Innate barriers to viral infection

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Innate immunity represents the foremost barrier to viral infection. In order to infect a cell efficiently, viruses need to evade innate immune effectors such as interferons and inflammatory cytokines. Pattern recognition receptors can detect viral components or pathogen-associated molecular patterns. These receptors then elicit innate immune responses that result in the generation of type I interferons and proinflammatory cytokines. Organized by the Society for General Microbiology, one session of this conference focused on the current state-of-the-art knowledge on innate barriers to infection of different RNA and DNA viruses. Experts working on innate immunity in the context of viral infection provided insight into different aspects of innate immune recognition and also discussed areas for future research. Here, we provide an overview of the session on innate barriers to infection.

The ‘Innate Barriers to Infection’ session at the Society for General Microbiology (SGM) workshop was organized by Ian Goodfellow (Imperial College London, UK) and Aine McKnight (Barts and The London School of Medicine and Dentistry, UK). This forum focused on the topic of host responses to viral infection and the virus counter-response. Eukaryotic cells have multiple inducible ways (‘innate immune responses’) to control viral infection, the most important of which is the type I interferon (IFN) pathway. An infected cell can alert the immune system through signaling driven by this pathway and at the same time induce the production of antiviral host defense proteins. These proteins minimize the impact of the virus on the infected cell by autocrine signaling and contain virus spread to bystander cells by paracrine signaling. Intrinsic antiviral resistance, such as innate immunity, is another nonadaptive immune host cell defense mechanism that is conferred by pre-existing cellular proteins.

Antiviral innate immune responses depend upon the detection of viral components (pathogen-associated molecular patterns [PAMPs]) by germ line-encoded pattern recognition receptors (PRRs), whose expression is not limited to professional antigen-presenting cells. The classes of PRR currently known are the transmembrane Toll-like receptors (TLRs) and C-type lectin receptors, the cytoplasmic RIG-I-like receptors, nucleotide-binding and oligomerization

leucine-rich repeat receptors (NLRs; also called NOD-like receptors), AIM2-like receptors and the cytosolic DNA receptors. Thus, PRR activation induces type I IFN and proinflammatory cytokines in response to PAMPs. By contrast, damage-associated molecular patterns are essentially noninfectious PAMPs that are derived from cellular nuclear and cytoplasmic proteins and nucleic acids. They may also be recognized by PRRs.

Viruses have evolved elaborate ways to evade or counteract antiviral innate and intrinsic immunity (e.g., to mitigate the effects of the type I IFN pathway). These immune responses and the viral defenses against them were the subjects of the session.

Activation & evasion of host pattern recognition receptor signaling by viruses

Andrew Bowie (Trinity College Dublin, Ireland)

Andrew Bowie gave the ‘state-of-the-art’ lecture on activation and evasion of host PRR signaling by viruses. He focused on the role and identity of cytosolic DNA sensors.

Several intracellular sensors that detect dsDNA encoded by DNA viruses were discussed. TLR9 is present in endosomes and can recognize CpG DNA, while DNA binding of dsDNA by DAI (a DNA-dependent activator of IFN-regulatory factors) can also bind DNA. AIM2 and IFI16 recognize dsDNA, and RNA polymerase III has been shown to convert AT-rich

Keywords

■ DNA virus ■ inflammatory cytokine ■ innate immunity ■ interferon ■ RNA virus

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DNA into a 5'-triphosphate-containing RNA, which then activates RIG-I to trigger an IFN response. Cytosolic CpG-A DNA is recognized by DHX36 as well.

Using a 70-nucleotide dsDNA element from vaccinia virus, Bowie's group showed that IFI16 can bind to this DNA element [1]. DNA sensing appears to depend on the length of the DNA, and the IFN- β response to cytosolic DNA is length-dependent but not sequence-dependent. Purified IFI16 protein can directly bind DNA *in vitro* and colocalizes with DNA in the cell [1]. IFI16 could recruit STING to induce IFN- β . Another virus, HSV, could also induce IFN- β in an IFI16-dependent manner. P204 is the murine ortholog of human IFI16 and appears to be essential for type I IFN, IRF3 and NF- κ B activation by HSV-1 infection or by synthesized dsDNA. IFI16 is a member of the PYHIN family, similar to AIM2, a cytosolic DNA receptor that forms an inflammasome [2].

