Active Caspase-1-Mediated Secretion of Retinoic Acid Inducible Gene-I

Min-Jung Kim and Joo-Yeon Yoo

*J Immunol* 2008; 181:7324-7331;

doi: 10.4049/jimmunol.181.10.7324

http://www.jimmunol.org/content/181/10/7324

---

**References**

This article cites 45 articles, 16 of which you can access for free at:

http://www.jimmunol.org/content/181/10/7324.full#ref-list-1

**Subscription**

Information about subscribing to *The Journal of Immunology* is online at:

http://jimmunol.org/subscription

**Permissions**

Submit copyright permission requests at:

http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts**

Receive free email-alerts when new articles cite this article. Sign up at:

http://jimmunol.org/alerts
Active Caspase-1-Mediated Secretion of Retinoic Acid Inducible Gene-I

Min-Jung Kim and Joo-Yeon Yoo

Caspase-1 is an inflammatory caspase that controls the activation and secretion of the inflammatory cytokines, IL-1β and IL-18. We observed that cellular levels of retinoic acid-inducible gene-I (RIG-I) were enhanced when the pan-caspase inhibitor Z-VAD-fmk or caspase-1-specific inhibitor Z-WEHD-fmk blocked caspase activity. Overexpression of caspase-1 reduced cellular levels of RIG-I and inhibited RIG-I-mediated signaling activity. Enzymatic activity of caspase-1 was necessary to control RIG-I, although it was not a substrate of proteolytic cleavage by caspase-1. Caspase-1 physically interacted with full length RIG-I, but not with mutant forms lacking either the amino- or carboxyl-terminal domains. RIG-I was present in the supernatant of cells transfected with active caspase-1 but not with caspase-4. Stimulating cells with LPS and ATP also induced secretion of endogenous RIG-I in macrophages. Our data suggest a novel mechanism that negatively regulates RIG-I-mediated signaling activity via caspase-1-dependent secretion of RIG-I protein. The Journal of Immunology, 2008, 181: 7324–7331.
cellular levels of RIG-I and RIG-I-mediated signaling activity can be negatively regulated by an IFN-inducible ISG15 conjugating system (24).

The amino-terminal CARD of RIG-I is required for activation of downstream signaling through interaction with adaptor proteins. Although it has two CARD domains, the role of caspase in the regulation of RIG-I or of RIG-I-mediated signaling activity has not been explored. Caspase-1 in the inflammasome complex is activated during viral infection, prompting us to examine the effect of caspase-1 upon RIG-I and the signaling pathways it mediates. In the present study, we demonstrate that caspase-1 negatively regulates cellular RIG-I via enhanced secretion.

