Innate DNA sensing moves to the nucleus

Leonie Unterholzner and Andrew G. Bowie

School of Biochemistry and Immunology, Trinity College Dublin, Dublin 2, Ireland

Correspondence: agbowie@tcd.ie

Summary:

It has been assumed that cells distinguish viral from cellular DNA due to the former's presence in the cytosol. However, in this issue, Kerur *et al*. propose that the DNA genome of Kaposi's sarcoma associated herpesvirus (KSHV) is recognized inside the nucleus by the DNA sensor IFI16 leading inflammasome activation.

Main text:

When a cell is infected with a virus, it responds by producing type I interferons (IFNs), pro-inflammatory cytokines and chemokines. These then alert neighbouring cells to the presence of a virus, and aid in the recruitment of cells of the innate and adaptive immune system to the site of infection. The production of type I IFNs, cytokines and chemokines is primarily regulated by transcription factors such as interferon regulatory factors (IRFs) and nuclear factor κB (NF-κB). A second level of regulation is required for the secretion of the pro-inflammatory cytokines interleukin-1β (IL-1β) and IL-18: these cytokines are synthesised as immature precursor proteins which need to be cleaved by the enzyme caspase-1 in order to be secreted as biologically active forms. Caspase-1 is itself activated by proteolytic cleavage, as a component of a multiprotein complex termed the inflammasome. Transcription factor activation follows the sensing of virus-derived nucleic acids, such as viral RNA or

DNA genomes, or RNA species generated during viral transcription or replication, by cellular pattern recognition receptors (PRRs). For example Toll-like receptors (TLRs) sense viral nucleic acids in endosomes. RIG-I-like receptors recognise viral RNA in the cytosol, leading to both transcription factor and caspase 1 activation (O'Neill and Bowie, 2010). In contrast to viral RNA detection, PRRs that sense intracellular DNA leading to gene induction and inflammasome activation are only beginning to be discovered. The first cytosolic DNA receptor identified was the protein DAI, which can sense DNA, including synthetic poly(dA-dT), and uses the essential signaling adaptor STING in order to induce type I IFN induction (Rathinam and Fitzgerald, 2011). DAI, like other recently described DNA sensors mediating gene induction such as the helicases DHX9 and DHX36, and Ku70 have either cell type- or gene-specific roles (Rathinam and Fitzgerald, 2011). A key inflammasome activating DNA sensor recently identified is AIM2, a cytosolic member of the PYHIN protein family, which contains a DNA-binding HIN domain at its C terminus and an N-terminal Pyrin domain which mediates its interaction with the inflammasome component ASC (Rathinam and Fitzgerald, 2011). Importantly, AIM2 has been shown to mediate caspase 1 activation in response to pathogen DNA in vivo (Fernandes-Alnemri et al., 2010; Rathinam et al., 2010).

Recently another human PYHIN protein related to AIM2, IFI16, has been shown to mediate DNA-induced transcription factor activation (Unterholzner et al., 2010). In contrast to AIM2, IFI16 has a predominantly nuclear localization. However, IFI16 directly bound to IFN-stimulating viral DNA, co-localised with transfected DNA in the cytosol, and recruited STING, causing type I IFN and cytokine induction (see Figure 1). IFI16, and the related murine protein p204, were essential for IFN induction mediated by infection of cells with Herpes Simplex Virus 1 (HSV-1), a

DNA virus that replicates in the nucleus (Unterholzner et al., 2010). In this issue of *Cell Host and Microbe*, Kerur et al. now describe a further function of IFI16 as an innate immune DNA sensor for a second DNA virus, and show that IFI16 can sense viral DNA in the nucleus, leading to inflammasome activation (Kerur et al., 2011).

The paper examines endothelial cells infected with the Kaposi's sarcoma-associated herpesvirus (KSHV), and shows that in these cells IFI16 is required for KSHV-mediated activation of the inflammasome, and consequently the processing of pro-IL-1β to active IL-1β during viral infection. KSHV is a DNA virus that establishes a latent infection, maintaining an episomal circular DNA genome in the nucleus of infected cells. Kerur et al. show that KSHV infection causes the activation of an ASC-containing inflammasome, with concomitant proteolytic processing of pro-caspase-1 and pro-IL-1β (figure 1). IFI16 associates with ASC in endothelial cells, and this association is increased between 2 and 48h of infection with KSHV, but not after exposure to UV-inactivated virus. Furthermore, while both IFI16 and ASC are predominantly nuclear in uninfected endothelial cells, they co-localize and move out of the nucleus to the peri-nuclear region in KSHV-infected cells. Using shRNAs targeting IFI16 or ASC, Kerur et al. demonstrate that both proteins are essential for the virus-induced processing of caspase-1. By over-expressing IFI16, ASC, pro-caspase 1 and pro-IL-1β in HEK293 cells, which normally lack IFI16 and inflammasome components, the authors are able to reconstitute KSHV-induced pro-IL-1β processing.