Bowie ended by posing several outstanding questions in the field that need to be addressed by future studies. For example, the presence of many different DNA sensors begs the question as to which ones are physiologically relevant in sensing viral infection. Is there a specificity to which DNA sensors recognize certain pathogens and do these DNA-sensing mechanisms detect DNA in the cytoplasm or can they detect DNA in the nucleus as well? Is viral dsDNA being recognized as a PAMP or a damage-associated molecular patterns? What makes it different from cellular DNA and is the nucleus an immune-privileged site as far as DNA sensing is concerned? Can host chromosomal DNA elicit an IFN response under certain circumstances? Overall, the role of nucleic acids (genomic or replication products) as PRR ligands has become increasingly evident, and in the case of understanding DNA recognition, progress is evolving rapidly.

The human *APOBEC3* cluster of viral DNA mutators, part of a much bigger picture

Simon Wain-Hobson (Institut Pasteur, France)

Simon Wain-Hobson discussed the *APOBEC3* (A3) cluster of genes and the enzymes they encode. The gene cluster is located on chromosome 22 and their enzymes, or 'DNA mutators', induce nucleotide base changes in ssDNA. In humans, there are at least seven *APOBEC* genes. They are expressed in most cell types and induce C→T transitions through cytidine deaminase activity, with a mutation rate of 0.1–0.8. One

of the most well-known members is AID, which participates in rearrangement and somatic hypermutation of the immunoglobulin genes. Two themes were covered in this talk: first, the impact of these proteins on the replication of the retroviruses and hepadnaviruses; second, the potential contribution of the A3 proteins to mutating the human genome and thereby contributing to cancer development.

It is through the study of HIV that the APOBEC proteins rose to prominence in the microbiology community when Michael Malim's group identified that this virus antagonizes A3G activity [3,4]. It is the Vif protein of HIV that antagonizes A3G, and indeed A3F, by ubiquitinating them. Without Vif, the cytidine deaminase mutates the HIV genome by C→T transition: cytosine I deamination to uracil (U) in the first strand of DNA during reverse transcription results in the substitution of adenine in the newly synthesized strand because U is recognized as thymine by the reverse transcriptase. Thus, in Wain-Hobson's words, HIV has solved the A3 problem, with Vif.

Given their mutational defense activities and that at least the A3A and A3G proteins are upregulated in response to IFN, what other virus genomes are vulnerable to A3 activities? Wain-Hobson discussed HBV as being affected by A3, as are HPVs, human T-cell lymphotropic virus, TT virus, HSV, EBV and some spumaviruses. A3 activity is thought to be unimportant for poxviruses, whereas the effects on adenoviruses and other herpesviruses are unknown.

Turning to the human genome as a target for A3 activity, Wain-Hobson cited his recent paper on A3A mutating nuclear and mitochondrial DNA [5]. A3 activity yielding U residues is sensed by cellular U DNA glycosylases (UNGs). Hence, in the healthy genome, there is a balance between A3 editing and UNG catabolism. He hypothesized that recurrent low-level mutation by A3A could catalyze the transition from a healthy to a cancer genome. Such a process might be more likely to occur under conditions of chronic immune activation, a known contributor to the development of at least some cancers, because IFNs can induce A3s. Where the frequency of C→T transitions is elevated, UNG-mediated base excision may be erroneous and generate genomic instability through, for example, double-stranded breaks, exacerbating the transition to the cancer genome. Wain-Hobson presented various lines of evidence in support of this hypothesis, including, for example, the analysis of cells of a very rare individual deleted

for UNG (*ung*^{-/-}), in which there was evidence of massive MYC exon 2 editing. He concluded by suggesting that there are four hallmarks of cancer genomes that provide evidence for A3 involvement (especially A3A) in the complex multistep process that leads to genome mutation and the development of at least some cancers:

- Chromothripsis: evidence of extensive double-strand breaks having occurred
- 5-methylcytosine hotspots
- Thousands of CG→TA transitions
- Predominance of CG→TA transitions among mutations

Tetherin/BST2 in the innate antiviral response to HIV-1

Stuart Neil (King's College London, UK)

Stuart Neil discovered the role of tetherin/Bst2 in the physical retention of virus particles on cell surfaces and its antagonism by the HIV-1 accessory protein Vpu [6]. In this session, he discussed his recent work in this area.

Tetherin is an IFN-induced glycoprotein that retains diverse enveloped viruses on the cell surface by cross-linking nascent virus to the plasma membrane. In this role, the structure of tetherin is fundamental. It consists of a short cytoplasmic tail, a transmembrane domain and an essentially helical extracellular domain containing three cysteine residues that are responsible for homodimerization. There is then a coiled-coil domain that is followed by a C-terminal glycosylphosphatidylinositol anchor that locates the protein into the plasma membrane. The two membrane anchors enable the protein to physically harness viruses to the cell by embedding one end in the virion membrane and the other in the plasma membrane [7].