Materials and Methods

Plasmid construction

Full-length human procaspase-1, -3 and -4 cDNAs were obtained from HepG2 cells by reverse transcriptase PCR, and were subsequently cloned into the pDEST-51 vector (Invitrogen). The following primer sequences were used for PCR: procaspase-1, 5'-GACTATGATGGGCGACAG TCTTGCTG-3' and 5'-CGGGATCCATGTCCTGGGAAGAGGTA-3'; procaspase-3, 5'-CGGGATCCGTGAAATATAATAGTGTC-3' and 5'-GGA CGTATAATGGACACATGAAAC-3; procaspase-4, 5'-GGA GTGATGGGCGACAG TCTTGCTG-3' and 5'-CGGGATCCATGGCACAG GAAAGGGTAGAA-3'. To generate procaspase-1 C285S, D297A, or D316A mutants, appropriate point mutations were introduced into procaspase-1 using the QuickChange site-directed mutagenesis kit. The following primer sequences were used for the construction of GST-fused procaspase-1: cDNA encoding procaspase-1 using the QuickChange site-directed mutagenesis kit (Stratagene). Sequences of the mutated clones were verified by automatic sequencing. PeptideCutter program (http://br.expasy.org/tools/peptidecutter) was used to search for caspase-dependent proteolysis of RIG-I. Previously, we observed that stimulation with poly(I:C) or IFNα/β resulted in a posttranslational decrease in RIG-I levels (24). To determine the underlying mechanism, we first examined the protease-dependent proteolyis of RIG-I. PeptideCutter program (http://br.expasy.org/tools/peptidecutter) was used to search for sites in the RIG-I protein sequence that could potentially be cleaved by proteases. The program predicted one site, YTD1221, in the helicase domain of RIG-I, as being potentially cleaved by caspase-1. To examine the involvement of caspase activity in the regulation of RIG-I, we treated Flag-RIG-I-transfected cells with a pan-caspase inhibitor, Z-VAD-fmk (Fig. 1A), or with a specific caspase-1 inhibitor, Z-WEHD-fmk (Fig. 1B), and levels of exogenous RIG-I protein were measured using an anti-Flag immunoblot assay. Blockage of caspase activity by both Z-VAD-fmk and Z-WEHD-fmk elicited a similar increase in the cellular levels of RIG-I protein, indicating that RIG-I might be regulated by caspase-related activity at the translational or posttranslational level. To identify the caspases responsible for this regulation, we cloned a procaspase-1 expression plasmid, transfected it into COS-7 cells, and monitored the resulting changes in RIG-I protein levels. As expected, levels of exogenous RIG-I were dramatically reduced in the cells transfected with the procaspase-1 expression plasmid (Fig. 1C). The effect of caspase-1 upon the level of endogenous RIG-I was confirmed in HepG2 cells, which express relatively high levels of RIG-I protein (Fig. 1D). The regulatory effect of caspase-1 was specific, as overexpression of procaspase-3 in HepG2 cells had no effect on the cellular levels of RIG-I protein (Fig. 1E). These results suggest that caspase-1 decreases cellular levels of RIG-I protein.
Enzymatic activity of caspase-1 is required to down-regulate RIG-I levels

Because it had been predicted that RIG-I contains a caspase-1 cleavage site, we hypothesized that it might also be a substrate for proteolytically active caspase-1. Cellular levels of exogenous RIG-I were compared in cells transfected with wild type or mutant forms of procaspase-1. Procaspase-1 mRNA encodes a 45 kDa cytosolic procaspase-1 protein that requires proteolytic cleavage to produce p20 and p10, which subsequently form a heterodimer, constituting the active caspase-1 complex (25). To inhibit proteolytic cleavage of procaspase-1, point mutations were introduced at the potential cleavage sites and the active cysteine residue of procaspase-1 are shown (adapted from ref. 25). Enzymatic activity of caspase-1 is required to control cellular levels of RIG-I proteins. A, top, Autocleavage sites and the active cysteine residue of procaspase-1 are shown (adapted from ref. 25). B, bottom, HepG2 cells were transfected with wild type or mutant forms of procaspase-1-V5 along with Flag-RIG-I. C, D, top, Potential sites on the RIG-I protein for cleavage by caspase-1 are shown. Point mutations at the potential cleavage sites are described. D, bottom, HepG2 cells were transfected with wild type or mutant forms of RIG-I along with procaspase-1-V5 expression plasmids. Cellular levels of exogenous RIG-I were determined by immunoblot assay.

The effect of caspase-1 on RIG-I down-regulation is enhanced by cytosolic poly(I:C)

