The Association of Receptor of Activated Protein Kinase C 1 (RACK1) with Infectious Bursal Disease Virus Viral Protein VP5 and Voltage-dependent Anion Channel 2 (VDAC2) Inhibits Apoptosis and Enhances Viral Replication*  

Wencheng Lin1**, Zhiqiang Zhang1**, Zhichao Xu1**, Bin Wang1**, Xiaqi Li1**, Hong Cao1**, Yongqiang Wang1**, and Shijun J. Zheng1**

From the 1State Key Laboratory of Agrobiotechnology, 2Key Laboratory of Animal Epidemiology and Zoonosis, Ministry of Agriculture, and 3College of Veterinary Medicine, China Agricultural University, Beijing 100193, China

**The abbreviations used are: IBD, infectious bursal disease; IBDV, infectious bursal disease virus; CEF, chicken embryo fibroblast; TCID_{50}, 50% tissue culture infective dose; TRITC, tetramethylrhodamine isothiocyanate.

Infectious bursal disease (IBD),3 also called Gumboro disease, is an acute, highly contagious, and immunosuppressive avian disease caused by IBD virus (IBDV). Our previous report indicates that IBDV VP5 induces apoptosis via interaction with voltage-dependent anion channel 2 (VDAC2). However, the underlying molecular mechanism is still unclear. We report here that receptor of activated protein kinase C 1 (RACK1) interacts with both VDAC2 and VP5 and that they could form a complex. We found that overexpression of RACK1 inhibited IBDV-induced apoptosis in DF-1 cells and that knockdown of RACK1 by small interfering RNA induced apoptosis. These results indicate that RACK1 inhibits apoptosis via interaction with VDAC2 and VP5, suggesting that VP5 sequesters RACK1 and VDAC2 in the apoptosis-inducing process.

Infectious bursal disease (IBD), also called Gumboro disease, is an acute, highly contagious disease in young chickens that occurs across the world (1). Its causative agent, IBD virus (IBDV), destroys its target cells, the B lymphocyte precursors (2–4). IBDV infection may cause mortality in naïve chickens and very high mortality in birds suffering from a severe immunosuppression that leads to an increased susceptibility to other pathogens (5).

IBDV belongs to the genus Avibirnavirus of the family Birnaviridae and has two segments of double-stranded genomic RNAs (A and B) (7). Segment B encodes VP1 (97 kDa), a RNA-dependent RNA polymerase (8), affecting viral replication and virulence (2, 9, 10). Segment A contains two partially overlapping ORFs. The first ORF encodes nonstructural viral protein 5 (VP5), and the second one encodes a pVP2-pVP4-VP3 precursor (110 kDa) that can be cleaved by the proteolytic activity of VP4 to form viral proteins VP2, VP3, and VP4 (7, 11, 12). VP2, a major structural protein (13), is involved in antigenicity, cell tropism, pathogenic phenotype, and apoptosis (14). VP3 also participates in the formation of viral particles and is involved in serotype specificity (15), viral assembly (11, 16–18), and apoptotic regulation (19). VP4, a viral protease, is able to cleave and is responsible for the interdomain proteolytic autoprocessing of the pVP2-pVP4-VP3 polypeptide into the pVP2 precursor (48 kDa) and VP4 (28 kDa) as well as VP3 (32 kDa) (6, 20). pVP2 is further processed at its C-terminal domain by VP4 to generate the mature capsid protein VP2 (41 kDa) and four small peptides (21). A recent report indicates that VP4 is responsible for IBDV-induced immune suppression (22). The nonstructural viral protein VP5 only exists in IBDV-infected cells and plays different roles in IBDV-induced apoptosis during IBDV infection. VP5 inhibits apoptosis early during infection (23, 24), whereas it induces apoptosis at a later stage of infection (4, 25, 26).

In a previous study, we found that VP5 induces apoptosis in DF-1 cells via interaction with voltage-dependent anion channel 2 (VDAC2) (25). However, the molecular mechanism underlying such an induction remains elusive. In this study, we expanded our investigation to search for the interacting proteins for VDAC2 by yeast two-hybrid screening, immunoprecipitation, and confocal microscopy assays. We found that receptor of activated protein kinase C 1 (RACK1) interacts with both VDAC2 and VP5 and that they can form a complex.
Importantly, overexpression of RACK1 suppressed IBDV-induced apoptosis. Furthermore, knockdown of RACK1 by siRNA markedly induced the activation of caspases 9 and 3 and suppressed IBDV growth.