All known primate lentiviruses encode antagonists of tetherin, although the identity of that protein varies. The existence of these antagonists is likely Darwinian because tetherin targets the one structural component of the virus that cannot be mutated: the lipid membrane. However, antagonism of tetherin is species-specific. Thus, HIV-1 Vpu is a transmembrane protein that promotes the release of virions from the cell but can only target human, chimpanzee and gorilla tetherin sequences. It does so through Vpu transmembrane domain sequences [8]. However, for most primate lentiviruses, the accessory protein Nef targets tetherin through a sequence that is absent in the human protein.

Vpu serves to lower cell surface levels of tetherin, reducing its chances of being incorporated into the HIV-1 virion. Vpu effects tetherin degradation, which is dependent on the endosomal sorting complex required for transport (ESCRT) machinery that traffics ubiquitylated proteins from endosomes to lysosomes. Neil described how UBAP1 is a novel ESCRT-I subunit essential for virus antagonist-mediated degradation of tetherin [9], but not antagonism *per se*.

This observation provides a mechanism to uncouple antagonism and degradation. However, in terms of the degradative mechanism of Vpu activity towards tetherin, Neil explained that the two proteins associate in the Golgi apparatus to enable a clathrin-driven mechanism of lysosomal degradation, although the precise cellular cofactors are currently unknown. Nevertheless, his laboratory has identified determinants within the second α -helical domain of the Vpu cytoplasmic domain that are required for cell-free release of HIV from tetherin-expressing cells. This motif, EXXXLV, is a putative trafficking motif that prevents ESCRT-mediated degradation of tetherin and Vpu complexes [10]. Importantly, the motif is not required for the physical interaction of Vpu with tetherin, emphasizing the separation of antagonism and degradation.

While tetherin restricts the release of cell-free Vpu-deficient HIV virions, paradoxically, it does not block cell–cell transmission of the virus. Rather, it enhances this mechanism of spread through virological synapses [11]. It may do so either by increasing the virion density at the cell surface or by regulating the integrity of the virological synapse. These data suggest the role of Vpu may be to circumvent cell–cell transfer of HIV-1 and increase virus dissemination, perhaps as an immune countermeasure.

The Darwinian nature of Vpu was also corroborated by Neil's laboratory, describing the sequences of Vpu alleles from HIV-1-infected individuals over time. These data pointed to functional conservation of these Vpu alleles in the context of tetherin antagonism despite evidence of considerable immune-driven sequence variation. Thus, this role for Vpu appears functionally important *in vivo*. Neil explained that the widely dispersed group M strains of HIV-1 encode tetherin-antagonizing Vpu proteins. By contrast, group O viruses cannot counteract human tetherin. Taken together, these data suggest that the incomplete adaptation of the Vpu of group O strains may account, at least in part, for the poor penetration of these strains.

The role of SUMO conjugation in mediating intrinsic antiviral defence during HSV-1 infection**Chris Boutell (University of Glasgow, UK)**

Chris Boutell focused on SUMO as an intrinsic antiviral defense against HSV. He discussed the concept of intrinsic immunity, which is also called 'intrinsic antiviral resistance'. This aspect of immunity is the first line of intracellular defense, and when compared with either adaptive or IFN-induced innate immunity, it is mediated by pre-existing cellular factors.

Boutell discussed HSV-1 evasion of intrinsic antiviral resistance. His work in this context has focused on the HSV-1 ICP0. Similar to all herpesviruses, HSV-1 can exist in either of two transcriptional states: latency, when a minimum number of virus gene products are expressed to ensure partition of the virus genome into daughter cells; and lytic replication, when new virus progeny are produced. ICP0 is an E3 ubiquitin ligase, belonging to the RING finger family, and is required for initiation of HSV-1 lytic infection and reactivation of latent genomes.

Previous work by Boutell and colleagues showed that ICP0 is located in the promyelocytic leukemia (PML) nuclear bodies (also called 'ND10 domains' or 'PODs'), and that PMLs migrate to sites of viral genomes in the absence of ICP0. ICP0 effects proteasomal degradation of PML components including PML modified by SUMO and SUMO-modified Sp100. This activity is functionally relevant because ICP0-deletion mutants of HSV-1 replicate to titers 1000-fold less than wild-type virus. However, depletion of PML or other PML proteins from the cell rescues the replication of the ICP0-deletion mutant by tenfold. This evidence suggests that ICP0 regulates proteasome-dependent degradation of PML proteins that repress HSV-1 replication. Indeed, the recruitment of the HSV-1-inhibitory PML components (PML, Sp100 and hDaxx) to sites on the viral genome is dependent upon their SUMO interaction motifs (SIMs) [12]. This SUMO-dependent cellular response is therefore an intrinsic antiviral defense mechanism, but it is antagonized by ICP0.

