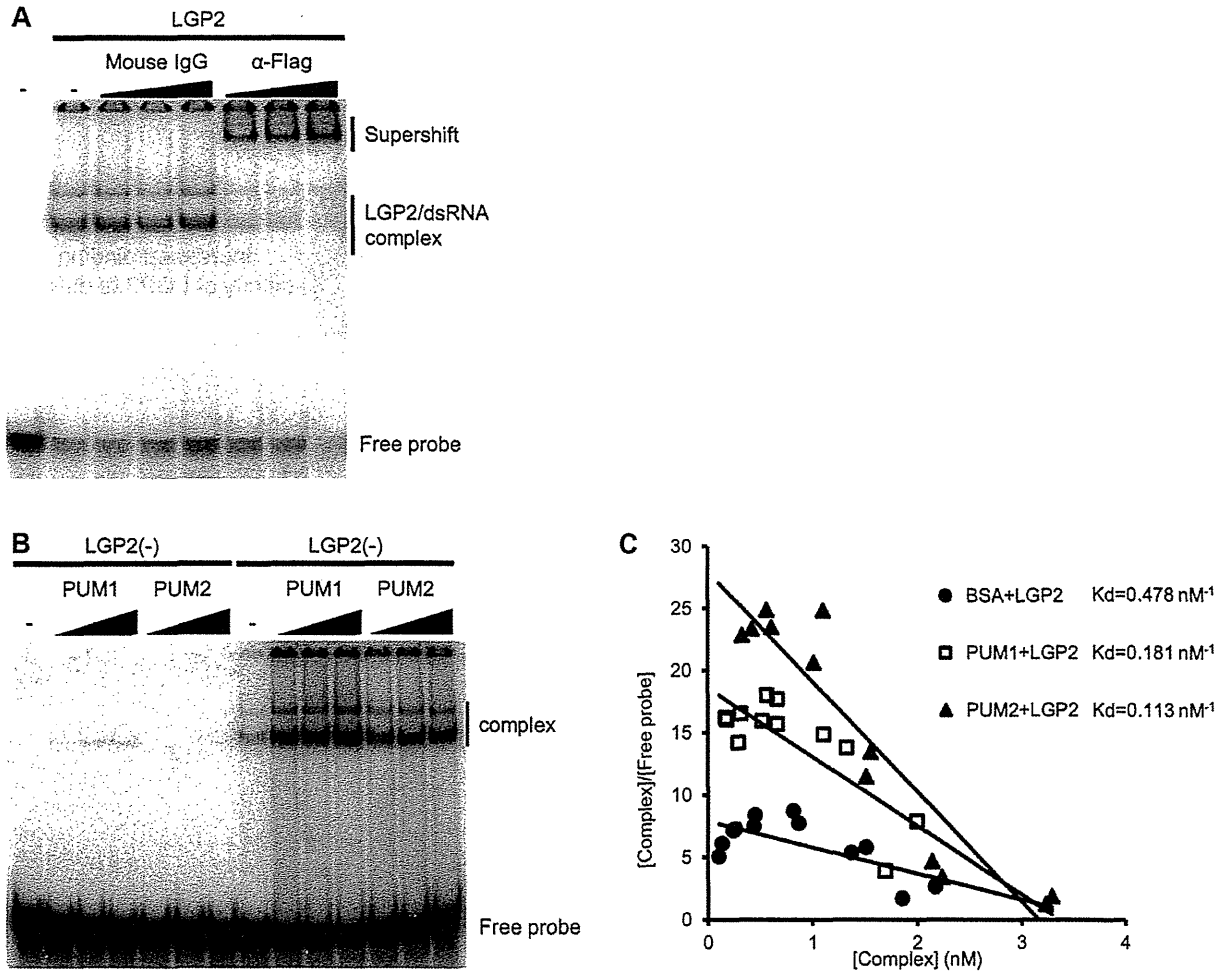

Figure 5. *In vitro* binding assay of dsRNA and LGP2. (A and B) Recombinant LGP2 proteins (0.125 μg) were mixed with ^{32}P-labeled dsRNA in the presence of a control mouse IgG or anti-Flag antibody (0.1, 0.2 or 0.4 μg) (A) or in the presence or absence of Pumilio proteins (0.1, 0.3 or 0.5 μg) (B). The mixture were separated by acrylamide gel and the radioactivity was analyzed. (C) LGP2 dsRNA binding affinities in the absence (filled circles) or presence of PUM1 (open square) or PUM2 (filled triangle) were analyzed and the Kd values were determined.
doi:10.1371/journal.ppat.1004417.g005

[46]. In light of these observations, it is probable that increased dsRNA binding of LGP2 facilitates RLR signaling.

Viral infection induces the formation of avSGs, including conventional SG markers, RIG-I, MDA5, LGP2, PKR, OAS, RNase L, DHX36, TRIM25, PUM1 and PUM2, some of which are critical in sensing non-self viral RNA and triggering antiviral signaling. Unlike SGs induced by physical stress, viral RNA is accumulated in virus-induced avSGs [34,35,37]. In summary, these results support the idea that avSGs act as a critical platform for sensing and discriminating viral RNA as a defense mechanism against viral infections. Although the IFN system is absent in plants, Pumilio proteins participate in the antiviral response in plants [47], suggesting that the principal mechanism of sensing non-self RNA is evolutionarily conserved.

Materials and Methods

Cell Culture and Reagents

L929 cells were maintained in minimal essential medium (MEM) (nacalai tesque,) containing 5% fetal bovine serum (FBS). HEK293T and HeLa cells were maintained in Dulbecco's modified Eagle's medium (DMEM) (nacalai tesque) containing 10% FBS.

Plasmid Constructs

The p-125 Luc, p-55C1B Luc, p-55A2 Luc, pU6i and pU6i-shLGP2 have been described previously [6,7]. pEF-Flag-PUM1 and PUM2 was obtained by subcloning cDNA into the empty vector pEF-BOS. Mutants were generated using the KOD -plus- Mutagenesis Kit (TOYOBO). TRIM25 cDNA was purchased from OriGene.

RNAi

Negative control siRNA and siRNA targeting PUM1 or PUM2 were purchased from BONAC. siRNAs were transfected using Lipofectamine RNAiMAX (Invitrogen) according to the manufacturer's protocol. After 48 h, the cells were stimulated as indicated.

Quantitative Real-Time PCR

Total RNA was isolated using Sepasol reagent (nacalai tesque), treated with DNase I (Roche) and subjected to reverse transcription