It has previously been reported that cytosolic antiviral RNAs, such as the influenza virus or synthetic poly(I:C), activate caspase-1 to mediate proteolytic cleavage of IL-1β and IL-18 in human primary macrophages (27). In HepG2 cells, we observed enhanced cleavage of procaspase-1 by intracellular poly(I:C) (Fig. 3A). HepG2 cells were transfected with synthetic dsRNA and poly(I:C), and cleavage of endogenous procaspase-1 was monitored by immunoblot assay. Cleavage of procaspase-1 to generate the p20 fragment was clearly visible after 6 h of cytosolic poly(I:C) stimulation. Cells were then transfected with procaspase-1-V5, and the degree of cleavage mediated by cytosolic poly(I:C) signals was examined (Fig. 3B). Levels of full-length procaspase-1-V5 protein were decreased by treatment with cytosolic poly(I:C), indicating that poly(I:C) mediated cleavage of procaspase-1 to form the active caspase-1 complex. Because cytosolic poly(I:C) stimulates the formation of the active caspase-1 complex, the next question was whether the caspase-1-mediated effect upon RIG-I protein levels can be enhanced by cytosolic poly(I:C)-mediated signals. For this purpose, cells were transfected with Flag-RIG-I and procaspase-1 expression plasmids, and cellular levels of exogenous RIG-I protein were compared in the presence of cytosolic poly(I:C) stimulation (Fig. 3C). As expected, the effect of those observed in wild type RIG-I-containing cells (Fig. 2B). Although enzymatic activity of caspase-1 was required for RIG-I regulation, this result indicates that RIG-I might not be a substrate of proteolytic cleavage by caspase-1.

FIGURE 1. Cellular levels of RIG-I protein are down-regulated by caspase-1 activity. Flag-RIG-I (1 μg plasmid/35 mm dish) transfected cells were treated with the pan-caspase inhibitor, Z-VAD-fmk (100 μM) (A) or with a caspase-1 specific inhibitor, Z-WEHD-fmk (100 μM) (B), for 16 h. Cellular levels of exogenous RIG-I was analyzed by anti-Flag immunoblot assay. C, Procaspase-1-V5 expression vectors were cotransfected along with Flag-RIG-I. Exogenous RIG-I level was assayed by anti-Flag Ab. D, Procaspase-1-V5 was transiently transfected into HepG2 cells and cellular levels of endogenous RIG-I was analyzed by anti-RIG-I immunoblot assay. E, HepG2 cells were transfected with procaspase-1-V5 or procaspase-3-V5 along with Flag-RIG-I expression plasmids. Protein levels of exogenous RIG-I were determined by immunoblot assay using anti-Flag Ab. For every membrane, anti-Flag or anti-RIG-I was first assayed, stripped, and then reprobed with anti-V5 or anti-GAPDH Abs.

FIGURE 2. Enzymatic activity of caspase-1 is required to control cellular levels of RIG-I proteins. A, top, Autocleavage sites and the active cysteine residue of procaspase-1 are shown (adapted from ref. 25). Bottom, HepG2 cells were transfected with wild type or mutant forms of procaspase-1-V5 along with Flag-RIG-I. B, top, Potential sites on the RIG-I protein for cleavage by caspase-1 are shown. Point mutations at the potential cleavage sites are described. Bottom, HepG2 cells were transfected with wild type or mutant forms of RIG-I along with procaspase-1-V5 expression plasmids. Cellular levels of exogenous RIG-I was determined by immunoblot assay.
caspase-1 upon RIG-I down-regulation was further enhanced by stimulation with cytosolic poly(I:C), as cellular levels of RIG-I protein were dramatically lowered by cotreatment with pro-caspase-1 and cytosolic poly(I:C). Similar results were repeatedly observed in transfected 293FT cells, confirming that cytosolic poly(I:C) activates caspase-1 to negatively control cellular levels of RIG-I protein.