What is the mechanism underlying ICP0 antagonism of this intrinsic antiviral defense mechanism to alleviate repression of HSV-1 gene expression? Boutell explained his group's recent findings that elucidate the process. They showed that the recruitment of ND10 proteins to sites of HSV-1 genomes depended upon the SUMO conjugation pathway, because when

the E2 SUMO-conjugating enzyme Ubc9 was depleted from cells, the replication of an ICP0-deleted mutant of HSV-1 was partially rescued [13].

Furthermore, SUMO-targeted ubiquitin ligases (STUbLs) are a class of cellular RING finger ubiquitin ligases containing SIMs. They regulate the levels of SUMO-modified substrate proteins by orchestrating their ubiquitination and proteasome-mediated degradation. Boutell and colleagues recognized the presence of multiple SIM-like sequences in ICP0, and on that basis, proceeded to demonstrate that the viral protein represents a viral STUbL [13]. Thus, ICP0 could affect the degradation of SUMO-modified PML isoforms in a RING finger- and proteasome-dependent mechanism. It could also cause the degradation of PML isoform I in a SUMO-independent manner. These data indicate that ICP0 functions through SUMO- and substrate-dependent activities. Boutell also demonstrated that ICP0 orthologs from bovine herpes virus-1, equine herpes virus-1 and pseudorabies virus all retain STUbL activity, suggesting that this activity is not unique to HSV-1. Boutell and colleagues have thus identified a new class of viral proteins, the viral STUbLs, and their role in the evasion of this SUMO-mediated intrinsic immune defense mechanism.

TRIM5 is an innate immune sensor for the capsid of HIV-1 & other retroviruses**Jeremy Luban (University of Geneva, Switzerland)**

Jeremy Luban spoke on the role of TRIM5 as a restriction factor that blocks retrovirus infection. TRIM5 has a B-box, RING and coiled-coil domain and is an E3 ubiquitin ligase. TRIM5 targets the capsid protein lattice in the virion that encapsidates the retroviral genomic RNA [14]. TRIM5, along with UBC13/UEV1A, can activate TAK1 (MAP3K7) kinase, NF- κ B and AP-1 signaling, leading to upregulation of inflammatory cytokines and chemokines [14]. The carboxy-terminal PRYSPRY or cyclophilin domain of TRIM5 interacts with the retroviral capsid, inducing the formation of lattice by TRIM5 with potent E3 ubiquitin activity. TRIM5 can synthesize K63-linked ubiquitin chains *in vitro* that are not attached to any protein. These ubiquitin chains induce the phosphorylation and activation of TAK1. Thus, Luban proposed that TRIM5 might be a PRR itself and be more specifically involved in recognizing retroviral infection [15].

There is also clinical significance of TRIM5 for HIV infection and disease progression.

Expression differences and TRIM5 polymorphisms influence rates of HIV-1 acquisition and/or disease progression [16]. Moreover, TRIM5 in nonhuman primates also plays an important role in limiting transmission of SIVs and in controlling the outcome of infection with these viruses [17].

IFN signaling pathways & viral infection

Richard Randall (University of St Andrews, UK)

Richard Randall talked about how defects in the type I IFN signaling pathways in human cells could affect their susceptibility to different virus infections. The V protein of parainfluenza virus 5 (PIV5) antagonizes IFN by inhibiting MDA-5 activation and indirectly inhibiting RIG-I activation [18]. PIV5 V protein blocks IFN signaling by targeting the STAT1 protein for proteasomal degradation [19]. During PIV5 replication, it is the defective interfering particles that are mostly responsible for IFN induction. IFN-stimulated genes that are induced during PIV5 replication are not the same as those that are induced during influenza replication.

Randall next discussed how cells from a patient with a primary immunodeficiency syndrome were defective in IFN signaling. When cells from this patient were infected with either PIV5, bunyavirus or measles virus, the plaque size was much larger as compared with the plaque size of cells from a normal individual that were infected with these same viruses. He correlated plaque size with the ability of cells to mount an IFN response. Hence, larger plaques correlated with a diminished IFN response. The larger plaques made by these viruses on the immunodeficient patient's cells were due to loss of STAT2 expression, as the patient had a mutation that affected the splicing of STAT2 mRNA. However, for the most part, STAT1 signaling was not affected in these cells. When the immunodeficient patient's cells were pretreated with IFN- α prior to viral infection, there was no protection from viral infection. However, when the patient's cells were pretreated with IFN- γ prior to viral infection, there was partial protection from viral infection, suggesting that the type I IFN pathway was primarily affected. Most surprisingly, despite the aforementioned defects in STAT2 signaling, the patient is alive and doing quite well. Randall said that this was in stark contrast to individuals with STAT1 deficiency, who usually die shortly after birth. Thus, STAT2 deficiency has a milder phenotype

as compared with STAT1 deficiency in humans, Randall concluded.