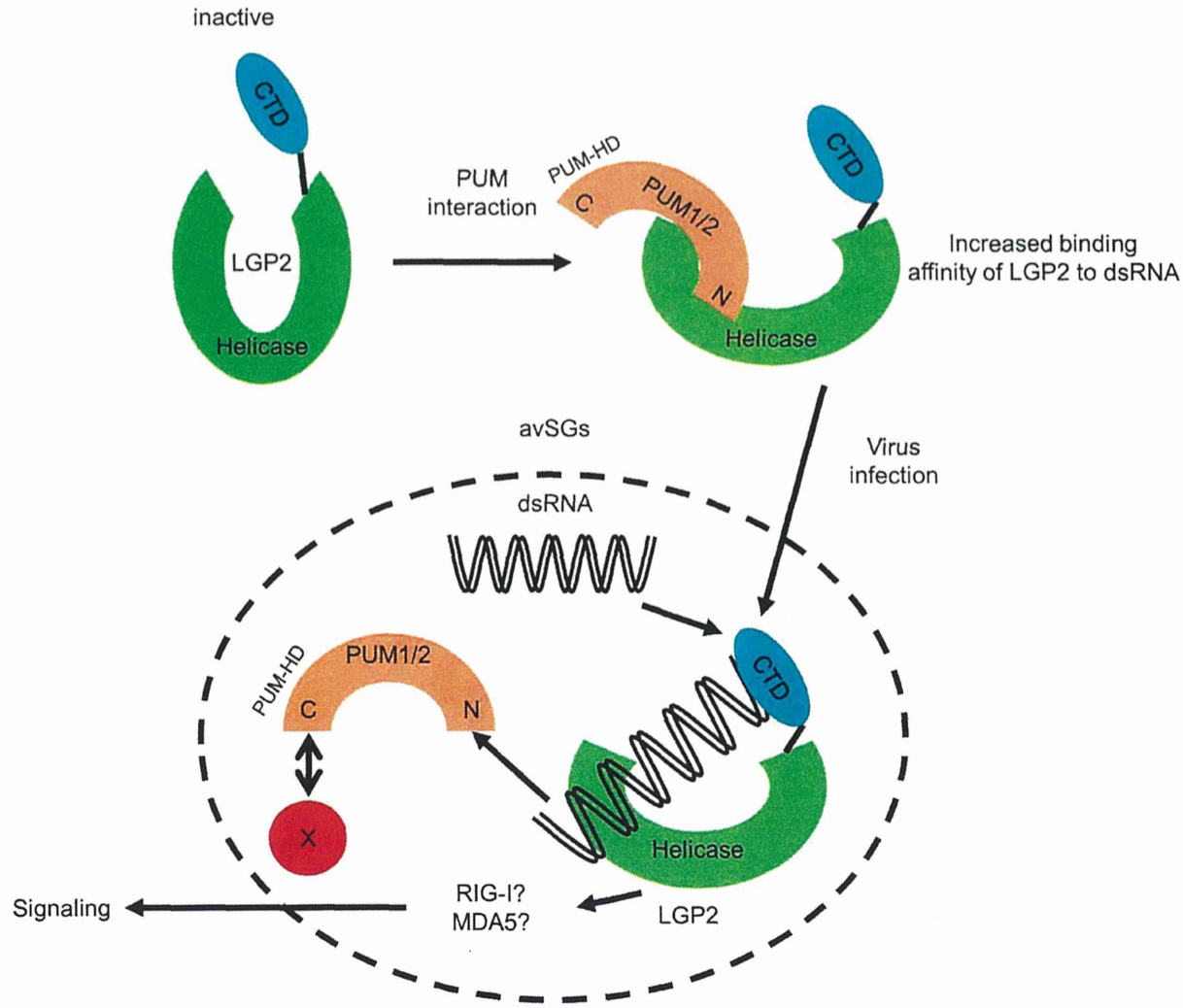

Figure 6. Hypothetical model for regulation of LGP2 by PUM1 and PUM2 in avSG. N-terminal domain of PUM1 and PUM2 possess intrinsic affinity to LGP2. This interaction confers higher binding affinity of LGP2 to viral dsRNA. Conformational change of LGP2 is one of the explanations for the increased affinity. Viral infection such as NDV induces avSGs and accumulation of viral dsRNA, LGP2, PUM1, PUM2 and other avSG markers into avSGs. Within avSG, dsRNA interacts with LGP2/PUM complex, producing LGP2/dsRNA complex and Pumilio proteins are released from the complex. Then, LGP2 triggers signals presumably in cooperation with RIG-I or MDA5. X: potential interacting partner of C-terminal domain of PUM1 and PUM2 determining their avSG localization.
doi:10.1371/journal.ppat.1004417.g006

using a High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems). mRNA levels were monitored with the StepOne plus Real Time PCR System and TaqMan Fast Universal Master Mix (Applied Biosystems). TaqMan primer and probe sets for 18S rRNA, human IFNB1 and human CXCL10 were purchased from Applied Biosystems. The RNA copy numbers of the gene of interest were normalized to that of internal 18S rRNA. NDV replication levels were monitored with Fast SYBR PCR Master Mix (Applied Biosystems) using the primers specific for the NDV F gene.

Antibodies

Anti-Flag and anti-HA antibody were purchased from Sigma and Cell Signaling Technology, respectively. Anti-GST anti-β-actin, anti-c-Myc, anti-Pumilio1, anti-Pumilio2 and anti-TIAR antibodies were from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Anti-IRF-3, anti-RIG-I, anti-MDA5 and anti-LGP2 antibody were described previously [34,48]. Alexa 488- and 594-conjugated anti-rabbit or anti-goat IgG antibodies (Invitrogen) were used as secondary antibodies.

Immunostaining

The cells were fixed with 4% paraformaldehyde (nacalai tesque) for 10 min, permeabilized with an acetone: methanol (1:1) solution, and blocked with 5 mg/ml bovine serum albumin (BSA) (nacalai tesque) for 30 min. The cells were incubated with the indicated primary antibodies overnight at 4°C, and then incubated with the relevant Alexa-conjugated antibodies at room temperature for 1 h. Nuclei were stained with DAPI (nacalai tesque). The cells were analyzed with a microscope (Leica microsystems).

Luciferase Assay

Luciferase assay was performed as described previously [7]. The Dual-Luciferase Reporter Assay System (Promega) was used according to the manufacturer's protocol.

Co-immunoprecipitation

The indicated plasmids were transfected with HEK293T cells using Lipofectamine 2000 (Invitrogen). The cell lysates were incubated with anti-Flag or anti-c-Myc antibody on ice for 30 min. The pre-washed Protein G Sepharose (GE Healthcare) was added to the mixture, which was rotated at 4°C overnight. After washing, the precipitates were eluted and separated by SPS-PAGE, followed by Western blotting.

Viruses

NDV was grown in the allantonic cavities of 9-day-old embryonated eggs. The cells were mock treated or infected with NDV at 37°C.

Native PAGE for IRF-3 Dimer Detection

The cell lysates were subjected to Native PAGE and Western blotting as described previously [6,48].

Enzyme-Linked Immunosorbent Assay (ELISA)

The cell culture supernatants were collected and subjected to ELISA with a human IFN-β ELISA kit (TORAY, Tokyo, Japan) according to the manufacturer's protocol.

Recombinant Proteins

Recombinant LGP2 was produced as 6xHis-LGP2 fusion using baculovirus and High Five cells. 6xHis-LGP2 was bound to Ni Sepharose 6 Fast Flow (GE Healthcare), and then eluted by elution buffer containing 50 mM Tris-HCl (pH 8.0), 150 mM NaCl, 1.5 mM DTT and 500 mM imidazole.

The intact PUM1 and PUM2 were amplified by PCR and inserted into a modified pGex-6p-1 vector (GE Healthcare). The C-terminal His6-tag was inserted using a KOD plus mutagenesis kit (TOYOBO) to produce N-terminal GST and C-terminal His6 tagged proteins. The vectors were transformed into an *E. coli* BL21 (DE3) strain. Bacteria were first grown at 37°C in LB medium containing 100 μg/ml ampicillin at 160 rpm. Protein expression was induced by the addition of 0.1 mM IPTG when the absorbance at 600 nm was approximately 0.4. The cells were then grown at 16°C for 16 h at 90 rpm. The cells were harvested by centrifugation and were suspended in a lysis buffer containing 50 mM Tris-HCl (pH 8.0), 500 mM NaCl, and 20 mM imidazole supplemented with protease Inhibitor Cocktail (Roche Diagnostics) and were lysed via sonication and centrifugation. The supernatant was suspended in Ni Sepharose 6 Fast Flow (GE Healthcare), then the resin was washed with lysis buffer, and the protein was eluted by elution buffer containing 50 mM Tris-HCl (pH 8.0), 500 mM NaCl, and 500 mM imidazole. The protein was diluted by phosphate-buffered saline (PBS) and mixed with Glutathione Sepharose 4B (GE Healthcare) for 16 h. The mixture was washed with PBS and proteins were eluted by a buffer containing 50 mM Tris-HCl (pH 8.0), 150 mM NaCl, and 20 mM reduced glutathione.

Electrophoresis Mobility Shift Assay (EMSA)

Recombinant LGP2 proteins were mixed with ^{32}P-labeled synthetic dsRNA (25/25c) [49] in a reaction mixture (20 mM Tris-HCl (pH 8.0), 1.5 mM $MgCl_2$, and 1.5 mM DTT) in the presence or absence of recombinant Pumilio proteins. After incubation at 37°C for 15 min, the reaction mixture was applied to a 15% acrylamide gel (TBE buffer) and the radioactivity was detected with an Image Analyzer (FUJIFILM, Tokyo, Japan).

GST-Pull Down Assay

Recombinant LGP2 proteins were mixed with Pumilio proteins in a reaction mixture (20 mM Tris-HCl (pH 8.0), 1.5 mM $MgCl_2$, and 1.5 mM DTT) in the presence or absence of synthetic dsRNA (25/25c) at 37°C for 15 min. Pre-washed Glutathione Sepharose 4B (GE Healthcare) was added to the mixture and incubated at room temperature for 1 h. After washing, the precipitates were separated by SDS-PAGE, followed by Western blotting.