**EXPERIMENTAL PROCEDURES**

**Cell Lines and Virus**—Both HEK293T and DF-1 (immortal chicken embryo fibroblast) cells were obtained from the ATCC. All cells were cultured in DMEM (Invitrogen) supplemented with 10% FBS in a 5% CO₂ incubator. Primary chicken embryo fibroblast (CEF) cells were prepared from 10-day-old specific pathogen-free chicken embryos. Lx, a cell culture-adapted IBDV strain, was provided by Dr. Jue Liu (Beijing Academy of Agriculture and Forestry, Beijing, China).

**Chemicals and Antibodies**—All restriction enzymes were purchased from New England Biolabs. The pRK5-FLAG, pDsRed-monomer-N1, pCMV-Myc, pEGFP-C1, and pEGFP-N1 vectors were obtained from Clontech. Anti-c-Myc (catalog no. sc-40), anti-GFP (catalog no. sc-9996), anti-RACK1 (catalog no. sc-17754), and anti-β-actin (catalog no. sc-1616-R) monoclonal antibodies were obtained from Santa Cruz Biotechnology. Rabbit anti-VDAC2 polyclonal antibodies (catalog no. ab47104) were purchased from Abcam. Anti-V5 monoclonal antibody (catalog no. EU0208) was purchased from CAEU Biological Co. (Beijing, China). Rabbit anti-GFP antibodies (catalog no. 2956S) were purchased from Cell Signaling Technology. Anti-FLAG (catalog no. F1804) antibody, propidium iodide, Annexin V-phycocerythrin (Annexin V-PE) and 7-amino-actinomycin D were purchased from Sigma. Optimem I, RNAiMAX, and Lipofectamine LTX were purchased from Invitrogen.

**Plasmid Construction**—Gallus RACK1 was cloned from DF-1 cells using the specific primers 5'-ATGACGGAGCAGAT-GACC-3' (sense) and 5'-TCATCTGTGTTCCAATGTG-3' (antisense) according to the published sequence in GenBank (accession No. AY393848.1). pRK5-FLAG-rack1, pCMV-Myc-rack1, pDsRed-rack1, and pEGFP-rack1 were constructed by standard molecular biology techniques. All primers were obtained from a commercial source (Sangon, Shanghai, China). pRK5-FLAG-vdac2, pEGFP-vdac2, pRK5-FLAG-vp5, and pEGFP-vp5 plasmids were kept in our laboratory.

**Yeast Two-hybrid Screening and Colony Lift Filter Assay**—Yeast two-hybrid screening was performed according to the protocol of the manufacturer (Matchmaker Two-Hybrid System 3). Briefly, the pGBK7-vdac2 plasmid expressing the fusion protein GAL4-BD-vdac2 was used as bait, and the bursa of Fabricius cDNA expression library fusion to the GAL4-activation domain in the pGADT7 plasmid was used as prey. Positive clones were selected on S.D./Ade/His/Leu/Trp medium and tested for β-galactosidase activity.

**Coimmunoprecipitation and Western Blot Analysis**—The coimmunoprecipitation approach used to analyze protein interaction has been described previously (25). Briefly, HEK293T cells or DF-1 cells were cotransfected with the indicated plasmids or empty vectors as controls. Twenty-four hours after transfection, cell lysates were subjected to immunoprecipitation with anti-Myc (or anti-FLAG) antibody at 4 °C for 3 h and then mixed with 20 μl of a 50% slurry of protein A/G plus agarose and incubated for another 3 h. Beads were washed three times with lysis buffer and boiled with 2X SDS loading buffer for 10 min. The samples were fractionated by SDS-PAGE and transferred onto polyvinylidene difluoride membrane. After blocking with 5% skimmed milk, the membranes were incubated with the indicated antibodies. Blots were developed using an ECL kit. For the endogenous pulldown assay, DF-1 cells were transfected with pEGFP-V5 or infected with IBDV. Twenty-four hours after transfection or 36 h after infection, the cell lysates were subjected to immunoprecipitation with anti-RACK1 antibody and immunoblotted with anti-VDAC2, anti-RACK1, anti-V5, or anti-GFP antibodies.