KSHV & innate immunity

Blossom Damania (University of North Carolina at Chapel Hill, NC, USA)

Blossom Damania focused on the innate immune response to Kaposi's sarcoma-associated herpesvirus (KSHV; also known as 'human herpesvirus 8') and the viral counter-response. KSHV is linked to several human malignancies. She discussed how the human host cell responded to KSHV primary infection by mounting a TLR or NLR response and how the virus counteracted these responses to evade host innate immunity.

Damania discussed how KSHV was recognized by the host immune system upon entry into monocytes and plasmacytoid dendritic cells (pDCs). Similar to other herpesviruses, KSHV establishes life-long latency in the human host. TLRs are one arm of the host innate immune system that are present either on the cytoplasmic membrane or within endosomes. KSHV primary infection of monocytes led to the upregulation of TLR3 and its downstream effectors, CXCL10 and IFN- β [20]. siRNA-mediated knockdown of TLR3 in primary human monocytes and a monocytic cell line led to decreased TLR3, IFN- β and CXCL10 expression levels following KSHV infection. Although TLR3 is an RNA sensor, it is likely that this PRR is recognizing viral RNAs present in the tegument of the KSHV virion.

Human pDCs do not express TLR3, but rather express TLR7 and TLR9. Damania discussed how KSHV primary infection of human pDCs led to activation of TLR9, a DNA sensor [21]. Inhibition of TLR9 in pDCs resulted in decreased IFN- α secretion following KSHV infection. She also described the establishment of latent KSHV-infected monocytes and the viral gene expression profile of these cells. Interestingly, she reported that certain cytokines and genes involved in the host immune response were downregulated during latent KSHV infection in monocytes, including CD80, CD86, TNF- α and IL-1 β [22]. She suggested that KSHV viral genes expressed in these latently infected monocytes could downregulate expression of these immune modulators.

The NLR family of proteins senses microbial infections and activates a multiprotein complex called the inflammasome. Activation of NLR-dependent inflammasomes leads to the production and activation of IL-1 β and IL-18. Damania reported that primary KSHV

infection of monocytes led to an increase in IL-1 β production, suggesting that the virus activated the inflammasome pathway during primary infection. However, knockdown of the NLR family member NLRP1 led to a decrease in IL-1 β secretion following KSHV infection. She found that the virus counteracted the inflammasome response by delivering a KSHV protein encoded by open reading frame (Orf)63 of the virus. KSHV Orf63 showed similarity to human NLRP1 in the nucleotide-binding domain and the leucine-rich repeat region, but unlike human NLRP1, KSHV Orf63 did not contain the Pyrin or CARD-activation domains. She went on to demonstrate that KSHV Orf63 blocked NLRP1-dependent caspase-1 activation and production of IL-1 β and IL-18 [23]. Furthermore, knockdown of Orf63 induced the secretion of IL-1 β from KSHV-infected cells. Finally, she showed that Orf63 was necessary for efficient reactivation and generation of progeny virus. Damania concluded by suggesting that NLRs and inflammasomes play a role in sensing KSHV and the virus has evolved a strategy to counteract this host innate immune response.

Coronaviruses RNA-processing enzymes: innate immune evasion by stealth

Volker Thiel (Cantonal Hospital St Gallen, Switzerland)

Volker Thiel discussed the innate immune recognition of coronaviruses, including SARS, human coronavirus 229E (HCoV-229E) and mouse hepatitis virus (MHV). Thiel and colleagues found that pDCs controlled both SARS and MHV replication through TLR7-mediated IFN induction [24]. Additionally, MDA-5, but not RIG-I, has been shown to be involved in the recognition of coronaviruses during primary infection.

Control of MHV replication depends primarily on IFN- α receptor expression on hematopoietic cells. Specifically, IFN- α receptor expression on LysM⁺ macrophages and CD11c⁺ dendritic cells was critical for restriction of MHV replication within secondary lymphoid organs, and also restricted lethal liver disease. Thus, Thiel demonstrated that the cross-talk between pDCs and conventional dendritic cells and macrophages was chiefly responsible for controlling MHV infection *in vivo* [25].

Another topic discussed by Thiel was the way coronaviruses evade host innate immunity. Higher eukaryotic mRNAs have 5'-cap structures that are methylated (ribose 2'-O-methylation).