Supporting Information

Figure S1 PUM1 and PUM2 positively regulate NDV-induced IFN induction.(A-C) HEK293T cells were transfected with control siRNA or siRNA targeting PUM1 or PUM2 for 48 h. The cells were mock-treated or infected with NDV for 7, 8 or 9 h. The cell lysates were separated by Native PAGE, followed by immunoblotting with anti-pIRF-3 (A) or anti-IRF-3 (B) antibodies. The cells were infected or transfected with the indicated nucleotides for 24 h. The culture media were collected and subjected to IFN-β ELISA (C).
(PDF)

Figure S2 The knockdown of Pumilio proteins did not affect the expression level of RLRs. HEK293T cells were transfected with control siRNA or siRNA targeting human PUM1 or PUM2 for 48 h. The cells were mock-treated or treated with human IFN-β (1000 U/ml) for 24 h. The cell lysates were subjected to SDS-PAGE, followed by immunoblotting with anti-RIG-I, anti-MDA5, anti-LGP2 or anti-β-actin antibodies.
(PDF)

Figure S3 Physical interaction between PUM1 and PUM2. HEK293T cells were transfected with a HA-tagged PUM2 together with Flag-tagged PUM1. The cell lysates were subjected to IP with anti-Flag, followed by Western blotting.
(PDF)

Figure S4 PUM1 and PUM2 interacted with LGP2 through its helicase domain. HEK293T cells were transfected with a HA-tagged LGP2 full-length, helicase domain (dCTD) or CTD together with Flag-tagged PUM1 or PUM2. The cell lysates were subjected to IP with anti-Flag, followed by Western blotting.
(PDF)

Figure S5 PUM1 and PUM2 are not required for NDV-induced avSG formation. (A and B) HeLa cells were transfected with control siRNA or siRNA targeting PUM1 or PUM2. After 48 h, the cells were mock-infected or infected with NDV for 9 h. The cells were then fixed and stained with anti-TIAR and anti-NDV NP (A) or anti-TIAR and anti-LGP2 (B) antibodies. (C) LGP2 WT or KO cells were infected with NDV for 9 h. The cells were fixed and stained with anti-PUM1 and anti-PUM2 (Upper) or anti-PUM1 and anti-TIAR (Lower) antibodies. (D) HEK293T cells were transfected with Flag-tagged PUM1dC or PUM2dC for 48 h and infected with NDV for 9 h. The cells were fixed and stained with anti-Flag and anti-TIAR antibodies.
(PDF)

Figure S6 *In vitro* binding assay of dsRNA and LGP2 in the presence or absence of PUM1dC or PUM2dC. (A) Recombinant LGP2 (0.125 μg) proteins were mixed with ^{32}P-labeled dsRNA in the presence or absence of Pumilio proteins lacking PUM-HD (PUM1dC and PUM2dC, 0.5 μg). The mixture was separated by acrylamide gel and the radioactivity was analyzed. (B) LGP2 dsRNA binding affinities in the absence (filled circles) or presence

of PUM1dC (open square) or PUM2dC (filled triangle) were analyzed and the Kd values were determined.
(PDF)

Figure S7 Association between LGP2 with PUM1 or PUM2 in the presence or absence of dsRNA. Recombinant LGP2 proteins (0.5 μg) were mixed with Pumilio proteins (0.5 μg) in the presence or absence of dsRNA (25/25c, 0.4 μg). The mixture (10 μl) was then incubated with Glutathione Sepharose. After washing, the precipitates were eluted and separated by SDS-PAGE, followed by Western blotting.
(PDF)

References

1. Kawai T, Akira S (2011) Toll-like receptors and their crosstalk with other innate receptors in infection and immunity. Immunity 34: 637–650.
2. Yoneyama M, Fujita T (2009) RNA recognition and signal transduction by RIG-I-like receptors. Immunological reviews 227: 54–65.
3. Yan N, Chen ZJ (2012) Intrinsic antiviral immunity. Nature immunology 13: 214–222.
4. Ireton RC, Gale M, Jr. (2011) RIG-I like receptors in antiviral immunity and therapeutic applications. Viruses 3: 906–919.
5. Schlee M, Hartmann G (2010) The chase for the RIG-I ligand—recent advances. Molecular therapy: the journal of the American Society of Gene Therapy 18: 1254–1262.
6. Yoneyama M, Kikuchi M, Natsukawa T, Shinobu N, Imaizumi T, et al. (2004) The RNA helicase RIG-I has an essential function in double-stranded RNA-induced innate antiviral responses. Nature immunology 5: 730–737.
7. Yoneyama M, Kikuchi M, Matsumoto K, Imaizumi T, Miyagishi M, et al. (2005) Shared and unique functions of the DExD/H-box helicases RIG-I, MDA5, and LGP2 in antiviral innate immunity. Journal of immunology 175: 2851–2858.
8. Wu B, Peisley A, Richards C, Yao H, Zeng X, et al. (2013) Structural basis for dsRNA recognition, filament formation, and antiviral signal activation by MDA5. Cell 152: 276–289.
9. Peisley A, Wu B, Yao H, Walz T, Hur S (2013) RIG-I forms signaling-competent filaments in an ATP-dependent, ubiquitin-independent manner. Molecular cell 51: 573–583.
10. Xu LG, Wang YY, Han KJ, Li LY, Zhai Z, et al. (2005) VISA is an adapter protein required for virus-triggered IFN-beta signaling. Molecular cell 19: 727–740.
11. Seth RB, Sun L, Ea CK, Chen ZJ (2005) Identification and characterization of MAVS, a mitochondrial antiviral signaling protein that activates NF-kappaB and IRF 3. Cell 122: 669–682.
12. Meylan E, Curran J, Hofmann K, Moradpour D, Binder M, et al. (2005) Cardif is an adaptor protein in the RIG-I antiviral pathway and is targeted by hepatitis C virus. Nature 437: 1167–1172.
13. Kawai T, Takahashi K, Sato S, Coban C, Kumar H, et al. (2005) IPS-1, an adaptor triggering RIG-I- and Mda5-mediated type I interferon induction. Nature immunology 6: 981–988.
14. Kumar H, Kawai T, Kato H, Sato S, Takahashi K, et al. (2006) Essential role of IPS-1 in innate immune responses against RNA viruses. The Journal of experimental medicine 203: 1795–1803.
15. Sun Q, Sun L, Liu HH, Chen X, Seth RB, et al. (2006) The specific and essential role of MAVS in antiviral innate immune responses. Immunity 24: 633–642.
16. Fitzgerald KA, McWhirter SM, Faia KL, Rowe DC, Latz E, et al. (2003) IKKepsilon and TBK1 are essential components of the IRF3 signaling pathway. Nature immunology 4: 491–496.
17. Sato M, Suemori H, Hata N, Asagiri M, Ogasawara K, et al. (2000) Distinct and essential roles of transcription factors IRF-3 and IRF-7 in response to viruses for IFN-alpha/beta gene induction. Immunity 13: 539–548.
18. Kato H, Takeuchi O, Sato S, Yoneyama M, Yamamoto M, et al. (2006) Differential roles of MDA5 and RIG-I helicases in the recognition of RNA viruses. Nature 441: 101–105.
19. Kato H, Takeuchi O, Mikamo-Satoh E, Hirai R, Kawai T, et al. (2008) Length-dependent recognition of double-stranded ribonucleic acids by retinoic acid-inducible gene-I and melanoma differentiation-associated gene 5. The Journal of experimental medicine 205: 1601–1610.
20. Pichlmair A, Schulz O, Tan CP, Naslund TI, Liljestrom P, et al. (2006) RIG-I-mediated antiviral responses to single-stranded RNA bearing 5'-phosphates. Science 314: 997–1001.
21. Hornung V, Ellegast J, Kim S, Brzozka K, Jung A, et al. (2006) 5'-Triphosphate RNA is the ligand for RIG-I. Science 314: 994–997.
22. Schlee M, Roth A, Hornung V, Hagmann CA, Wimmenauer V, et al. (2009) Recognition of 5' triphosphate by RIG-I helicase requires short blunt double-stranded RNA as contained in panhandle of negative-strand virus. Immunity 31: 25–34.
23. Satoh T, Kato H, Kumagai Y, Yoneyama M, Sato S, et al. (2010) LGP2 is a positive regulator of RIG-I- and MDA5-mediated antiviral responses. Proceedings of the National Academy of Sciences of the United States of America 107: 1512–1517.