**Confocal Laser-scanning Microscopy Assay**—HEK293T cells transfected with pDsRed-rack1 and pEGFP-vdac2 (or pEGFP-vp5) were fixed with 4% paraformaldehyde, and the nuclei were stained with DAPI. For endogenous protein staining, mock- or IBDV-infected DF-1 cells were fixed with 4% paraformaldehyde, permeabilized with Triton X-100, blocked with bovine serum albumin, and then probed with the indicated antibodies. The cells were stained for nuclei with DAPI. The samples were analyzed with a laser confocal scanning microscope (Nikon C1 standard detector).

**Apoptosis Assay**—DF-1 or CEF cells were transfected with pEGFP-rack1 or pEGFP-N1 plasmids by Lipofectamine LTX before infection with IBDV at an m.o.i. of 1. Twenty-four hours after infection, cells were harvested and stained with propidium iodide (1 μg/ml) or PE-conjugated Annexin V in a binding buffer for 15 min. GFP-positive cells were gated for apoptosis analysis. Fluorescence-activated cell sorter data were analyzed with CellQuest software (BD Biosciences).

DF-1 cells receiving RACK1-specific siRNA or control siRNA were harvested and washed twice with PBS, resuspended in a binding buffer, and incubated with PE-conjugated Annexin V and 7-amino-actinomycin D for 15 min at room temperature. Samples were subjected to flow cytometry analysis as described above.

**Knockdown of RACK1 by RNAi**—siRNAs designed by Genechem Co. (Shanghai, China) were used to knock down RACK1 in DF-1 cells. The sequences of siRNA for targeting RACK1 in DF-1 cells included RNAi#1 (sense, 5'-CCCGAGAUGAGACCAACUAtt-3'; antisense, 5'-UAGUUGGUCUCAUCUGGtt-3'), RNAi#2 (sense, 5’-CCCGAGAAGAGACCAACUAtt-3'; antisense, 5'-UAGUUGGUCUCAUCUGGttGtt-3'), RNAi#3 (sense, 5'-UCAAAUCUGAAGACCAACUtt-3'; antisense, 5’-AAAGUGUUCAGAGUUAAGtt-3'), and a negative siRNA control (sense, 5'-UUCUCGGAAGUGUGUGGUttGtt-3'; antisense, 5’-ACGUGACACGUAAGUGGUttGtt-3'). DF-1 cells were transfected with siRNA using RNAiMAX reagent according to the instructions of the manufacturer (Invitrogen). Triple transfections were performed at a 24-h interval. Twenty-four hours after the third transfection, cells were harvested for further analysis.

**Caspase 3 and 9 Activity Assays**—DF-1 cells receiving RACK1-specific siRNA or control siRNA were lysed in chilled cell lysis buffer, the cell lysates were centrifuged, and the supernatants were collected to measure caspase 3 and 9 activities according to the instructions of the manufacturer (BioVision). The results of all experiments are reported as mean ± S.D.
**RESULTS**

**RACK1 Interacts with VDAC2 and IBDV VP5**—Our previous report indicated that VP5 induced apoptosis in DF-1 cells via interaction with VDAC2, which affected the viral release (25). To elucidate the mechanism of such an induction, we set out to search for the cellular targets of VDAC2. We used VDAC2 as a bait in a yeast two-hybrid system to screen a cDNA library generated from the chicken bursa of Fabricius. Among the proteins that potentially interacted with VDAC2, RACK1 proteins were identified twice. The interaction between VDAC2 and RACK1 was further verified with a colony lift filter assay (Fig. 1).

Because RACK1 is related to apoptosis (28–30), we proposed that RACK1 might be involved in VDAC2-mediated apoptosis. Therefore, we constructed a plasmid that allowed the expression of Myc-RACK1 and GFP-VDAC2 to be immunoprecipitated with Myc antibody, GFP-VDAC2 was detected in the precipitate, indicating that RACK1 interacted with exogenous VDAC2 in HEK293T cells (Fig. 2A). Similar results were obtained in an experiment using DF-1 cells (Fig. 2B), indicating that the interaction observed between these two proteins is not cell type-specific. Considering the binding capacity of RACK1 as a signaling hub (31), we expanded our investigation to study whether RACK1 could interact with IBDV VP5. Lysates of cells expressing Myc-RACK1 and FLAG-VP5 were immunoprecipitated with FLAG antibody, and Myc-RACK1 was detected in the precipitate, indicating that RACK1 interacted with ectopically expressed VP5 in HEK293T cells (Fig. 2C). Similar results were obtained in an experiment using DF-1 cells (Fig. 2D).