Many viruses that replicate in the cytoplasm encode 2'-O-methyltransferases to modify their viral mRNAs, including SARS, HCoV-229E and MHV. 2'-O-methylation of viral mRNA is critical for hindering the induction of type I IFN. Both HCoV-229E and MHV mutants lacking 2'-O-methyltransferase activity triggered a much higher expression of type I IFN than wild-type viruses, and these mutant viruses were highly sensitive to IFN. The induction of type I IFN was dependent on the RIG-I-like receptor family member, MDA-5 [26,27]. Thiel concluded by highlighting how coronaviruses evade the MDA-5-triggered innate immune response.

Function & mechanism of RNA silencing suppression by RNA viruses

Shou-Wei Ding (University of California, Riverside, CA, USA)

Shou-Wei Ding presented his work on RNA-silencing suppression mechanisms by RNA viruses. He focused on different viral suppressors of RNAi functions encoded by viruses capable of infecting different species. These included viruses that infect plants (e.g., cucumber mosaic virus [CMV]), viruses that infect insects (e.g., flock house virus [FHV]) and viruses that infect mammals (e.g., influenza virus).

Ding showed that the 2b protein of CMV inhibits the accumulation of viral siRNAs produced by the infected host cells. A CMV 2b-deletion mutant can replicate in plant cells that do not express Dicer, but cannot replicate in wild-type plant cells [28]. The 2b protein interacts directly with Ago1 and 4 proteins in the nucleolus. This viral protein has a dsRNA binding domain that is needed for RNAi suppression *in vivo*. Additionally, the CMV 2b protein appears to repress post-transcriptional gene silencing as well as RNA-directed DNA methylation by binding and sequestering siRNA and the long dsRNA precursor [29].

A similar situation occurs in insects. Replication of RNA viruses in *Drosophila* and mosquitoes produces viRNAs that lower virus accumulation through RNAi. Replication of FHV results in the production of a 400-bp dsRNA that is acted upon by cellular Dicer-2 to generate the viRNAs [30]. These viRNAs bind Ago2 and are methylated. To counteract this host defense mechanism, FHV encodes an RNAi suppressor named B2 that interacts with both viral dsRNA and the RNA replicase, thereby inhibiting the generation of viRNAs [30]. While wild-type FHV can replicate in adult

flies, a B2-deletion FHV mutant virus is hindered for replication in adult flies. However, this mutant virus could replicate in Dicer-2- and Ago2-mutant flies [31].

As noted above, the antiviral response in *Drosophila* is mediated by the RNAi pathway. Human influenza viruses encode an NS1 protein that suppresses this pathway in *Drosophila*. Influenza NS1 is a dsRNA-binding protein that inhibits the innate antiviral response and is essential for pathogenesis. The dsRNA-binding domain of NS1 is required for innate immune suppression [32].

However, unlike the situation in insects [33], there is no evidence that viral siRNAs are produced in RNA virus-infected mammalian cells. Ding also spoke about the impact that influenza replication in mammalian cells has on cellular miRNAs. He reported that influenza virus replication in mammalian cells results in a reduction in the biogenesis of some miRNAs by binding of the NS1 protein to the miRNA precursors. NS1's ability to inhibit the biogenesis of these miRNAs appears to confer a replicative and survival advantage on the virus.

Antiviral pathways in insects & how viruses suppress RNAi

Raul Andino (University of California at San Francisco, CA, USA)

Raul Andino spoke of the various antiviral functions of the RNAi pathway in insects. Cricket paralysis virus (CrPV) in Ago2- and Dicer-2-knockout insect cells leads to decreased cell survival and increased viral replication. The CrPV suppressor protein CrPV-1A interacts and colocalizes with Ago2 and inhibits its activity [34]. However, CrPV-1A does not inhibit the miRNA Ago1-mediated silencing pathway.

Andino next spoke about the ability of multicellular organisms to respond to infection at the site of infection, as well as distal uninfected sites. RNAi is important for antiviral immunity in flies at the site of infection. However, a dsRNA-uptake pathway is required for systemic antiviral RNAi immunity in adult flies. Flies with deletions in the dsRNA-uptake pathway were very sensitive to *Drosophila* C virus (DCV) and Sindbis virus infection, with high mortality rates [35]. Andino stated that the spread of dsRNA to distal, uninfected sites is required for antiviral immunity in flies. Thus, there is a systemic signal involved in antiviral immunity in flies, a concept that is similar to protein-based immunity in vertebrates. To counteract these antiviral host immune

responses, DCV encodes a suppressor protein, DCV-1A, which is a dsRNA-binding protein [36]. DCV-1A inhibits RISC assembly and copurifies with RISC. Andino summarized his talk by stating that RNAi is a major antiviral system in insects, and there is a newly discovered systemic antiviral pathway mediated by dsRNA. To counter these responses, insect viruses induce the expression of certain host miRNAs to moderate the antiviral response, and viral suppressor proteins target the RNAi machine in the host cell.

