A.G., Reid, H.H., Gras, S., Lucet, I.S., et al. (2009). Immunity *31*, 47–59.

Sullivan, B.A., Nagarajan, N.A., Wingender, G., Wang, J., Scott, I., Tsuji, M., Franck, R.W., Porcelli, S.A., Zajonc, D.M., and Kronenberg, M. (2010). J. Immunol. *184*, 141–153.

Wun, K.S., Borg, N.A., Kjer-Nielsen, L., Beddoe, T., Koh, R., Richardson, S.K., Thakur, M., Howell, A.R., Scott-Browne, J.P., Gapin, L., et al. (2008). J. Exp. Med. *205*, 939–949.

Wun, K.S., Cameron, G., Patel, O., Pang, S.S., Pellicci, D.G., Sullivan, L.C., Keshipeddy, S., Young, M.H., Uldrich, A.P., Thakur, M.S., et al. (2011). Immunity *34*, this issue, 327–339.

Yu, K.O., Im, J.S., Molano, A., Dutronc, Y., Illarionov, P.A., Forestier, C., Fujiwara, N., Arias, I., Miyake, S., Yamamura, T., et al. (2005). Proc. Natl. Acad. Sci. USA *102*, 3383–3388.

Caspase-8: Clipping off RIG-I Signaling

Susanne Roth[1] and Jürgen Ruland[1,2,*]
[1]Institut für Molekulare Immunologie, Klinikum rechts der Isar, Technische Universität München, 81675 Munich, Germany
[2]Laboratory of Signaling in the Immune System, Helmholtz Zentrum München - German Research Center for Environmental Health, 85764 Neuherberg, Germany
*Correspondence: jruland@lrz.tum.de
DOI 10.1016/j.immuni.2011.03.011

Activation of the RIG-I signaling molecule is essential for antiviral immunity but mechanisms downmodulating the response are ill defined. In this issue of *Immunity*, Rajput et al. (2011) describe caspase-8-mediated RIP1 cleavage as a key step for restricting RIG-I signaling.

The innate immune system senses microbial pathogens through pattern recognition receptors (PRRs) that recognize pathogen-associated molecular patterns (Takeuchi and Akira, 2010). PRR ligation triggers conserved intracellular signaling pathways that drive proinflammatory responses, which are crucial for productive innate and adaptive immunity and required for host protection. Still, inflammatory responses cannot last permanently and innate immune signaling needs to be negatively regulated and terminated to avoid tissue damage and autoimmune disease. Although the last decade has seen a rapid progress in deciphering the pathways that activate innate immunity, the mechanisms that limit these responses to secure homeostasis are not well defined.

The recognition of viruses by the innate immune system depends mainly on the ability to discriminate viral nucleic acids from host RNA or DNA. The major PRRs for virus-derived RNA, originating from either genomic RNA or replication intermediates, are the retinoic acid inducible gene I (RIG-I)-like helicases (RLHs) including RIG-I, MDA5, and LGP2 (Yoneyama and Fujita, 2009). RIG-I itself is required for the detection of specific RNA viruses including Newcastle disease virus, Sendai virus (SeV), vesicular stomatitis virus, influenza virus, and Japanese encephalitis virus. This receptor possesses a central DExD/H-box RNA helicase domain and a C-terminal repressor domain for ligand binding together with two caspase recruitment domains (CARDs) for signal propagation. RNA sensing by RIG-I induces an intramolecular conformational change that exposes the CARDs to allow association of RIG-I with the mitochondrial antiviral signaling adaptor (MAVS) (Yoneyama and Fujita, 2009). These events enable the formation of a large mitochondria-associated complex that contains several conserved signaling proteins, which also participate in other innate and inflammatory pathways. These factors include the serine-threonine kinase receptor interacting protein 1 (RIP1) together with TRADD, FADD, and caspase-8, all of which have additional well-recognized roles in TNF-receptor (TNFR) signaling pathways (Yoneyama and Fujita, 2009). Further recruitment of the regulatory IKK subunit NEMO to the activated RIG-I complex is essential for downstream signaling to interferon regulatory factors (IRFs) and NF-κB via IKK-related kinases.