The results are somewhat surprising since IFI16 had previously been excluded as potential inflammasome activator, as, in contrast to AIM2, it does not constitutively activate the inflammasome when over-expressed (Hornung et al., 2009). However Kerur et al demonstrate that it is IFI16, rather than AIM2, which is

responsible for KSHV-induced activation of the inflammasome, as shRNA targeting AIM2 had no effect on pro-caspase-1 processing in KSHV-infected endothelial cells. They do however observe a transient association between AIM2 and ASC 2h after KSHV infection, suggesting that AIM2 may contribute to KSHV sensing at that time, possibly while the virus particle transits through the cytoplasm before the establishment of latency in the nucleus. IFI16 appears to have a dual function in the KSHV-induced production of pro-inflammatory cytokines in this system, as in addition to being required for inflammasome activation, pro-IL-1β and IL-6 transcription were also IFI16-dependent. As pro-IL-1β and IL-6 are NF-κB-dependent genes, this is consistent with the previously described function of IFI16 as an activator of this transcription factor during infection with the herpesvirus HSV-1 (Unterholzner et al., 2010). Apart from NF-κB activation, HSV-1 required IFI16 for IRF3 activation and type I IFN induction, so it will be interesting to determine if this is true for KSHV, and/or other herpesviruses. Furthermore, it has yet to be determined whether the function of IFI16 as an inflammasome activator can be extended to other cell types, apart from endothelial cells, or other DNA viruses, apart from KSHV.

A further, particularly intriguing hypothesis presented by Kerur *et al*. is that IFI16 senses KSHV DNA in the nucleus, and that it may activate a nuclear pool of ASC before exiting the nucleus to cause pro-IL-1β processing in the cytosol (figure 1). The authors present several lines of evidence that support this notion: They observe co-localization of KSHV genomic DNA with IFI16 in the nucleus of infected endothelial cells, and co-localization of nuclear ASC and IFI16 at early times after infection. Also, they show that a predominantly nuclear form of ASC containing a nuclear localization signal can be activated by IFI16 to cause pro-caspase-1 processing when the system is reconstituted in HEK293 cells. Furthermore, the

function of IFI16 as an inflammasome activator may be particular to DNA viruses such as KSHV, which replicate and maintain their genomes in the nucleus, since IFI16 did not affect inflammasome activation following infection with vaccinia virus, a DNA virus which replicates in the cytosol, and which has previously been shown to activate an AIM2-containing inflammasome (Rathinam et al., 2010).

If IFI16 indeed recognizes the presence of viral DNA in the nucleus, the question arises: How does IFI16 distinguish viral genomic DNA from the abundance of cellular DNA in this location? While it had previously been assumed that it is the presence of DNA in the cytosol that acts as a distinguishing feature between self and exogenous DNA, this has never been formally demonstrated. Also, at least in the case of IFI16, DNA sensing leading to innate immune activation is independent of the sequence composition of the DNA (Unterholzner et al., 2010). It is possible that cellular DNA is protected from IFI16-mediated recognition due to its association with histones and other protective host proteins. An alternative, and particularly tantalizing explanation may be that IFI16 senses DNA damage before initiating its innate immune signaling functions. Many viruses, including herpesviruses such as KSHV cause a DNA damage response during the replication of their genome (Lilley et al., 2007), and this may alert IFI16 to the presence of a DNA virus in the nucleus. In fact, IFI16 is known to associate with components of the DNA damage response, such as BRCA1, and has been shown to assemble on genomic sites of DNA damage in a BRCA1-dependent manner (Aglipay et al., 2003). Also, IFI16 can translocate out of the nucleus following UV-mediated DNA damage in epithelial cells (Costa et al., 2010), in analogy to the nuclear export of IFI16 and ASC in response to KSHV infection observed by Kerur et al. Thus, it is tempting to speculate that IFI16 might be involved in the detection of both ‘stranger’, namely intracellular viral DNA, and

‘danger’ such as damaged DNA. Overall, the idea of innate immune detection of viral DNA in the nucleus is intriguing, and this study should provoke further investigation in order to test this hypothesis.