To further substantiate the binding of RACK1 to VDAC2 or IBDV VP5, we expressed RACK1 or VDAC2 in DF-1 cells and examined their interaction with endogenous partners using a pulldown assay. The binding of FLAG-RACK1 with endogenous VDAC2 was readily detectable in cells expressing RACK1 protein (Fig. 3A), and the binding of FLAG-VDAC2 with endogenous RACK1 was also detectable (Fig. 3B). These results demonstrate that VDAC2 interacts with RACK1 in host cells. When lysates of DF-1 cells expressing GFP-VP5 were immuno-
precipitated with RACK1 antibody, GFP-VP5 was detected in the precipitate, indicating that VP5 interacted with endogenous RACK1 in host cells (Fig. 3C). These data suggest that RACK1 interacts with both VDAC2 and VP5 proteins.

**VDAC2, RACK1, and IBDV VP5 Form a Complex**—Because RACK1 interacted with both VDAC2 and VP5 and was considered to play a role in complex assembly, we expanded our investigation to study whether RACK1, VDAC2, and IBDV VP5 form a complex. Lysates of HEK293T cells expressing FLAG-RACK1, GFP-VP5, and GFP-VDAC2 were immunoprecipitated with FLAG antibody, and both GFP-VP5 and GFP-VDAC2 were detected in the precipitate (Fig. 4A), indicating that ectopically expressed VP5, RACK1, and VDAC2 formed a complex. To further examine the complex of VDAC2 and RACK1 to VP5, we expressed VP5 in DF-1 cells and examined the complex using a pulldown assay. Lysates of DF-1 cells expressing GFP-VP5 were immunoprecipitated with RACK1 antibody, and both VP5 and VDAC2 were detected in the precipitate (Fig. 4B), indicating that VP5, endogenous RACK1, and VDAC2 formed a complex in host cells. To determine whether the IBDV VP5, VDAC2, and RACK1 could form a complex under physiological conditions, we infected DF-1 cells and performed a pulldown assay using anti-RACK1 antibodies. As a result, both VP5 and VDAC2 could be detected in the precipitate of the lysates from IBDV-infected cells but not from mock-infected controls (Fig. 4C). These data clearly show that VDAC2, RACK1, and IBDV VP5 formed a complex in host cells.

A Domain That Spans Residues 100–145 of VP5 Is Involved in Interacting with RACK1—To determine the region of VP5 responsible for interacting with RACK1, we constructed a series of VP5 truncated mutants fused to the GFP tag (Fig. 5A). These VP5 derivatives were coexpressed individually with FLAG-RACK1 in HEK293T cells, and lysates of cells were immunoprecipitated with FLAG antibody. Our results indicated that, with the exception of mutants (H90043 and H90044) lacking residues 100–145, the VP5 mutant with residues 100–145 (H90042 mutant) retained the ability to interact with RACK1 (Fig. 5B), suggesting that the region with 100–145 of VP5 is important for its interaction with RACK1. Because we have established previously that a domain that spans residues 1–50 of VP5 interacts with VDAC2 (25), and because our data from this study show that a domain with residues 100–145 of VP5 interacts with RACK1 and that VDAC2 binds to RACK1, it is very likely that VP5, RACK1, and VDAC2 form a complex in cells (Fig. 5C).