In the present work, Rajput et al. (2011) describe a mechanism for how RIG-I signaling is limited in both duration and magnitude. This intriguing study was prompted by the surprising and incidental finding that a lentivirus, which specifically expresses a caspase-8 siRNA, potently inhibited the proliferation of fibroblast cell lines. These growth-inhibitory effects were directly caused by hyperactive IRF3 and were associated with an increased expression of interferon-stimulated genes. In contrast to lentiviral infection, transfection of oligonucleotide caspase-8 siRNA affected neither IRF3 activity nor cell proliferation. The authors therefore hypothesized that the infection with the lentivirus would trigger an IRF3 response, which is substantially enhanced in the absence of caspase-8. Consistently, SeV infection or transfection of specific RIG-I ligands resulted in a massively enhanced IRF3 signal in fibroblasts or primary keratinocytes from caspase-8-deficient mice. Moreover, SeV-induced IRF3 signaling was strongly augmented in conditional caspase-8-deficient hepatocytes in vivo.

To understand the molecular basis for these observations, Rajput et al. (2011) studied functional interactions of caspase-8 and RIG-I. They revealed that caspase-8 can directly bind to RIG-I and in addition observed a virus-induced cotranslocation of caspase-8 and RIP1 to the mitochondrial RIG-I complex. Importantly, RIP1 was gradually cleaved

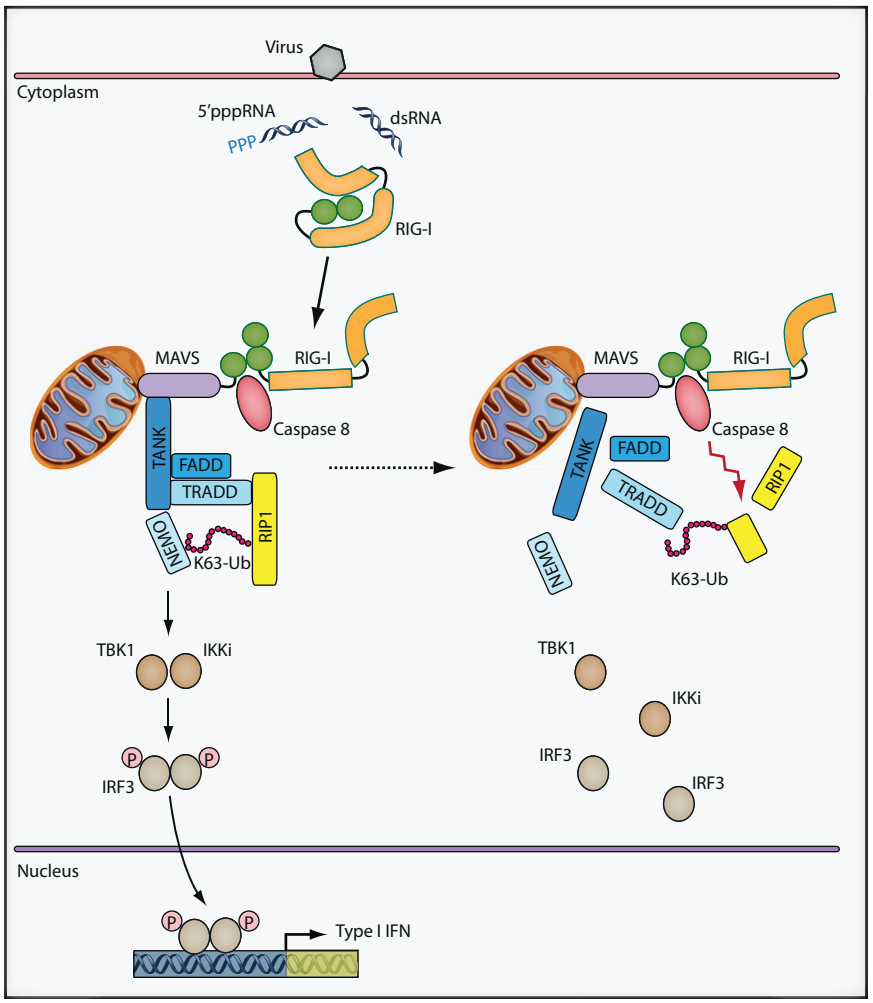

Figure 1. Positive and Negative Regulation of Virus-Induced IRF3 Activation by RIP1 and Caspase-8

RNA recognition by RIG-I leads to the formation of a mitochondria-associated signaling complex for the induction of type I interferon responses. Lysine 63-linked polyubiquitinated RIP1 is required for maximal IRF3 activity and serves subsequently as a prerequisite for caspase-8-mediated cleavage of RIP1. The proteolysis of RIP1 destabilizes the RIG-I complex and generates a short IRF3 inhibiting RIP1 fragment to downmodulate the inflammatory response. Abbreviations: FADD, Fas-associated protein with death domain; IFN, interferon; IKKi, inhibitor of NF-κB (IκB) kinase-i; IRF3, interferon regulatory factor 3; K63-Ub, lysine 63-linked polyubiquitin; MAVS, mitochondrial antiviral signaling adaptor; NEMO, NF-κB essential modulator; RIG-I, retinoic acid inducible gene I; dsRNA, double-stranded RNA; 5′pppRNA, 5′-triphosphate RNA; RIP1, receptor interacting protein 1; TANK, TNFR-associated factor (TRAF)-associated NF-κB activator; TBK1, TANK-binding kinase 1; TRADD, TNFR1-associated death domain protein.