Conclusion

These international leaders focused on many different aspects of innate and intrinsic immunity in their contributions to the 'Innate Barriers to Infection' session at the spring 2012 SGM meeting. Different innate immune pathways that were activated upon RNA and DNA virus infection were described in plant, insect and mammalian cells. New host innate immune modulators were described that were capable of recognizing specific components of these viruses. Additionally, various mechanisms by which viral-encoded suppressors could down-regulate these innate immune pathways in order to evade host immunity were discussed.

Two take-home messages were apparent: first, that studying the interactions of viruses with their hosts has inherent value by contributing fundamental knowledge on the laws of nature by which viruses operate; second, that by studying virus counter defense mechanisms, we gain better understanding of the mechanisms and machinations of the innate and intrinsic immune systems. With current technologies and intellects, we are in the midst of an enthralling epoch for understanding virus host immune system interactions. Surely we will not have long to wait for the next exciting development.

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References

- Unterholzner L, Keating SE, Baran M *et al.* IFI16 is an innate immune sensor for intracellular DNA. *Nat. Immunol.* 11(11), 997–1004 (2010).
- Hornung V, Ablasser A, Charrel-Dennis M *et al.* AIM2 recognizes cytosolic dsDNA and forms a caspase-1-activating inflammasome with ASC. *Nature* 458(7237), 514–518 (2009).
- Harris RS, Bishop KN, Sheehy AM *et al.* DNA deamination mediates innate immunity to retroviral infection. *Cell* 113(6), 803–809 (2003).
- Sheehy AM, Gaddis NC, Choi JD, Malim MH. Isolation of a human gene that inhibits HIV-1 infection and is suppressed by the viral Vif protein. *Nature* 418(6898), 646–650 (2002).
- Suspene R, Aynaud MM, Guetard D *et al.* Somatic hypermutation of human mitochondrial and nuclear DNA by APOBEC3 cytidine deaminases, a pathway for DNA catabolism. *Proc. Natl Acad. Sci. USA* 108(12), 4858–4863 (2011).
- Neil SJ, Zang T, Bieniasz PD. Tetherin inhibits retrovirus release and is antagonized by HIV-1 Vpu. *Nature* 451(7177), 425–430 (2008).
- Perez-Caballero D, Zang T, Ebrahimi A *et al.* Tetherin inhibits HIV-1 release by directly tethering virions to cells. *Cell* 139(3), 499–511 (2009).
- Vigan R, Neil SJ. Determinants of tetherin antagonism in the transmembrane domain of the human immunodeficiency virus type 1 Vpu protein. *J. Virol.* 84(24), 12958–12970 (2010).
- Agromayor M, Soler N, Caballe A *et al.* The UBAP1 subunit of ESCRT-I interacts with ubiquitin via a SOUBA domain. *Structure* 20(3), 414–428 (2012).
- Kueck T, Neil SJ. A cytoplasmic tail determinant in HIV-1 Vpu mediates targeting of tetherin for endosomal degradation and counteracts interferon-induced restriction. *PLoS Pathog.* 8(3), e1002609 (2012).
- Jolly C, Booth NJ, Neil SJ. Cell–cell spread of human immunodeficiency virus type 1 overcomes tetherin/BST-2-mediated restriction in T cells. *J. Virol.* 84(23), 12185–12199 (2010).
- Cuchet-Lourenco D, Boutell C, Lukashchuk V *et al.* SUMO pathway dependent recruitment of cellular repressors to herpes simplex virus type 1 genomes. *PLoS Pathog.* 7(7), e1002123 (2011).
- Boutell C, Cuchet-Lourenco D, Vanni E *et al.* A viral ubiquitin ligase has substrate preferential SUMO targeted ubiquitin ligase activity that counteracts intrinsic antiviral defence. *PLoS Pathog.* 7(9), e1002245 (2011).
- Pertel T, Hausmann S, Morger D *et al.* TRIM5 is an innate immune sensor for the retrovirus capsid lattice. *Nature* 472(7343), 361–365 (2011).
- Lukic Z, Hausmann S, Sebastian S *et al.* TRIM5 α associates with proteasomal subunits in cells while in complex with HIV-1 virions. *Retrovirology* 8, 93 (2011).
- Van Manen D, Rits MA, Beugeling C, Van Dort K, Schuitemaker H, Kootstra NA. The effect of *Trim5* polymorphisms on the clinical course of HIV-1 infection. *PLoS Pathog.* 4(2), e18 (2008).