Acknowledgments

We thank T. Sakaguchi (Hiroshima University) for the anti-NP antibody.

Author Contributions

Conceived and designed the experiments: RN HK TF. Performed the experiments: RN KT EM EH SPY MY. Analyzed the data: RN MY HK TF. Wrote the paper: RN HK TF.

24. Takahasi K, Kumeta H, Tsuduki N, Narita R, Shigemoto T, et al. (2009) Solution structures of cytosolic RNA sensor MDA5 and LGP2 C-terminal domains: identification of the RNA recognition loop in RIG-I-like receptors. The Journal of biological chemistry 284: 17465–17474.
25. Kato H, Takahasi K, Fujita T (2011) RIG-I-like receptors: cytoplasmic sensors for non-self RNA. Immunological reviews 243: 91–98.
26. Gack MU, Shin YC, Joo CH, Urano T, Liang C, et al. (2007) TRIM25 RING-finger E3 ubiquitin ligase is essential for RIG-I-mediated antiviral activity. Nature 446: 916–920.
27. Gao D, Yang YK, Wang RP, Zhou X, Diao FC, et al. (2009) REUL is a novel E3 ubiquitin ligase and stimulator of retinoic-acid-inducible gene-I. PloS one 4: e5760.
28. Oshiumi H, Matsumoto M, Hatakeyama S, Seya T (2009) Riplet/RNF135, a RING finger protein, ubiquitinates RIG-I to promote interferon-beta induction during the early phase of viral infection. The Journal of biological chemistry 284: 807–817.
29. Arimoto K, Takahashi H, Hishiki T, Konishi H, Fujita T, et al. (2007) Negative regulation of the RIG-I signaling by the ubiquitin ligase RNF125. Proceedings of the National Academy of Sciences of the United States of America 104: 7500–7505.
30. Lin R, Yang L, Nakhaei P, Sun Q, Sharif-Askari E, et al. (2006) Negative regulation of the retinoic acid-inducible gene I-induced antiviral state by the ubiquitin-editing protein A20. The Journal of biological chemistry 281: 2095–2103.
31. Kayagaki N, Phung Q, Chan S, Chaudhari R, Quan C, et al. (2007) DUBA: a deubiquitinase that regulates type I interferon production. Science 318: 1628–1632.
32. Friedman CS, O'Donnell MA, Legarda-Addison D, Ng A, Cardenas WB, et al. (2008) The tumour suppressor CYLD is a negative regulator of RIG-I-mediated antiviral response. EMBO reports 9: 930–936.
33. Jiang X, Kinch LN, Brautigam CA, Chen X, Du F, et al. (2012) Ubiquitin-induced oligomerization of the RNA sensors RIG-I and MDA5 activates antiviral innate immune response. Immunity 36: 959–973.
34. Onomoto K, Jogi M, Yoo JS, Narita R, Morimoto S, et al. (2012) Critical role of an antiviral stress granule containing RIG-I and PKR in viral detection and innate immunity. PloS one 7: e43031.
35. Ng CS, Jogi M, Yoo JS, Onomoto K, Koike S, et al. (2013) Encephalomyocarditis virus disrupts stress granules, the critical platform for triggering antiviral innate immune responses. Journal of virology 87: 9511–9522.
36. Fung G, Ng CS, Zhang J, Shi J, Wong J, et al. (2013) Production of a dominant-negative fragment due to G3BP1 cleavage contributes to the disruption of mitochondria-associated protective stress granules during CVB3 infection. PloS one 8: e79546.
37. Yoo JS, Takahasi K, Ng CS, Ouda R, Onomoto K, et al. (2014) DHX36 enhances RIG-I signaling by facilitating PKR-mediated antiviral stress granule formation. PLoS pathogens 10: e1004012.
38. Murata Y, Wharton RP (1995) Binding of pumilio to maternal hunchback mRNA is required for posterior patterning in Drosophila embryos. Cell 80: 747–756.
39. Forbes A, Lehmann R (1998) Nanos and Pumilio have critical roles in the development and function of Drosophila germline stem cells. Development 125: 679–690.
40. Parisi M, Lin H (1999) The Drosophila pumilio gene encodes two functional protein isoforms that play multiple roles in germline development, gonadogenesis, oogenesis and embryogenesis. Genetics 153: 235–250.
41. Dubnau J, Chiang AS, Grady L, Barditch J, Gossweiler S, et al. (2003) The staufen/pumilio pathway is involved in Drosophila long-term memory. Current biology: CB 13: 286–296.
42. Chen D, Zheng W, Lin A, Uyhazi K, Zhao H, et al. (2012) Pumilio 1 suppresses multiple activators of p53 to safeguard spermatogenesis. Current biology: CB 22: 420–425.
43. Wang X, McLachlan J, Zamore PD, Hall TM (2002) Modular recognition of RNA by a human pumilio-homology domain. Cell 110: 501–512.
44. Friend K, Campbell ZT, Cooke A, Kroll-Conner P, Wickens MP, et al. (2012) A conserved PUF-Ago-eEF1A complex attenuates translation elongation. Nature structural & molecular biology 19: 176–183.

45. Vessey JP, Vaccani A, Xie Y, Dahm R, Karra D, et al. (2006) Dendritic localization of the translational repressor Pumilio 2 and its contribution to dendritic stress granules. The Journal of neuroscience: the official journal of the Society for Neuroscience 26: 6496–6508.
46. Saito T, Hirai R, Loo YM, Owen D, Johnson CL, et al. (2007) Regulation of innate antiviral defenses through a shared repressor domain in RIG-I and LGP2. Proceedings of the National Academy of Sciences of the United States of America 104: 582–587.
47. Huh SU, Kim MJ, Paek KH (2013) Arabidopsis Pumilio protein APUM5 suppresses Cucumber mosaic virus infection via direct binding of viral RNAs. Proceedings of the National Academy of Sciences of the United States of America 110: 779–784.
48. Mori M, Yoneyama M, Ito T, Takahashi K, Inagaki F, et al. (2004) Identification of Ser-386 of interferon regulatory factor 3 as critical target for inducible phosphorylation that determines activation. The Journal of biological chemistry 279: 9698–9702.
49. Takahasi K, Yoneyama M, Nishihori T, Hirai R, Kumeta H, et al. (2008) Nonself RNA-sensing mechanism of RIG-I helicase and activation of antiviral immune responses. Molecular cell 29: 428–440.

Cytokine & Growth Factor Reviews 25 (2014) 739–743

Contents lists available at ScienceDirect

Cytokine & Growth Factor Reviews

journal homepage: www.elsevier.com/locate/cytogfr

Mini review

Autoimmunity caused by constitutive activation of cytoplasmic viral RNA sensors

Hiroki Kato [a,b], Takashi Fujita [a,*]

[a] Laboratory of Molecular Genetics, Institute for Virus Research, Kyoto University, Kyoto, Japan
[b] PRESTO, Tokyo, Japan

ARTICLE INFO

Article history:
Available online 19 August 2014

ABSTRACT

RIG-I-like receptors (RLRs) are well-known viral sensors that trigger the antiviral interferon (IFN) response by recognizing the non-self signatures of viral RNAs. The proper induction of the IFN response is known to play a crucial role in protecting against viral infections, whereas aberrant activation can lead to autoimmune disorders. We herein provided an overview of the antiviral IFN response and autoimmunity, with a focus on recent studies describing autoimmunity caused by mutations in the cytoplasmic viral RNA sensor, melanoma differentiation-associated gene 5 (MDA5).