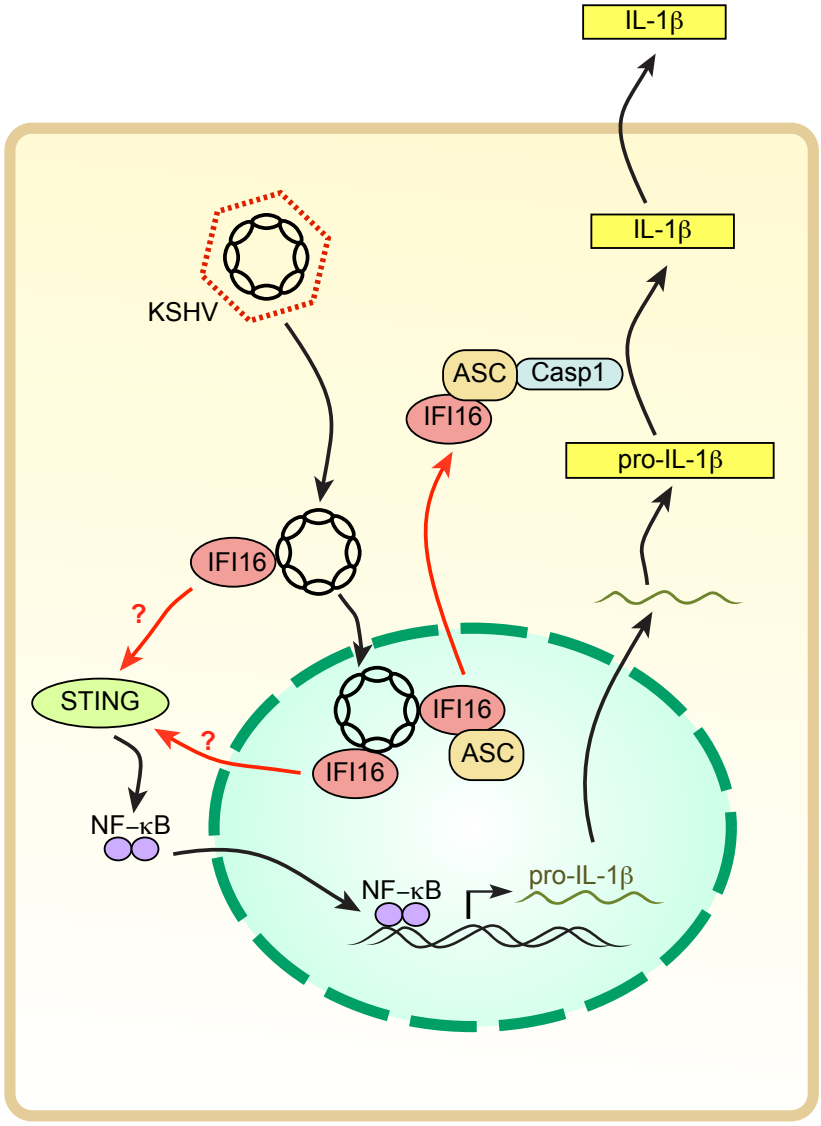

Figure legend:

Dual function of IFI16 in endothelial cells infected with Kaposi sarcoma-associated herpesvirus (KSHV). IFI16 can sense viral DNA and activate transcription factors such as NF-κB via the adaptor protein STING. This leads to the transcription of proinflammatory cytokines such as pro-interleukin-1β (pro-IL-1β). Kerur et al. demonstrate that, following the sensing of KSHV DNA in the nucleus, IFI16 can also associate with the inflammasome component ASC. This leads to the translocation of the IFI16-ASC complex to the cytosol, and the activation of caspase-1 (Casp-1), which processes pro-IL-1β to its active form IL-1β.

References

Aglipay, J.A., Lee, S.W., Okada, S., Fujiuchi, N., Ohtsuka, T., Kwak, J.C., Wang, Y., Johnstone, R.W., Deng, C., Qin, J.*, et al.* (2003). Oncogene *22*, 8931-8938.

Costa, S., Borgogna, C., Mondini, M., De Andrea, M., Meroni, P.L., Berti, E., Gariglio, M., and Landolfo, S. (2010). Br J Dermatol *164*, 282-290.

Fernandes-Alnemri, T., Yu, J.W., Juliana, C., Solorzano, L., Kang, S., Wu, J., Datta, P., McCormick, M., Huang, L., McDermott, E.*, et al.* (2010). Nat Immunol *11*, 385-393.

Hornung, V., Ablasser, A., Charrel-Dennis, M., Bauernfeind, F., Horvath, G., Caffrey, D.R., Latz, E., and Fitzgerald, K.A. (2009). Nature *458*, 514-518.

Kerur, N., Veettil, M.V., Sharma-Walia, N., Bottero, V., Sadagopan, S., Otageri, P., and Chandran, B. (2011). Cell Host and Microbe, this issue.

Lilley, C.E., Schwartz, R.A., and Weitzman, M.D. (2007). Trends Microbiol *15*, 119-126.

O'Neill, L.A., and Bowie, A.G. (2010). Curr Biol *20*, R328-333.

Rathinam, V.A., and Fitzgerald, K.A. (2011). Virology *411*, 153-162.

Rathinam, V.A., Jiang, Z., Waggoner, S.N., Sharma, S., Cole, L.E., Waggoner, L., Vanaja, S.K., Monks, B.G., Ganesan, S., Latz, E.*, et al.* (2010). Nat Immunol *11*, 395-402.

Unterholzner, L., Keating, S.E., Baran, M., Horan, K.A., Jensen, S.B., Sharma, S., Sirois, C.M., Jin, T., Latz, E., Xiao, T.S.*, et al.* (2010). Nat Immunol *11*, 997-1004.