**Caspase-1 attenuates RIG-I-mediated signaling activity**

We next examined the effect of inflammatory caspase-1 upon RIG-I-mediated signaling events. Activated RIG-I transmits signals to produce type I IFN, which activates JAK/STAT signaling pathways to induce transcription of IFN stimulated genes (4, 28). RIG-I-mediated signaling activity was assayed using pPRDIII-I-luc reporter constructs derived from the IFN promoter, while IFN-mediated signaling activity was assayed using a pISRE-luc reporter construct. Luciferase assays were performed in HepG2 cells that had been transfected with pISRE-luc or pPRDIII-I-luc constructs along with Flag-RIG-I and Procaspase-1-V5 expression vectors. Overexpression of RIG-I markedly increased IFN promoter activity and IFN-mediated signaling activity, both of which were completely abolished in the cells cotransfected with procaspase-1 (Fig. 4A). RIG-I-mediated signaling activity was positively correlated with cellular RIG-I, as its protein level was increased 3-fold upon IFN stimulation, as cellular levels of RIG-I were dramatically lowered by cotreatment with pro-caspase-1 and cytosolic poly(I:C). However, the enhanced luciferase activity resulting from the overexpression of RIG-I was significantly decreased by coexpression of procaspase-1, suggesting that procaspase-1 specifically targets the RIG-I protein and attenuates RIG-I-mediated signaling activity.

**Caspase-1 physically interacts with RIG-I**

To determine whether procaspase-1 binds to RIG-I, we performed a GST-fusion protein pull-down of procaspase-1 from lysates of Flag-RIG-I and GST-procaspase-1 and Procaspase-1-V5 expression vectors. We next examined the effect of inflammatory caspase-1 upon RIG-I-mediated signaling events. Activated RIG-I transmits signals to produce type I IFN, which activates JAK/STAT signaling pathways to induce transcription of IFN stimulated genes (4, 28). RIG-I-mediated signaling activity was assayed using pPRDIII-I-luc reporter constructs derived from the IFN promoter, while IFN-mediated signaling activity was assayed using a pISRE-luc reporter construct. Luciferase assays were performed in HepG2 cells that had been transfected with pISRE-luc or pPRDIII-I-luc constructs along with Flag-RIG-I and Procaspase-1-V5 expression vectors. Overexpression of RIG-I markedly increased IFN promoter activity and IFN-mediated signaling activity, both of which were completely abolished in the cells cotransfected with procaspase-1 (Fig. 4A). RIG-I-mediated signaling activity was positively correlated with cellular RIG-I, as its protein level was increased 3-fold upon IFN stimulation, as cellular levels of RIG-I were dramatically lowered by cotreatment with pro-caspase-1 and cytosolic poly(I:C). However, the enhanced luciferase activity resulting from the overexpression of RIG-I was significantly decreased by coexpression of procaspase-1, suggesting that procaspase-1 specifically targets the RIG-I protein and attenuates RIG-I-mediated signaling activity.

**Caspase-1 physically interacts with RIG-I**

To determine whether procaspase-1 binds to RIG-I, we performed a GST-fusion protein pull-down of procaspase-1 from lysates of Flag-RIG-I and GST-procaspase-1-transfected cells. GST-procaspase-1, but not GST alone, coprecipitated with RIG-I, indicating that these proteins physically interact (Fig. 5A). The GST pull-down assay was repeated using mutant forms of RIG-I to identify the domains responsible for the physical interaction with procaspase-1. RIG-I (Exon2 del) interacted with procaspase-1 as efficiently as wild type RIG-I (Fig. 5A). To determine whether procaspase-1 interacts with RIG-I, we performed a GST-fusion protein pull-down of procaspase-1 from lysates of Flag-RIG-I and GST-procaspase-1-transfected cells. GST-procaspase-1, but not GST alone, coprecipitated with RIG-I, indicating that these proteins physically interact (Fig. 5A). The GST pull-down assay was repeated using mutant forms of RIG-I to identify the domains responsible for the physical interaction with procaspase-1. RIG-I (Exon2 del) interacted with procaspase-1 as efficiently as wild type RIG-I (Fig. 5A). The GST pull-down assay was repeated using mutant forms of RIG-I to identify the domains responsible for the physical interaction with procaspase-1. RIG-I (Exon2 del) interacted with procaspase-1 as efficiently as wild type RIG-I (Fig. 5A). To determine whether procaspase-1 interacts with RIG-I, we performed a GST-fusion protein pull-down of procaspase-1 from lysates of Flag-RIG-I and GST-procaspase-1-transfected cells. GST-procaspase-1, but not GST alone, coprecipitated with RIG-I, indicating that these proteins physically interact (Fig. 5A). The GST pull-down assay was repeated using mutant forms of RIG-I to identify the domains responsible for the physical interaction with procaspase-1. RIG-I (Exon2 del) interacted with procaspase-1 as efficiently as wild type RIG-I (Fig. 5A).
reported (4). However, CARD domain in the procaspase-1 was not required for interaction, as the mutant form of procaspase-1 that lacks an N-terminal CARD domain still interacted with RIG-I as efficiently as full-length procaspase-1 (Fig. 5D). This result indicated that an active form of caspase-1 was sufficient for physical interaction with RIG-I. We also examined the effect of cytosolic poly(I:C) on physical interaction between procaspase-1 and RIG-I. Because we observed that down-regulation of RIG-I by pro-caspase-1 was further enhanced by cytosolic poly(I:C) (Fig. 3C), we postulated that cytosolic poly(I:C) might stabilize the interaction between procaspase-1 and RIG-I. However, to our surprise, the observed interaction was diminished by stimulation with cytosolic poly(I:C) (Fig. 5E). Because it’s been previously reported that poly(I:C) treatment decreases cellular levels of exogenous RIG-I (24), this result might be caused by diminished Flag-RIG-I, rather than disrupted interaction between RIG-I and procaspase-1. We also attempted, but failed, to detect interaction between endogenous RIG-I and caspase-1 after cytosolic poly(I:C) (data not shown).