Introduction

Host cells evoke antiviral innate immune responses, including the production of type I interferon (IFN), to viral infections by recognizing viral nucleic acids for the elimination of intracellular invaders. Toll-like receptors (TLRs), RIG-I like receptors (RLRs), and cytosolic DNA sensors including cGAS have been identified as key sensors that recognize viral DNAs and RNAs and trigger the induction of IFN [1–3]. IFN secreted from virus-infected cells activates non-infected cells by binding with cell surface receptors and promotes the expression of antiviral IFN-stimulated genes (ISGs) including 2′-5′-oligoadenylate synthetases, RNase L, PKR, and IFITs [4]. The production of IFN is generally tightly regulated: It is undetectable in uninfected cells and is rapidly induced upon infections through positive feedback regulation. However, the production of IFN is programmed to be transient by the actions of multiple negative regulators. On the other hand, several lines of evidence have shown that aberrant antiviral signaling caused by a failure in the clearance of endogenous nucleic acids can lead to autoimmune disorders [5–7]. More recent studies described the relationship between MDA5, one of the RLRs, and autoimmunity in mouse and human systems [8,9]. In this review, we focused on the involvement of MDA5 in different autoimmune disorders and their possible causative mechanisms.

Viral RNA sensing and antiviral signaling mediated by RLRs

RLRs are DEAD/H box RNA helicases localized in the cytoplasm and consist of three family molecules: retinoic-acid inducible gene-I (RIG-I), melanoma differentiation-associated gene 5 (MDA5), and laboratory of genetics and physiology 2 (LGP2). RLRs are known to play a critical role in eliciting antiviral responses, including the production of type I interferon (IFN) and inflammatory cytokines, to eliminate invading viruses from the host [10,11]. Domain and structural analyses of these helicases revealed that RLRs were composed of three structural domains: the caspase activation and recruitment domain (CARD) at the N-terminus for signal transduction, central DExD/H box RNA helicase domain with RNA-dependent ATPase activity, and C-terminal domain (CTD). The helicase domain and CTD participate in the recognition of viral RNA. Although LGP2 lacks CARD, a loss-of-function analysis revealed that it acts as a positive regulator for signaling [12,13]. Therefore, LGP2 is presumed to cooperate with either RIG-I and/or MDA5 to trigger the antiviral response. The determination of crystal structures revealed that the linker region (also known as the pincer) of RIG-I, which is located between the helicase domain and CTD, was critical for maintaining RIG-I in a repressed conformation, in which CARD is masked by an interaction with the helicase domain [14]. Mutagenesis of the linker conferred the constitutive activity of RIG-I and loss of responsiveness to viral RNA [15].

Several steps have been proposed for the activation of RLRs, leading to antiviral signal transduction as shown in Fig. 1. The first step involves a physical association between viral RNA and RLRs.

* Corresponding author. Tel.: +81 75 751 4031; fax: +81 75 751 4031.
E-mail address: tfujita@virus.kyoto-u.ac.jp (T. Fujita).

http://dx.doi.org/10.1016/j.cytogfr.2014.08.003
1359-6101/

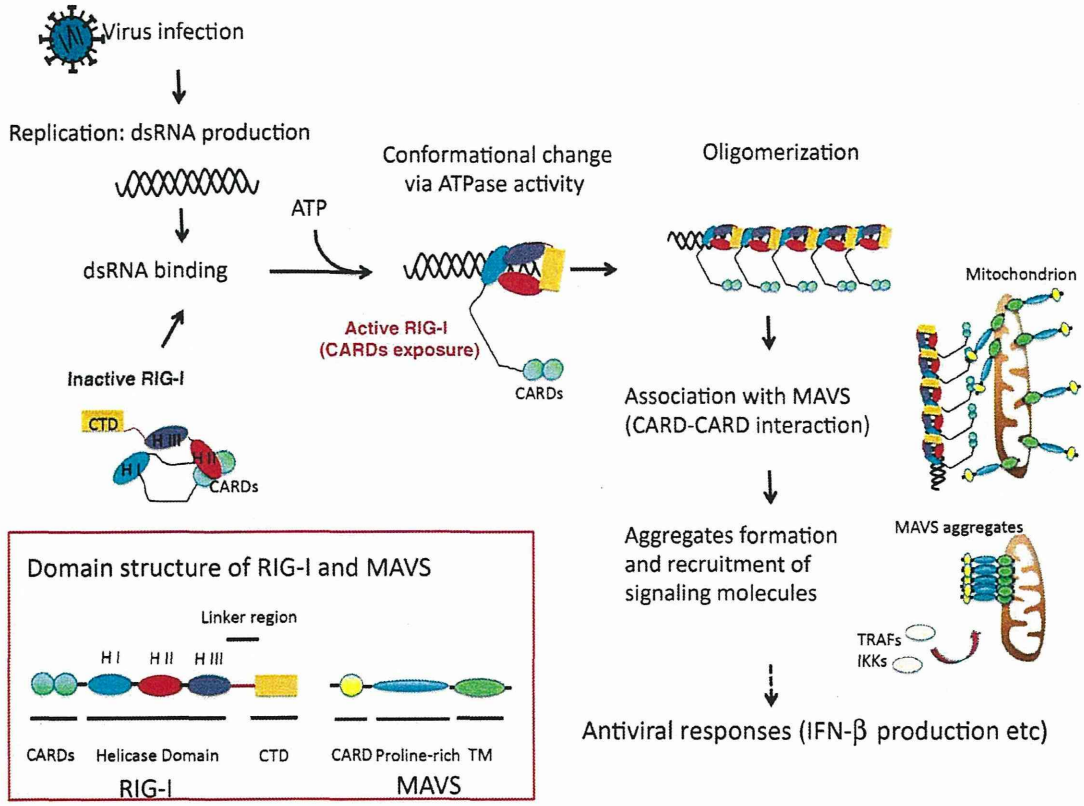

Fig. 1. Activation steps of RIG-I in the cytoplasm.
Domain structure of RIG-I and MAVS and activation steps of RIG-I are indicated. RIG-I is composed of three structural domains: The caspase activation and recruitment domain (CARD) at the N-terminus for signal transduction, central DExD/H box RNA helicase domain with RNA-dependent ATPase activity, and C-terminal domain (CTD). MAVS also possesses CARD for the interaction with RIG-I. TM: transmembrane domain. The first step for RIG-I activation involves a physical association between viral RNA and RIG-I. The next step is conformational alterations in RIG-I via ATPase activity to expose CARD. Once the CARD of RIG-is exposed, the signal from RIG-I oligomers is relayed to another CARD-containing protein, MAVS via CARD–CARD interactions.

Initial binding studies revealed that RLRs bound to double-stranded RNA and binding affinities to single-stranded RNA and dsDNA were very low [10,16]. RIG-I was previously reported to specifically sense the 5′-ppp structure of the primary viral transcript [17,18]; however, this finding overlooked the possibility of the in vitro transcript having a copy back structure; partial transcription using product RNA as a template occurs after template transcription, producing a partial dsRNA structure. Subsequent studies revealed that the dsRNA structure was a prerequisite and the 5′-ppp structure enhanced signaling by RIG-I [19,20]. Influenza A virus and Sendai virus produce panhandle and defective interfering RNA (with copy back), respectively, and these activate RIG-I [21,22]. Although several early studies showed that RIG-I specifically bound to the 5′-ppp structure, the involvement of a copy back dsRNA structure was not considered. Therefore, there is no evidence to suggest that RIG-I solely recognizes 5′-ppp in the absence of a dsRNA structure. A previous study clearly demonstrated that RIG-I and MDA5 recognized short and long dsRNA molecules and also that the virus specificities of RIG-I and MDA5 could be roughly correlated with the sizes of dsRNA produced by the respective viruses [23].

The next step is conformational alterations in RLRs to expose CARD. The ATP hydrolysis activities (ATPase) of RIG-I and MDA5 may be involved in these conformational changes because a mutation at the ATP binding site (Walker's A motif) was shown to inactivate these sensors. Although ATPase activity was required for dsRNA unwinding (helicase activity), the helicase-resistant substrate (dsRNA with a 5′-overhang), but not the helicase-sensitive substrate (dsRNA with a 3′-overhang) induced signaling, which suggested that helicase activity may be irrelevant for antiviral signaling [24]. Once the CARD of RIG-I or MDA5 is exposed, the signal is relayed to another CARD-containing protein, MAVS (also termed IPS-1, VISA, or Cardif) via CARD–CARD interactions [25–30]. MAVS is localized on the mitochondrial outer membrane; therefore, RLR signaling from the cytosol is transmitted to an insoluble compartment in the cytoplasm. Recent in vitro reconstitution studies revealed that MDA5 formed a filament-like complex (also RIG-I-oligomer formation) on its ligand dsRNA and also that these MDA5 filaments promoted the prion-like aggregation of MAVS [31,32]. However, ATP hydrolysis induced filament disassembly, suggesting that these filaments may be unstable under physiological conditions. Mitochondrial fusion and fission are known to be essential for the aggregation of MAVS [33]. In addition to mitochondria, several groups reported that virus-induced stress granules (SGs) functioned as critical loci for the activation of RLR. In several viral infections, viral dsRNA activates PKR and induces the formation of SGs, including SG components, RLRs, and viral RNAs, to promote the activation of RLR [34,35].