**RACK1 Colocalizes with VDAC2 and IBDV VP5**—To determine the subcellular localization of VDAC2 and RACK1, we performed confocal microscopy assays with HEK293T cells
Because we have shown previously that VP5 colocalized with VDAC2 in the mitochondrion of host cells (25) and because VP5-VDAC2-RACK1 formed a complex, as demonstrated by immunoprecipitation assays, we performed a confocal microscopy assay to examine the colocalization of VP5 with RACK1 in cells. As shown in Fig. 6, L–N, the colocalization of VP5 with exogenous RACK1 was found primarily in the cytoplasm of cells transfected with both pDsRed-RACK1 and pEGFP-VP5. When cells were transfected with pEGFP-VP5 only, endogenous RACK1 colocalized with VP5 (Fig. 6, O–Q). In addition, transfection of DF-1 cells with pDsRed-RACK1 indicated that RACK1 was also located in the mitochondria of DF-1 cells (Fig. 6, R–T). All these data strongly suggested that VP5, VADC2, and RACK1 formed a complex in the cytoplasm of cells and that they were all located in the mitochondria.

**Overexpression of RACK1 Inhibits IBDV-induced Apoptosis and Enhances Viral Growth in Host Cells**—Because IBDV VP5 protein was mainly responsible for IBDV-induced apoptosis via interaction with VDAC2 (25) and because RACK1 was associated physically with both VP5 and VDAC2, RACK1 was assumed to affect IBDV-induced apoptosis. To test this hypothesis, DF-1 cells were transfected with pEGFP-rack1 or pEGFP-N1 as a control, followed by mock infection or infection with IBDV. As shown in Fig. 7A, exogenous RACK1 was expressed very well in both mock- and IBDV-infected cells. Interestingly, we found that overexpression of RACK1 markedly suppressed IBDV-induced apoptosis in DF-1 cells (Fig. 7, B and C). Similar results were obtained using primary cell culture (CEFs) (Fig. 7, D and E). These data suggest that RACK1 may play an antiapoptotic role in IBDV-induced apoptosis in host cells.

Because IBDV-induced apoptosis is associated with viral growth (25), we examined the viral growth in pEGFP-rack1-transfected DF-1 cells and CEFs at different time points after IBDV infection. As shown in Fig. 8A, overexpression of RACK1 enhanced IBDV growth in the cell culture compared with that of controls (p < 0.05), whereas there was no difference in viral loads in the supernatants of cell cultures between pEGFP-rack1-transfected cells and controls (Fig. 8B). Similarly, overexpression of RACK1 in CEF cells enhanced viral loads in both supernatants and cell cultures compared with those of controls in CEF cells (Fig. 8, C and D). These data suggest that RACK1-induced suppression of apoptosis favors IBDV growth in host cells.

**Knockdown of RACK1 Induces Apoptosis and Inhibits IBDV Growth**—Our previous report indicated that VP5 induced apoptosis via interaction with VDAC2 at a later stage of IBDV infection, which might facilitate IBDV release (25). It was intriguing to examine apoptosis and viral growth in RACK1 knockdown cells. We knocked down RACK1 expression in DF-1 cells by RNAi and examined apoptosis and IBDV growth in these cells. We made three RACK1 RNAi constructs and found that one (RNAi #1) can effectively lower the cellular level of RACK1 (Fig. 9, A and B). Therefore, the RACK RNAi #1 construct was used to knock down endogenous RACK1 in the following assays. Surprisingly, we found that knockdown of endogenous RACK1 by siRNA significantly induced apoptosis associated with activation of caspases 9 and 3 (p < 0.01) (Fig. 9, A and B).
RACK1 Inhibits IBDV-induced Apoptosis

The nonstructural viral protein VP5 is mainly responsible for IBDV-induced apoptosis (25). VP5 inhibited apoptosis early during infection (23, 24) but induced apoptosis at a later stage of infection (25), suggesting that VP5 may play different roles at different stages of IBDV infection. Our previous report indicated that VP5 induced apoptosis and that this induction resulted from interaction with VDAC2 in the mitochondrial membrane and is involved in apoptosis (36–39). In this report, we attempted to elucidate the molecular mechanism underlying VP5-induced apoptosis by searching for the immediate binding proteins of VDAC2 that are involved in apoptotic signaling transduction. We first determined that RACK1 specifically interacted with VDAC2 and IBDV VP5 under all test conditions. Our data also showed that overexpression of RACK1 markedly suppressed IBDV-induced apoptosis in host cells, indicating an antiapoptotic role of RACK1 in host cells. To the contrary, knockdown of RACK1 induced considerable apoptosis associated with acti-
vation of caspases 9 and 3, suggesting that intrinsic apoptosis might be triggered by knockdown of RACK1. Therefore, RACK1 might act as an antiapoptotic protein in cells. Furthermore, we found that overexpression of RACK1 favored viral growth. In contrast, knockdown of RACK1 reduced viral loads in IBDV-infected cells, which might result from cell death induced by RACK1 knockdown.