**RIG-I is secreted by active caspase-1**

Active caspase-1 was found to down-regulate RIG-I and its signals, but this effect did not involve any caspase-related cleavage of...
RIG-I. To investigate the underlying mechanism of caspase-1-mediated down-regulation of RIG-I, the relationship between cellular localization of RIG-I and caspase-1 was examined. Recently, caspase-1-mediated unconventional secretion of leaderless proteins has been reported in various cell types including mouse macrophages, human primary keratinocytes, and COS-1 cells (16). Therefore, we tested whether RIG-I can be secreted to extracellular compartments in a caspase-1 activity-dependent manner. For this purpose, HepG2 cells were transiently transfected and maintained in medium containing low levels of serum (0.5% FBS) for 24 h, before the conditioned medium was collected for RIG-I analysis. In the lysates of cells cotransfected with Flag-RIG-I and Pro-caspase-1-V5, RIG-I protein levels were dramatically reduced, as observed previously. Simultaneously, RIG-I was detected in the supernatant of procaspase-1 transfected cells, along with caspase-1 (Fig. 6A). Secretion of RIG-I was dependent on the enzymatic activity of caspase-1, as a mutant form of procaspase-1 (C285S) did not support secretion of RIG-I (Fig. 6B). Neither RIG-I nor caspase-1 was found in the conditioned medium of procaspase-1 (C285S) transfected cells. Notably, the cleaved form of caspase-1 was found in the supernatant of enzymatically active form of caspase-1 transfected cells, indicating that secretion of RIG-I might be dependent on the enzymatic activity of caspase-1. To exclude the possibility that RIG-I secretion was a result of pyroptosis, a cell-death pathway that depends on caspase-1 activity (30), we checked degree of cell lysis by measuring the ratio between the activity of cytosolic lactate dehydrogenase (LDH) in the supernatant and total cell lysates (Fig. 6C). Overexpression of Flag-RIG-I and procaspase-1 didn’t change the degree of LDH release, suggesting that the nonspecific cell lysis was not the cause of RIG-I secretion in our system. Along with RIG-I, a cleaved form of active caspase-1 was also found in the supernatant. Furthermore, when cells were stimulated with cytosolic poly(I:C) to activate caspase-1, secretion of exogenous Flag-RIG-I (Fig. 6D) and endogenous RIG-I (Fig. 6E) were similarly observed in the supernatant. All together, these results strongly suggest that RIG-I is secreted to extracellular compartment in the active caspase-1-dependent manner. Finally, we examined secretion of endogenous RIG-I in the activated macrophage. In addition to PAMP signals, such as bacterial LPS, it has been previously reported that activation of the purinergic P2X7 receptor by exogenous ATP is necessary to induce caspase-1 dependent secretion of IL-1β in macrophages (30–32). To examine the secretion of RIG-I, RAW264.7 cells were treated with LPS for 12 h, followed by stimulation with exogenous ATP for 20 min (Fig. 6F). Treatment with LPS alone induced expression of RIG-I in the cells, but RIG-I was not detected in the medium, indicating that PAMP signal alone was not sufficient to induce RIG-I secretion in macrophage. In the presence of exogenous ATP, however, secretion of RIG-I to extracellular compartment was clearly visible, suggesting that both PAMP and exogenous ATP signals are required to induce RIG-I secretion in macrophages.