Signaling molecules including ubiquitin ligases (TRAFs) and kinase complexes (TBK1/IKK and IKKa/b/g) are recruited after the formation of MAVS aggregates and eventually activate the transcription factors IRF3/7 and NF-κB, leading to the production of type I IFNs and inflammatory cytokines, such as IL-6. Furthermore, either RIG-I or MDA5 induce the production of IL-1β through IPS-1/CARD9/NF-κB signaling in certain cell types upon viral infection. Unlike MDA5, RIG-I is capable of triggering inflammatory responses by the direct formation of a signal

complex with ASC and caspase-1 in a NLRP3-independent manner, suggesting a distinctive role of RIG-I in inflammatory responses [36].

Relationship between RLRs and autoimmune diseases

To control the replication and spread of invading viruses, IFN responses need to be triggered by the proper activation of RLRs as described above. Previous studies clearly demonstrated using gene targeting that a deficiency in IFN signal transduction markedly increased susceptibility to infected viruses, which could result in high lethality. On the other hand, recent studies revealed that the sustained or inappropriate activation of RLR-mediated signaling led to the onset of autoimmune diseases as described in Fig. 2.

Systemic lupus erythematosus (SLE)

Systemic lupus erythematosus (SLE) is known to cause various symptoms, with the most common being joint pain, skin rash, and tiredness. Nephritis has been reported in severe cases. Elevated levels of type I IFN, termed the IFN signature, have been reported in patients with SLE and the central role of type I IFN in disease pathogenesis has been suggested [7]. SNPs in several molecules involved in the production of IFN have been strongly correlated with the onset of SLE.

TLR7 and 9 are receptors that are essential for eliciting the production of IFN from plasmacytoid dendritic cells (pDC) in response to nucleic acids [37–39]. Previous studies on Y-linked autoimmune accelerator (Yaa) mice, which harbor a duplication in the TLR7 gene [40,41], suggested its role in an amplification loop for the production of IFN and activation of B cells in SLE.

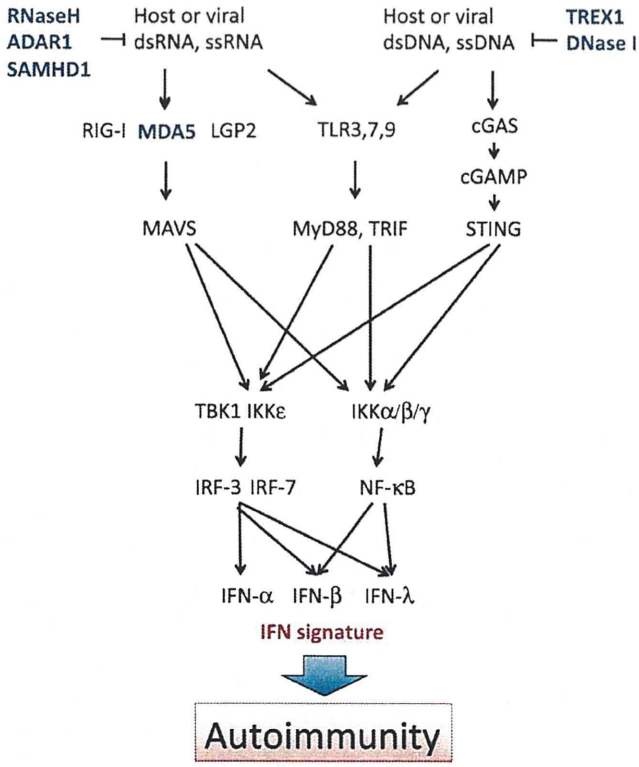

Fig. 2. Nucleic acids sensing pathways.
Nucleic acids sensing pathways that trigger type I and III IFNs are indicated. Defect in indicated RNase or Dnase (TREX1, Dnase I, RNaseH etc.) will cause abnormal activation of these pathways. Also auto-activation of sensors such as MDA5 has been shown to lead aberrant signaling, leading to the onset of autoimmune diseases.

DNase I is a secreted protein that can be detected in sera, saliva, urine, and intestinal juices. Serum DNase I activity was shown to be lower in SLE patients and a correlation was reported between several SNPs of DNase I and the pathogenesis of SLE. DNase I knockout mice also develop SLE-like symptoms, and this has been attributed to a failure in the clearance of endogenous DNA leading to abnormal IFN responses [42], suggesting a critical role of DNase I in the prevention of SLE.

A previous study reported that human SNPs in *IFIH1* encoding MDA5 such as rs1990760 (A946T) were correlated with an increase in the susceptibility to SLE [43], suggesting that the atypical activation of RLR signaling may lead to SLE. Fubabiki et al. recently reported that mice with the *Ifih1* missense mutation spontaneously developed lupus-like symptoms including nephritis and skin rash [8]. This is the first study to have directly demonstrated that mutations in RLRs directly led to an autoimmune disease. The up-regulation of type I IFNs, IFN-inducible genes, and inflammatory cytokines including IL-6 and TNF-a was detected in multiple organs in this mutant mouse, reflecting the ubiquitous expression of MDA5. This missense mutation enhanced the basal activation level of IFN by MDA5, but abrogated responsiveness to viral infection as well as the ATPase activity induced by dsRNA. More importantly, the autoimmune phenotype was not observed in the mouse background of Mavs−/−. These findings suggested that the mutant MDA5 may confer constitutive activity rather than being hypersensitive to endogenous or viral RNA. A simple increase in the wild type *Ifih1* gene dosage was insufficient to cause spontaneous nephritis [44], suggesting that a dysregulation in MDA5 by a mutation may be essential for triggering autoimmunity.

Aicardi-Goutieres syndrome (AGS)

Aicardi-Goutieres syndrome (AGS) is an inflammatory disease that particularly affects the brain and skin. AGS patients exhibit profound intellectual disabilities and dystonia, and lethality by the age of 17 years has been reported in approximately 25% of patients. It's been shown that SNPs in the genes functioning in nucleic acid metabolism, including *TREX1, SAMHD1, ADAR1, RNASEH2A, RNASEH2B, and RNASEH2C*, were strongly linked to AGS [45].

The exodeoxyribonuclease TREX1 is known to eliminate viral and aberrant cellular DNA in the cytoplasm, which potentially activate the DNA sensor cGAS to transduce IFN signaling via the essential adaptor molecule, STING [46,47]; thus, TREX1 has been identified as a negative regulator of the cGAS-STING-dependent IFN signaling pathway. *TREX1* mutant alleles that encode inactive exonuclease have been detected in AGS patients [48], whereas the mis-localization of TREX1 has been associated with SLE [49]. *Trex1* knockout mice spontaneously develop SLE-like symptoms, but not AGS. These findings strongly suggest a link between the aberrant production of IFN and SLE and AGS.

In 2014, Crow's group (Rice et al.) identified mutations in *IFIH1* in AGS patients [50]. These mutations conferred the constitutive activity of MDA5, but occurred at different positions from the mouse *Ifih1* mutation reported by Fubabiki et al. Human MDA5 mutants exhibited hyper-responsiveness to ligand stimulation, thereby suggesting the possible involvement of endogenous and/or viral RNA in the onset of AGS.

Another group (Oda et al.) more recently identified *IFIH1* heterozygous missense mutations in AGS patients [51]. The encoded MDA5 mutants exhibited constitutive activity, but failed to respond to a viral stimulus similar to the mouse MDA5 mutation. These findings indicated a link between constitutive MDA5 activity and AGS; however, responsiveness to viral infections remains controversial, particularly the same mutation (G2336A:R779H) included in these studies. This discrepancy needs to be reexamined using a common assay. The early onset of

autoimmunity in mutant mice and AGS suggests the autonomous cause of the activation of MDA5 rather than a strong association with a particular viral infection. Considering the high expression of IFN-inducible genes detectable in AGS patients, the so-called interferon signature, mutations in the deoxynucleoside triphosphate triphosphohydrolase SMAHD1, the dsRNA editing enzyme ADAR1, and the degradation enzyme of RNA:DNA heteroduplexes RNASEHs are likely to directly activate DNA and/or RNA sensors (including cGAS and RLRs), leading to the production of type I IFN.