Besides RACK1, VDAC2 and p85α of PI3K have been found to interact with IBDV VP5 (24, 25). On the basis of results from yeast two-hybrid screening, there are other proteins (Hsp70, E74-like factor 1, β2-microglobulin, and eEF1A1) that may act as the binding partners for VP5 (data not shown), but further evidence is required to confirm their interaction. We attempted to search for the target proteins of IBDV VP5 using different assays, such as pulldown and chemical cross-linking assays, but we did not have any solid evidence for the presence of more specific binding proteins of VP5. However, it is still possible that VP5 may interact with more proteins than expected at different stages of IBDV infection.

RACK1, also named guanine nucleotide binding protein subunit β 2 like 1 (GNB2L1), has been identified as a WD40 motif protein that exists ubiquitously in the macromolecular complexes (40). The conserved seven-blade propeller structure gives RACK1 a strong protein binding capacity. RACK1 is highly conserved in all species, such as human, mice, chicken, canine, and swine (41). It has been reported that RACK1 can interact with multiple proteins, such as Src (42), Hif-1 α (43), androgen receptor (44), IAKs (45), and STATs (46), suggesting that RACK1 may act as a scaffolding protein, recruiting various proteins, providing a platform for protein interaction, and playing a critical role in different aspects of cell regulation (40).

RACK1 has been found to play different roles in apoptosis (28–30, 47–49). In this study, we found that RACK1, forming a complex with VDAC2 and VP5, played an antiapoptotic role in

**FIGURE 7. Overexpression of RACK1 inhibits IBDV-induced apoptosis.** A, determination of GFP-RACK1 expression in DF-1 cells by Western blot analysis. B, overexpression of RACK1 inhibits IBDV-induced apoptosis in DF-1 cells. DF-1 cells transfected with pEGFP-rack1 or empty vector were mock-infected or infected with IBDV at an m.o.i. of 1. Twenty-four hours after infection, cells were harvested for examination of GFP-RACK1 by Western blot analysis (A) or stained with propidium iodide, followed by flow cytometry analysis (B). GFP-positive cells were gated for further analysis of propidium iodide (PI)-positive cells using CellQuest software (BD Biosciences). C, the percentages of death cells (as shown in B) from each group were compared. Error bars indicate mean ± S.D. from three independent experiments. **, p < 0.01. D, overexpression of RACK1 inhibits IBDV-induced apoptosis in CEF cells. CEF cells isolated from 10-day-old specific pathogen-free chicken embryos were transfected with pEGFP-rack1 or empty vector. Twenty-four hours after transfection, cells were mock-infected or infected with IBDV at an m.o.i. of 1. Twenty-four hours after infection, cells were stained with Annexin V-PE, followed by flow cytometry analysis. E, the percentages of apoptotic CEF cells (as shown in D) from each group were compared. Error bars indicate mean ± S.D. from two independent experiments. *, p < 0.05.
IBDV-induced apoptosis because overexpression of RACK1 suppressed IBDV-induced apoptosis in host cells. In contrast, knockdown of RACK1 induced apoptosis associated with activation of caspases 9 and 3. Therefore, our data support the role of RACK1 as an antiapoptotic protein in cells. Several groups reported that RACK1 can promote apoptosis (28, 29). Controversially, RACK1 has been reported to suppress apoptosis in multiple types of cells, such as PC-12 cells (30), W7.2 T cells (47, 48), and HeLa cells (49). These controversial reports indicate that RACK1 may play dual roles in apoptosis. However, the underlying molecular mechanism of apoptotic regulation of RACK1 is still unclear. It has been reported that PKC activation could be involved in the antiapoptotic effects of RACK1 in W7.2 T cells, whereas activation of other PKC isoforms could be obscuring the effects of PKCβ (48). Therefore, PKC might be involved in the inhibition of IBDV-induced apoptosis. Furthermore, RACK1 has been reported as a negative modulator of p73, and overexpression of RACK1 in human osteosarcoma SAOS-2 directly suppresses p73-mediated transcription and inhibits p73-dependent apoptosis (50). These observations clearly show that RACK1 is involved in the regulation of apoptosis. In addition, it has been reported that the NF-κB transcription factor promotes the expression of RACK1 and that RACK1 acts downstream of NF-κB in facilitating cell survival (30), whereas NF-κB could be activated during IBDV infection (23). Therefore, it is very likely that NF-κB is involved in the RACK1 inhibition of IBDV-induced apoptosis in DF-1 cell lines. More effort will be required to determine the related molecules downstream of RACK1-mediated signaling transduction.