after SeV infection, resulting in the appearance of a truncated 38 kDa RIP1 fragment. This processing of RIP1 was strictly dependent on proteolytic caspase-8 activity, which was confirmed in experiments with caspase-8 inhibitory peptides and genetic reconstitutions of caspase-8-deficient cells with enzymatically inactive caspase-8 variants. Previous work in the TNFR1 signaling pathway has established that caspase-8 can directly cleave RIP1 at Asp-324 (Lin et al., 1999). Likewise, SeV infection induced a proteolytic RIP1 processing directly at Asp-324.

Intriguingly, the short RIP1 fragment was not observed in the absence of caspase-8. Instead, a prominent high-molecular-weight form of RIP1 appeared upon SeV infection of caspase-8-deficient cells, representing lysine 63-linked polyubiquitinated RIP1. Similar to TNF-α stimulation, virus infection triggered RIP1 polyubiquitination specifically at Lys-377 (Ea et al., 2006; Wu et al., 2006). Interestingly, this polyubiquitinated RIP1 form accumulated selectively in caspase-8-deficient cells but not in wild-type cells. Moreover, a K377R mutation that prevented RIP1 ubiquitination also prohibited caspase-8-mediated RIP1 cleavage. Therefore, Rajput et al. (2011) concluded that virus-induced polyubiquitination of RIP1 is a crucial prerequisite for its processing by caspase-8.

Cell stimulation with TNF-α leads to a direct recruitment of NEMO to polyubiquitinated RIP1 with a subsequent activation of IKK (Ea et al., 2006; Wu et al., 2006). To study potential cell-activating roles for RIP1 polyubiquitination in the RIG-I pathway, RIP1-deficient cells were reconstituted with the K377R RIP1 mutants. Infection experiments demonstrated maximal virus-induced IRF3 signaling only upon lysine 63-linked polyubiquitination of RIP1. Additional reconstitution studies were performed with D324A RIP1 mutants, which are resistant to caspase-8-mediated cleavage. These cells exhibited strongly enhanced IRF responses upon SeV infection that were not affected by caspase-8 deficiency. These results indicated that caspase-8 negatively regulates RIG-I signaling through RIP1 cleavage. This hypothesis was further supported by the fact that the enforced overexpression of the 38 kDa RIP1 cleavage fragment actively inhibited IRF3 activation.

Together, these intriguing findings suggest the following model (Figure 1): viral RNA recognition by RIG-I and its subsequent interaction with MAVS induces a rapid recruitment of RIP1 to the mitochondrial-associated complex leading to RIP1 polyubiquitination. Mitochondria-associated polyubiquitinated RIP1 then stabilizes the assembly of a large signalosome that contains NEMO and the activated IKK-related kinases TBK1 and IKKi together with TANK to initiate IRF3 activation. After enabling maximal IRF3 activity, polyubiquitinated RIP1 is somehow recognized and processed by caspase-8. This signal-induced cleavage leads to a destabilization of the RIG-I signalosome and downmodulation of the inflammatory response. Thus, virus-induced polyubiquitination of RIP1 coordinates first the maximal innate cell activation and subsequently causes directly its caspase-8-mediated restriction. Although RIP1 and caspase-8 have antagonistic functions, caspase-8 limits antiviral responses only

after their initial RIP1-dependent activation.

This study establishes a paradigm for a temporally coordinated negative regulatory mechanism in the innate immune system. It demonstrates a selective cleavage of an activated signaling molecule by the multifunctional caspase-8 enzyme. Importantly, RIP1 or its family members RIP2 and RIP3 are involved in not merely the RIG-I or TNFR1 systems. These molecules play broad roles in inflammation and regulate multiple signaling cascades including the TLR3, NOD1, and NOD2 pathways (Meylan and Tschopp, 2005). Thus, it will be very important to investigate whether proteolytic cleavage of activated and ubiquitinated RIP family members by caspase-8 or other proteases could be a general mechanism for restricting innate immunity. Previous studies with conditional caspase-8-deficient mice have already reported active functions for caspase-8 in preventing rampant inflammation in vivo (Ben Moshe et al., 2007; Kovalenko et al., 2009). Specific deletion of caspase-8 in keratinocytes resulted in an excessive activation of TBK1 and IRF3 (Kovalenko et al., 2009), which is consistent with the data presented here. In the future it will be very interesting to determine whether the anti-inflammatory functions of caspase-8 are restricted to RIP1 cleavage and the interferon regulatory system or whether additional molecules and other proinflammatory pathways are also directly affected. Finally, multiple human chronic diseases like lupus erythematosus, rheumatoid arthritis, and inflammatory bowel disease are caused by deregulated activities of proinflammatory cascades. Future translational studies are therefore needed to investigate whether defective caspase-8 regulation and subsequent failures to restrict innate immunity might contribute to human inflammatory disease.