**Caspase-mediated RIG-I secretion is a specific process**

To verify the role of caspase-1 to RIG-I secretion, we first examined the effect of a specific caspase-1 inhibitor, Z-WEHD-fmk, in HepG2 cells. Secretion mediated by overexpression of procaspase-1 (Fig. 7A) or by cytosolic poly(I:C) (Fig. 7B) was specifically inhibited by Z-WEHD-fmk. To determine whether RIG-I secretion can be induced by any kind of inflammatory caspases, HepG2 cells were transfected with procaspase-1 or procaspase-4, along with Flag-RIG-I, and secretion of RIG-I was examined in the supernatant of transfected cells (Fig. 7C). RIG-I was not detected in the supernatant of procaspase-4 overexpressed cells, suggesting that secretion of RIG-I was specific to activity of caspase-1. We previously observed that cellular levels RIG-I decreased when enzymatic activity of 26S proteasome was inhibited in HepG2 cells (24). Therefore, we examined whether blockage of proteasome activity enhances the secretion processes of RIG-I in HepG2 cells. When procaspase-1 transfected cells were treated with MG132, a 26S-proteasome inhibitor, intracellular levels of RIG-I was further decreased. However, secretion of RIG-I was not affected by MG132 treatment (Fig. 7D). Finally, we tested whether MDA5 can be secreted. Because MDA5 shares multiple functions with RIG-I, such as dsRNA binding and MAVS activation, it was reasonable to speculate its secretion. However, protein levels of MDA5 was not altered by coexpression of procaspase-1 in the cells. Furthermore, MDA-5 was not found in the supernatant of cotransfected cells (Fig. 7E). These results suggested that active caspase-1 specifically controls RIG-I secretion, and RIG-I mediated anti-viral signaling pathways.

**Discussion**

The present study demonstrates that caspase-1 controls intracellular levels of RIG-I protein, hence down-regulates RIG-I-mediated antiviral signaling responses. Assays using reporter constructs containing an IFN-responsive promoter indicated that caspase-1 inhibited both RIG-I-mediated production of IFN, and IFN-mediated signaling activity. There are several possibilities for the physiological significance of caspase-1’s negative regulation of RIG-I-mediated signaling activity. First, it might provide leverage to balance the cytokines produced during the response to microbial detection. Second, it might function as a checkpoint for the full response against microbial infection; when caspase-1 is activated by DAMPs without microbial infection, it might act to prevent IFN overproduction.
The intracellular presence of foreign nucleic acids, such as microbial DNA, adenosine viral, or viral RNA, and host RNA in the cytoplasm stimulates an immune response involving the production of type I IFN or proinflammatory cytokines such as IL-1β or TNF-α (33–35). Although the production of type I IFN is controlled mainly by transcriptional induction of the IFNβ gene, the production of the inflammatory cytokines IL-1β and IL-18 requires both transcriptional induction and inflammasome-mediated maturation (1, 13, 14, 36). Although both type I IFN and proinflammatory cytokines are reported to function during the antiviral or antimicrobial innate immune response, several lines of evidence indicate that there might be negative crosstalk between type I IFN-mediated signaling pathways and proinflammatory cytokine-mediated signaling pathways. Notably, caspase-mediated cleavage of STAT1, a transcription factor that transmits type I IFN signals, has been reported to occur in HeLa cells activated with dsRNA (37). Pretreatment of human FS-4 fibroblasts with IL-1β inhibits the IFNβ production that normally occurs in response to poly(I:C) stimulation (38). In addition, pretreatment with IFNs inhibited the secretion of IL-1β that normally occurs in response to Staphylococcal α-toxin (39).