It has been reported that RACK1 interacts with the ion-gated channels and ligand-gated channels such as GABA$_\alpha$ receptor (51, 52), conductance Ca$^{2+}$-activated potassium (BK) channel (53), and NMDA receptor (54). In addition, an interesting study showed that RACK1 slowed the activation of the channel in Xenopus oocytes (53). VDAC2, an ion channel component, is involved in the release of cytochrome $c$ during IBDV infection (25). It is possible that overexpression of RACK1 might slow the activation of VDAC2 and inhibit the release of cytochrome $c$, leading to the suppression of apoptosis induced by IBDV infection. More effort will be required to elucidate the exact role of RACK1 in VDAC2-mediated apoptosis.

Up to now, it has been reported that RACK1 can be hijacked by viruses to affect viral replication, such as Epstein-Barr virus (55), mumps virus (56), sarcoma virus (57), and hepatitis C virus (58). Consistent with these reports, in this study we also found that knockdown of RACK1 suppressed IBDV growth. In addition, we found that knockdown of RACK1 induced apoptosis. These results are consistent with earlier observations that deletion of the RACK1 gene is embryonically lethal (41). We believe that a certain level of cell death in RACK1 knockdown cells partially affected
viral replication, which suggests that the apoptosis induced by RACK1 knockdown does not favor IBDV growth. Clearly RACK1 plays a critical role in suppression of IBDV-induced apoptosis, which might provide favorable conditions for viral replication.

Of note is that our findings were obtained primarily from experiments using DF-1 cells and CEFs. RACK1 plays an anti-apoptotic role in IBDV-induced apoptosis and enhances viral growth in cells. In contrast, in our study, knockdown of RACK1 could induce considerable apoptosis and suppress IBDV growth, indicating that RACK1 plays a critical role in the suppression of IBDV-induced apoptosis. Because we have found previously that VP5-induced apoptosis markedly enhanced viral release (25), inhibition of VP5-induced apoptosis by RACK1 might be a protective measure employed by the host to restrict virus spread and to avoid tissue damage. However, the virus might take advantage of this for its own benefit (viral replication). Therefore, RACK1, as an antiapoptotic molecule, may protect cells from apoptosis and restrict viral spread but could also favor IBDV growth in cells. The restriction of virus spread by RACK1 could mitigate tissue damage and allow time for an effective immune response. The role of RACK1 in IBDV infection might exceed our imagination. It has been reported that IBDV infection could induce the activation of the JNK signaling pathway.
pathway and the activation of the proapoptotic protein Bax (59). Bax and JNK are binding partners of RACK1 (28, 60). Currently it is still not clear how these proteins interact and act during IBDV infection. On the other hand, IBDV could activate the PI3K/Akt signaling pathway via interaction of VP5 protein with the p85α subunit of PI3K (24), whereas RACK1 could promote the activation of the PI3K/Akt/Rac1 pathway (61).

RACK1 also suppressed IBDV-induced apoptosis in this study. These results provide clues that RACK1 might play an important role during IBDV infection.

Because RACK1 interacts with VDAC2 in mitochondria under physiological conditions during IBDV infection, we propose that, when host cells are infected with IBDV, the nonstructural protein VP5 moves to the mitochondria and sequesters RACK1 and VDAC2, therefore causing cytochrome c release and activation of caspases 9 and 3 and inducing apoptosis and virus release.

In summary, our results revealed that RACK1 formed a complex with VDAC2 and IBDV VP5. The observation that overexpression of RACK1 inhibited IBDV-induced apoptosis and knockdown of RACK1 induced apoptosis and suppressed viral growth suggests that RACK1 plays a critical role during IBDV infection. These findings provide insights for further studies of the molecular mechanism of IBDV infection.

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