- Grutter MG, Luban J. TRIM5 structure, HIV-1 capsid recognition, and innate immune signaling. *Curr. Opin. Virol.* 2(2), 142–150 (2012).
- Childs K, Stock N, Ross C *et al.* MDA-5, but not RIG-I, is a common target for paramyxovirus V proteins. *Virology* 359(1), 190–200 (2007).
- Precious BL, Carlos TS, Goodbourn S, Randall RE. Catalytic turnover of STAT1 allows PIV5 to dismantle the interferon-induced anti-viral state of cells. *Virology* 368(1), 114–121 (2007).
- West J, Damania B. Upregulation of the TLR3 pathway by Kaposi's sarcoma-associated herpesvirus during primary infection. *J. Virol.* 82(11), 5440–5449 (2008).
- West JA, Gregory SM, Sivaraman V, Su L, Damania B. Activation of plasmacytoid dendritic cells by Kaposi's sarcoma-associated herpesvirus. *J. Virol.* 85(2), 895–904 (2011).
- Gregory SM, Wang L, West JA, Dittmer DP, Damania B. Latent Kaposi's sarcoma-associated herpesvirus infection of monocytes downregulates expression of adaptive immune response costimulatory receptors and proinflammatory cytokines. *J. Virol.* 86(7), 3916–3923 (2012).
- Gregory SM, Davis BK, West JA *et al.* Discovery of a viral NLR homolog that inhibits the inflammasome. *Science* 331(6015), 330–334 (2011).
- Cervantes-Barragan L, Zust R, Weber F *et al.* Control of coronavirus infection through plasmacytoid dendritic-cell-derived type I interferon. *Blood* 109(3), 1131–1137 (2007).
- Cervantes-Barragan L, Kalinke U, Zust R *et al.* Type I IFN-mediated protection of macrophages and dendritic cells secures control of murine coronavirus infection. *J. Immunol.* 182(2), 1099–1106 (2009).
- Zust R, Cervantes-Barragan L, Habjan M *et al.* Ribose 2'-O-methylation provides a molecular signature for the distinction of self and non-self mRNA dependent on the RNA sensor MDA5. *Nat. Immunol.* 12(2), 137–143 (2011).
- Daffis S, Szretter KJ, Schriewer J *et al.* 2'-O methylation of the viral mRNA cap evades host restriction by IFIT family members. *Nature* 468(7322), 452–456 (2010).
- Diaz-Pendon JA, Li F, Li WX, Ding SW. Suppression of antiviral silencing by cucumber mosaic virus 2b protein in *Arabidopsis* is associated with drastically reduced accumulation of three classes of viral small interfering RNAs. *Plant Cell* 19(6), 2053–2063 (2007).
- Duan CG, Fang YY, Zhou BJ *et al.* Suppression of *Arabidopsis* ARGONAUTE1-mediated slicing, transgene-induced RNA silencing, and DNA methylation by distinct domains of the cucumber mosaic virus 2b protein. *Plant Cell* 24(1), 259–274 (2012).
- Aliyari R, Wu Q, Li HW *et al.* Mechanism of induction and suppression of antiviral immunity directed by virus-derived small RNAs in *Drosophila*. *Cell Host Microbe* 4(4), 387–397 (2008).
- Han YH, Luo YJ, Wu Q *et al.* RNA-based immunity terminates viral infection in adult *Drosophila* in the absence of viral suppression of RNA interference: characterization of viral small interfering RNA populations in wild-type and mutant flies. *J. Virol.* 85(24), 13153–13163 (2011).
- Li WX, Li H, Lu R *et al.* Interferon antagonist proteins of influenza and vaccinia viruses are suppressors of RNA silencing. *Proc. Natl Acad. Sci. USA* 101(5), 1350–1355 (2004).
- Abdul-Sater AA, Said-Sadier N, Lam VM *et al.* Enhancement of reactive oxygen species production and chlamydial infection by the mitochondrial NOD-like family member NLRX1. *J. Biol. Chem.* 285(53), 41637–41645 (2010).
- Nayak A, Berry B, Tassetto M *et al.* Cricket paralysis virus antagonizes Argonaute 2 to modulate antiviral defense in *Drosophila*. *Nat. Struct. Mol. Biol.* 17(5), 547–554 (2010).
- Saleh MC, Tassetto M, Van Rij RP *et al.* Antiviral immunity in *Drosophila* requires systemic RNA interference spread. *Nature* 458(7236), 346–350 (2009).
- Van Rij RP, Saleh MC, Berry B *et al.* The RNA silencing endonuclease Argonaute 2 mediates specific antiviral immunity in *Drosophila melanogaster*. *Genes Dev.* 20(21), 2985–2995 (2006).