Type 1 diabetes mellitus (T1DM)

Type 1 diabetes mellitus (T1DM) is a well-known disease in which the production of insulin by pancreatic β cells is impaired by genetic and immune factors. The involvement of viral infections and antiviral host responses has been suggested in the early stage of T1DM. Even though type I IFN plays an antiviral role, it can be deleterious by acting as a trigger of autoimmune responses against β cells. Nejentsev et al. reported that two canonical splice site variants in *IFIH1*, one nonsense variant and the other a missense substitution, were protective against type 1 diabetes mellitus (T1DM) [52]. They speculated that these loss-of-function mutations in *IFIH1* may attenuate innate immune responses against viruses. Although the rubella virus, cytomegalovirus, rotavirus, and retroviruses have been linked to the development of T1DM, enteroviruses, especially coxsackievirus B4, have been strongly implicated in its development [53]. MDA5 acts as a selective sensor for detecting the *Picornaviridae* family, which includes enteroviruses. However, the link between hyper-responsive mutations in *IFIH1* and T1DM has not yet been elucidated. Further studies on *IFIH1* will provide a deeper understanding on how viral infections and antiviral responses are linked to the pathogenesis of T1DM.

Future perspectives

In addition to MDA5, RIG-I and LGP2 belong to the cytoplasmic viral RNA sensor family. However, GWAS showed a link between MDA5 and autoimmunity. It is possible that active RIG-I or LGP2, through genomic mutations, may cause autoimmunity because they share the common signaling adaptor, MAVS, the loss-of-function variant of which has been implicated in the increase in susceptibility to SLE. Although artificial mutagenesis of RIG-I can confer constitutive activity [15], corresponding mutations in the human genome remain unknown.

Funabiki et al. clearly demonstrated that a single missense mutation in *Ifih1* caused spontaneous SLE-like nephritis [8], while Crampton et al. reported that *Ifih1* transgenic mice show by accelerated SLE-like nephritis, but did not develop spontaneous nephritis in spite of chronically elevated levels of type I IFN [44]. This finding suggested that the chronic activation of IFN may be insufficient to trigger autoimmune diseases. Although quantitative comparisons of IFN levels between these mice are critical, qualitative differences between mutations and gene multiplication may account for the triggering of these diseases. The *IFIH1* mutations detected in humans and mice cause amino acid substitutions within the helicase domain of MDA5; however, no obvious hot spot has been identified to date. These mutations may commonly induce conformational changes, resulting in the unmasking of CARD for constitutive activity. Some mutations may also enhance the detection of ligand RNA derived from host cells (chronic viral infections). In this regard, other causative mutations in AGS may be implicated in the loss-of-function of nucleic acid clearance (*TREX, RNASEH2A, B, C* and *ADAR*). The endogenous ligand RNAs that activate RLRs and how this is prevented by homeostatic mechanisms remain unknown. Therefore, the detailed molecular phenotypes of these mutations need to be categorized in relation to the different disease phenotypes, such as SLE and AGS.

RLR research is advancing to the next stage. The activation and suppression mechanisms of RLRs in virus-infected cells have been extensively investigated. However, important questions still remain including how RLRs recognize each virus and how each virus evades the activation of RLR. Furthermore, new questions have recently emerged such as how excess innate immunity causes autoimmunity.

References

[1] Kawai T, Akira S. Toll-like receptors and their crosstalk with other innate receptors in infection and immunity. Immunity 2011;34:637–50.
[2] Yoo JS, Kato H, Fujita T. Sensing viral invasion by RIG-I like receptors. Curr Opin Microbiol 2014;20C:131–8.
[3] Xiao TS, Fitzgerald KA. The cGAS-STING pathway for DNA sensing. Mol Cell 2013;51:135–9.
[4] Diamond MS, Farzan M. The broad-spectrum antiviral functions of IFIT and IFITM proteins. Nat Rev Immunol 2013;13:46–57.
[5] Hooks JJ, Moutsopoulos HM, Notkins AL. The role of interferon in immediate hypersensitivity and autoimmune diseases. Ann NY Acad Sci 1980;350:21–32.
[6] Higgs BW, Liu Z, White B, Zhu W, White WI, et al. Patients with systemic lupus erythematosus, myositis, rheumatoid arthritis and scleroderma share activation of a common type I interferon pathway. Ann Rheum Dis 2011;70:2029–36.
[7] Crow MK. Advances in understanding the role of type I interferons in systemic lupus erythematosus. Curr Opin Rheumatol 2014;26:467–74.
[8] Funabiki M, Kato H, Miyachi Y, Toki H, Motegi H, et al. Autoimmune disorders associated with gain of function of the intracellular sensor MDA5. Immunity 2014;40:199–212.
[9] Miner JJ, Diamond MS. MDA5 and autoimmune disease. Nat Genet 2014;46: 418–9.
[10] Yoneyama M, Kikuchi M, Natsukawa T, Shinobu N, Imaizumi T, et al. The RNA helicase RIG-I has an essential function in double-stranded RNA-induced innate antiviral responses. Nat Immunol 2004;5:730–7.
[11] Kato H, Takeuchi O, Sato S, Yoneyama M, Yamamoto M, et al. Differential roles of MDA5 and RIG-I helicases in the recognition of RNA viruses. Nature 2006;441:101–5.
[12] Satoh T, Kato H, Kumagai Y, Yoneyama M, Sato S, et al. LGP2 is a positive regulator of RIG-I- and MDA5-mediated antiviral responses. PNAS 2010;107: 1512–7.
[13] Venkataraman T, Valdes M, Elsby R, Kakuta S, Caceres G, et al. Loss of DExD/H box RNA helicase LGP2 manifests disparate antiviral responses. J Immunol 2007;178:6444–55.
[14] Luo D, Ding SC, Vela A, Kohlway A, Lindenbach BD, et al. Structural insights into RNA recognition by RIG-I. Cell 2011;147:409–22.
[15] Kageyama M, Takahasi K, Narita R, Hirai R, Yoneyama M, et al. 55 Amino acid linker between helicase and carboxyl terminal domains of RIG-I functions as a critical repression domain and determines inter-domain conformation. Biochem Biophys Res Commun 2011;415:75–81.
[16] Yoneyama M, Kikuchi M, Matsumoto K, Imaizumi T, Miyagishi M, et al. Shared and unique functions of the DExD/H-box helicases RIG-I, MDA5, and LGP2 in antiviral innate immunity. J Immunol 2005;175:2851–8.
[17] Hornung V, Ellegast J, Kim S, Brzozka K, Jung A, et al. 5′-Triphosphate RNA is the ligand for RIG-I. Science 2006;314:994–7.
[18] Pichlmair A, Schulz O, Tan CP, Naslund TI, Liljestrom P, et al. RIG-I-mediated antiviral responses to single-stranded RNA bearing 5′-phosphates. Science 2006;314:997–1001.
[19] Schlee M, Roth A, Hornung V, Hagmann CA, Wimmenauer V, et al. Recognition of 5′ triphosphate by RIG-I helicase requires short blunt double-stranded RNA as contained in panhandle of negative-strand virus. Immunity 2009;31:25–34.
[20] Schmidt A, Schwerd T, Hamm W, Hellmuth JC, Cui S, et al. 5′-triphosphate RNA requires base-paired structures to activate antiviral signaling via RIG-I. PNAS 2009;106:12067–72.
[21] Rehwinkel J, Tan CP, Goubau D, Schulz O, Pichlmair A, et al. RIG-I detects viral genomic RNA during negative-strand RNA virus infection. Cell 2010;140: 397–408.
[22] Patel JR, Jain A, Chou YY, Baum A, Ha T, et al. ATPase-driven oligomerization of RIG-I on RNA allows optimal activation of type-I interferon. EMBO Rep 2013;14:780–7.
[23] Kato H, Takeuchi O, Mikamo-Satoh E, Hirai R, Kawai T, et al. Length-dependent recognition of double-stranded ribonucleic acids by retinoic acid-inducible gene-I and melanoma differentiation-associated gene 5. J Exp Med 2008;205: 1601–10.
[24] Takahasi K, Yoneyama M, Nishihori T, Hirai R, Kumeta H, et al. Nonself RNA-sensing mechanism of RIG-I helicase and activation of antiviral immune responses. Mol Cell 2008;29:428–40.
[25] Seth RB, Sun L, Ea CK, Chen ZJ. Identification and characterization of MAVS, a mitochondrial antiviral signaling protein that activates NF-kappaB and IRF 3. Cell 2005;122:669–82.
[26] Kawai T, Takahashi K, Sato S, Coban C, Kumar H, et al. IPS-1, an adaptor triggering RIG-I- and Mda5-mediated type I interferon induction. Nat Immunol 2005;6:981–8.