REFERENCES

Ben Moshe, T., Barash, H., Kang, T.B., Kim, J.C., Kovalenko, A., Gross, E., Schuchmann, M., Abramovitch, R., Galun, E., and Wallach, D. (2007). Hepatology *45*, 1014–1024.

Ea, C.K., Deng, L., Xia, Z.P., Pineda, G., and Chen, Z.J. (2006). Mol. Cell *22*, 245–257.

Kovalenko, A., Kim, J.C., Kang, T.B., Rajput, A., Bogdanov, K., Dittrich-Breiholz, O., Kracht, M., Brenner, O., and Wallach, D. (2009). J. Exp. Med. *206*, 2161–2177.

Lin, Y., Devin, A., Rodriguez, Y., and Liu, Z.G. (1999). Genes Dev. *13*, 2514–2526.

Meylan, E., and Tschopp, J. (2005). Trends Biochem. Sci. *30*, 151–159.

Rajput, A., Kovalenko, A., Bogdanov, K., Yang, S.-H., Kang, T.-B., Kim, J.-C., Du, J., and Wallach, D. (2011). Immunity *34*, this issue, 340–351.

Takeuchi, O., and Akira, S. (2010). Cell *140*, 805–820.

Wu, C.J., Conze, D.B., Li, T., Srinivasula, S.M., and Ashwell, J.D. (2006). Nat. Cell Biol. *8*, 398–406.

Yoneyama, M., and Fujita, T. (2009). Immunol. Rev. *227*, 54–65.

Viperin Links Lipid Bodies to Immune Defense

Xiaomo Jiang[1] and Zhijian J. Chen[1,2,*]
[1]Department of Molecular Biology
[2]Howard Hughes Medical Institute
University of Texas Southwestern Medical Center Dallas, TX 75390-9148, USA
*Correspondence: zhijian.chen@utsouthwestern.edu
DOI 10.1016/j.immuni.2011.03.012

Viperin is an interferon-stimulated gene that exerts antiviral effects. In this issue of *Immunity*, Saitoh et al. (2011) uncovered an unexpected function of Viperin and lipid bodies in interferon induction by Toll-like receptors, specifically in plasmacytoid dendritic cells.

Interferons (IFNs) and cytokines are important mediators of immune defense against infections. An effective IFN response involves two phases: an early phase of IFN production triggered by pattern recognition receptors (PRRs) that detect pathogens and a late phase of IFN signaling mediated by the type I IFN receptor (IFNR), which activates the JAK-STAT signaling pathway to induce hundreds of interferon stimulated genes (ISGs). ISGs collectively suppress viral infection, replication, and assembly, but the mechanisms underlying the antiviral functions of most ISGs remain largely unknown. In this issue of *Immunity*, Saitoh et al. (2011) reveal a surprising role of an ISG, Viperin (virus inhibitory protein, endoplasmic reticulum-associated, IFN-inducible), in the first phase of IFN production mediated by Toll-like receptor 7 (TLR7) and TLR9 in plasmacytoid dendritic cells (pDCs). Importantly, through investigating the mechanism of IFN regulation by Viperin, the authors uncover an interesting connection between lipid storage organelles called lipid bodies (also known as lipid droplets) and antiviral immune defense.

Most mammalian cells have the ability to secrete and respond to IFNs. However, pDCs are specialized to produce copious amounts of IFNα, in part through constitutive expression of endosomal TLRs (TLR7 and TLR9) and IRF7, a master transcription factor for IFN-α. In addition, TLR ligands (e.g, CpG DNA) capable of triggering IFN production in pDCs are retained in specialized endosomal vesicles, and TLRs on the cytosolic surface of these vesicles recruit signaling proteins including MyD88, TRAF6, TRAF3, IRAK4, and IRAK1 (Honda et al., 2005) (Figure 1).