphorylation by the IKK-α/MYD88/TRAF6 kinase complex that normally phosphorylates IRF7. Several paramyxoviruses also encode proteins that directly disrupt activation of the IFN-β promoter. In addition to blocking IFN induction, paramyxoviruses are able to interfere with IFN signaling [24]. The nucleoprotein of some arenaviruses can block the phosphorylation and nuclear translocation of IRF3—key steps in IFN induction—and can suppress expression from the IFN-β and IRF3 promoters [25]. Considering its large coding capacity, it is perhaps not surprising that herpes simplex virus encodes a variety of

proteins that interfere with or help the virus to evade innate immunity [26]. To cite just one example, ICP0 (infected cell protein 0) is a RING-finger ubiquitin ligase that interferes with nuclear translocation of IRF3 and also sequesters this key protein in nuclear complexes. ICP0 also disrupts NF-κB activation by subverting the activity of the cellular deubiquitylating enzyme USP7. The β-herpesvirus HCMV blocks IFN-induced phosphorylation of several key effectors including STAT1 and STAT2, activates cellular phosphatases that dephosphorylate these proteins, or induces their sequestration in the nucleus [27]. HCMV also encodes a protein that is reported to block IFN-β expression by preventing NF-κB binding to the IFN-β promoter. HCMV also disrupts the function of several ISGs, including dsRNA-activated protein kinase (PKR), 2′5′-oligoadenylate synthetase, and RIG-I. Finally, HCMV has evolved the capacity to usurp the activity of several ISGs and convert these normally antiviral proteins to factors that actually promote virus eplication. As described in the Langevin chapter, although still poorly understood, fish viruses have also evolved mechanisms to escape from the innate immune responses of their hosts [28].

We hope that the thought-provoking articles presented in this special issue will both inform the readership of the *Journal of Molecular Biology* and stimulate further progress in the fascinating and rapidly moving field of innate antiviral immunity. We thank all authors who contributed to this project, our many colleagues who served as reviewers for the papers assembled here, and the editorial staff of the *Journal of Molecular Biology.*

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