[27] Xu LG, Wang YY, Han KJ, Li LY, Zhai Z, et al. VISA is an adapter protein required for virus-triggered IFN-beta signaling. Mol Cell 2005;19:727–40.
[28] Meylan E, Curran J, Hofmann K, Moradpour D, Binder M, et al. Cardif is an adaptor protein in the RIG-I antiviral pathway and is targeted by hepatitis C virus. Nature 2005;437:1167–72.
[29] Sun Q, Sun L, Liu HH, Chen X, Seth RB, et al. The specific and essential role of MAVS in antiviral innate immune responses. Immunity 2006;24:633–42.
[30] Kumar H, Kawai T, Kato H, Sato S, Takahashi K, et al. Essential role of IPS-1 in innate immune responses against RNA viruses. J Exp Med 2006;203:1795–803.
[31] Wu B, Peisley A, Richards C, Yao H, Zeng X, et al. Structural basis for dsRNA recognition, filament formation, and antiviral signal activation by MDA5. Cell 2013;152:276–89.
[32] Hou F, Sun L, Zheng H, Skaug B, Jiang QX, et al. MAVS forms functional prion-like aggregates to activate and propagate antiviral innate immune response. Cell 2011;146:448–61.
[33] Onoguchi K, Onomoto K, Takamatsu S, Jogi M, Takemura A, et al. Virus-infection or 5'ppp-RNA activates antiviral signal through redistribution of IPS-1 mediated by MFN1. PLoS Pathog 2010;6:e1001012.
[34] Onomoto K, Jogi M, Yoo JS, Narita R, Morimoto S, et al. Critical role of an antiviral stress granule containing RIG-I and PKR in viral detection and innate immunity. PLoS One 2012;7:e43031.
[35] Yoo JS, Takahasi K, Ng CS, Ouda R, Onomoto K, et al. DHX36 enhances RIG-I signaling by facilitating PKR-mediated antiviral stress granule formation. PLoS Pathog 2014;10:e1004012.
[36] Poeck H, Bscheider M, Gross O, Finger K, Roth S, et al. Recognition of RNA virus by RIG-I results in activation of CARD9 and inflammasome signaling for interleukin 1 beta production. Nat Immunol 2010;11:63–9.
[37] Hemmi H, Takeuchi O, Kawai T, Kaisho T, Sato S, et al. A Toll-like receptor recognizes bacterial DNA. Nature 2000;408:740–5.
[38] Heil F, Hemmi H, Hochrein H, Ampenberger F, Kirschning C, et al. Species-specific recognition of single-stranded RNA via toll-like receptor 7 and 8. Science 2004;303:1526–9.
[39] Diebold SS, Kaisho T, Hemmi H, Akira S, Reis e Sousa C. Innate antiviral responses by means of TLR7-mediated recognition of single-stranded RNA. Science 2004;303:1529–31.
[40] Deane JA, Pisitkun P, Barrett RS, Feigenbaum L, Town T, et al. Control of toll-like receptor 7 expression is essential to restrict autoimmunity and dendritic cell proliferation. Immunity 2007;27:801–10.
[41] Pisitkun P, Deane JA, Difilippantonio MJ, Tarasenko T, Satterthwaite AB, et al. Autoreactive B cell responses to RNA-related antigens due to TLR7 gene duplication. Science 2006;312:1669–72.
[42] Napirei M, Karsunky H, Zevnik B, Stephan H, Mannherz HG, et al. Features of systemic lupus erythematosus in Dnase1-deficient mice. Nat Genet 2000;25:177–81.
[43] Robinson T, Kariuki SN, Franek BS, Kumabe M, Kumar AA, et al. Autoimmune disease risk variant of IFIH1 is associated with increased sensitivity to IFN-alpha and serologic autoimmunity in lupus patients. J Immunol 2011;187:1298–303.
[44] Crampton SP, Deane JA, Feigenbaum L, Bolland S. Ifih1 gene dose effect reveals MDA5-mediated chronic type I IFN gene signature, viral resistance, and accelerated autoimmunity. J Immunol 2012;188:1451–9.
[45] Crow YJ, Rehwinkel J. Aicardi-Goutieres syndrome and related phenotypes: linking nucleic acid metabolism with autoimmunity. Hum Mol Genet 2009;18:R130–6.
[46] Sun L, Wu J, Du F, Chen X, Chen ZJ. Cyclic GMP-AMP synthase is a cytosolic DNA sensor that activates the type I interferon pathway. Science 2013;339:786–91.
[47] Ishikawa H, Ma Z, Barber GN. STING regulates intracellular DNA-mediated, type I interferon-dependent innate immunity. Nature 2009;461:788–92.
[48] Crow YJ, Hayward BE, Parmar R, Robins P, Leitch A, et al. Mutations in the gene encoding the 3'–5' DNA exonuclease TREX1 cause Aicardi-Goutieres syndrome at the AGS1 locus. Nat Genet 2006;38:917–20.
[49] Lee-Kirsch MA, Gong M, Chowdhury D, Senenko L, Engel K, et al. Mutations in the gene encoding the 3'–5' DNA exonuclease TREX1 are associated with systemic lupus erythematosus. Nat Genet 2007;39:1065–7.
[50] Rice GI, del Toro Duany Y, Jenkinson EM, Forte GM, Anderson BH, et al. Gain-of-function mutations in IFIH1 cause a spectrum of human disease phenotypes associated with upregulated type I interferon signaling. Nat Genet 2014;46:503–9.
[51] Oda H, Nakagawa K, Abe J, Awaya T, Funabiki M, et al. Aicardi-Goutieres syndrome is caused by IFIH1 mutations. Am J Hum Genet 2014;95:121–5.
[52] Nejentsev S, Walker N, Riches D, Egholm M, Todd JA. Rare variants of IFIH1, a gene implicated in antiviral responses, protect against type 1 diabetes. Science 2009;324:387–9.
[53] Jaidane H, Hober D. Role of coxsackievirus B4 in the pathogenesis of type 1 diabetes. Diabete Metab 2008;34:537–48.

Takashi Fujita is a Professor of Molecular Genetics in Institute for Virus Research, Kyoto University Japan. He obtained his Ph.D. from the Waseda University, Tokyo 1982 on studies on interferon priming. He joined Dr. T. Taniguchi's laboratory at Cancer Institute, then later at Osaka University as a postdoctoral fellow until 1990, where he worked on interferon-b gene and identified virus-inducible enhancer element and cloned IRF-1. He joined Prof. D. Baltimore's laboratory as a postdoctoral fellow at Whitehead Institute and Rockefeller University until 1993 and worked on transcriptional regulation by NF-kB. He started his own laboratory in Tokyo Metropolitan Institute for medical Sciences in 1993. His group discovered IRF-3 as a key regulator for interferon genes. In 2004, his group including Dr. M. Yoneyama, discovered RIG-I and related sensors for viral RNA. In 2005, his group moved to Kyoto University.

Hiroki Kato started his research in the field of innate immunity during a Ph.D. course in Prof. Akira Shizuo's lab (Osaka University), generated knockout-mice of RIG-I like receptors (RLRs) and examined the functional role of RLRs in antiviral responses. After getting Ph.D., he worked with Prof. Craig Mello (University of Massachusetts Medical School) as a postdoctoral fellow and investigated a possible role of RLRs in RNA silencing pathway. Now he is an Associate Professor in Kyoto University. Currently his major interest is the involvement of RLRs in miRNA and RNA silencing pathways.