Activation mechanism of inflammasome and caspase-1 has been well established in the macrophage and dendritic cells (11, 12, 40). Generally, two kinds of signals are required to induce secretion of IL-1β by caspase-1; one is the signal that activates TLR-mediated signaling pathways, such as LPS, CpG dinucleotides, and R848. The other is danger-related signals, such as perturbation of ionic efflux and membrane blebbing caused by exogenous ATP or nigericin (11). On the contrary to this two-signal hypothesis, secretion of RIG-I was observed in the hepatocyte stimulated with cytosolic poly(I:C) in our study. We do not yet know whether cytosolic poly(I:C) generates two signals that are necessary to activate caspase-1 inflammasome in the hepatocytes, or secretion of RIG-I requires two signals. It is noteworthy to mention that inflammasome also senses cytoplasmic DNA in the TLR-independent manner and activates IL-1β secretion (41). In the earlier data we presented (Fig. 1A), we also observed caspase-1 activity without obvious caspase-1 activating agents used in the study. We interpreted this result by the activation of caspase-1 via transfected plasmid.

Because enzymatic activity of caspase-1 was required for down-regulation of RIG-I, we examined whether RIG-I might be proteolytically cleaved by caspase-1. However, no RIG-I cleavage products were found to occur specifically in association with caspase-1. Furthermore, mutations of the predicted caspase-1 cleavage sites in RIG-I had no effect on RIG-1 activity, strongly suggesting that RIG-I is not a substrate for caspase-1-mediated proteolysis. However, cellular localization of RIG-I was severely altered by the above-mentioned mutations, as RIG-I was found in the supernatant of procaspase-1 transfected cells. RIG-I is a cytosolic protein that does not contain a leader sequence, therefore it cannot be secreted through conventional ER-Golgi trafficking pathways. There are several unconventional mechanisms via which RIG-I could be secreted. First, it might form complexes with the inflammasome, and be transported into a subset of secreto-ry lysosomes along with caspase-1 and IL-1β (42, 43). It is noteworthy that secretion of Hsp70 upon microbial or viral infection also requires trafficking within lysosomal endosomes (44). Second, microvesicle shedding from the plasma membrane provides an alternative mechanism of RIG-I secretion, as reported in the case of IL-1β-containing microvesicles in human monocytes (45). Finally, the possibility that RIG-I travels directly through the plasma membrane cannot be excluded.

Accumulating evidence suggests that caspase-1-mediated secretion of RIG-I involves the inflammasome. We found that RIG-I physically associates with caspase-1, and it was detected in the supernatant along with caspase-1. Furthermore, RIG-I was detected in lysates immunoprecipitated with an anti-apoptosis-associated speck-like protein containing a CARD Ab (M. J. Kim, data not shown). Based on these findings, it is likely that RIG-I is secreted with caspase-1 in the inflammasome complex. However, the physiological significance of RIG-I secretion is not yet clear. It may simply serve as a mechanism to reduce cytosolic levels of RIG-I. Alternatively, secreted RIG-I might exert some function in the extracellular compartments. Because RIG-I has a strong affinity for binding to atypical foreign RNAs, future investigations of the role of extracellular RIG-I during antiviral or antimicrobial immune responses will be of great interest.

Acknowledgments
We thank Dr. Y. S. Gho for helpful discussions and Drs. T. Fujita and S. Goodburn for RIG-I and Mda5 expression plasmids.

Disclosures
The authors have no financial conflict of interest.

References


