Distinct Antiviral Roles for Human 2′,5′-Oligoadenylate Synthetase Family Members against Dengue Virus Infection

Ren-Jye Lin, Han-Pang Yu, Bi-Lan Chang, Wei-Chun Tang, Ching-Len Liao and Yi-Ling Lin

*J Immunol* 2009; 183:8035-8043; Prepublished online 18 November 2009;
doi: 10.4049/jimmunol.0902728
http://www.jimmunol.org/content/183/12/8035

References This article cites 46 articles, 20 of which you can access for free at:
http://www.jimmunol.org/content/183/12/8035.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

*The Journal of Immunology* is published twice each month by
The American Association of Immunologists, Inc.,
1451 Rockville Pike, Suite 650, Rockville, MD 20852
Copyright © 2009 by The American Association of Immunologists, Inc. All rights reserved.
Print ISSN: 0022-1767 Online ISSN: 1550-6606.
Distinct Antiviral Roles for Human 2′,5′-Oligoadenylate Synthetase Family Members against Dengue Virus Infection

Ren-Jye Lin,* Han-Pang Yu,* Bi-Lan Chang,† Wei-Chun Tang,*§ Ching-Len Liao,** and Yi-Ling Lin2*†‡§

The 2′,5′-oligoadenylate synthetase (OAS) and its downstream effector RNase L play important roles in host defense against virus infection. Oas1b, one of the eight Oas1 genes in the mouse genome, has been identified as a murine flavivirus-resistance gene. Four genes, OASI, OAS2, OAS3, and OAS-like (OASL), have been identified in the human OAS gene family, and 10 isoforms, including OAS1 (p42, p44, p46, p48, and p52), OAS2 (p69 and p71), OAS3 (p100), and OASL (p30 and p59) can be generated by alternative splicing. In this study, we determined the role of the human OAS/RNase L pathway in host defense against dengue virus (DEN) infection and assessed the antiviral potential of each isoform in the human OAS family. DEN replication was reduced by over-expression and enhanced by knockdown of RNase L expression, indicating a protective role for RNase L against DEN replication in human cells. The human OAS1 p42, OAS1 p46, and OAS3 p100, but not the other OAS isoforms, blocked DEN replication via an RNase L-dependent mechanism. Furthermore, the anti-DEN activities of these three OAS isoforms correlated with their ability to trigger RNase L activation in DEN-infected cells. Thus, OAS1 p42/p46 and OAS3 p100 are likely to contribute to host defense against DEN infection and play a role in determining the outcomes of DEN disease severity. The Journal of Immunology, 2009, 183: 8035–8043.

Copyright © 2009 by The American Association of Immunologists, Inc. 0022-1767/09/$2.00
allele at this single nucleotide polymorphism, which allows splicing to generate OAS1 p48 and p52 but not OAS1 p46, is increased in WNV seroconverters (20). Because WNV infection is less likely to result in seroconversion in individuals with the “G” genotype, which generates OAS1 p46 with higher OAS activity, OAS1 is likely to play an important antiviral role during the early phase of WNV infection.

IFN plays an important role in host defense against DEN infection in vitro and in vivo (21, 22). Using a microarray, IFN-related gene induction has been demonstrated in DEN-infected cells and in patients infected with DEN (23, 24). Furthermore, the majority of genes that are strongly up-regulated in the PBMCs of individuals with DF compared with PBMCs of individuals with DHF are antiviral genes, suggesting a protective role for the IFN system in defense against DEN infection (25). Despite clinical and experimental evidence, the role of the OAS/RNase L system in DEN infection is largely unknown. In our previous study, we have described the up-regulation of the IFN-induced genes, including OAS1, OAS2, OAS3, and OASL on DEN replication and to clarify the possible involvement of RNase L in this antiviral effect. Distinct antiviral potential of the 10 known human OAS isoforms of OAS1, OAS2, OAS3, and OASL on DEN replication and to clarify the possible involvement of RNase L in this antiviral effect.

Materials and Methods

Viruses, cell lines, chemicals, and Abs

The DEN-2 PLO46 strain (26) used in this study was propagated in the mosquito cell line C6/36, which was grown in RPMI 1640 medium containing 5% FBS. Baby hamster kidney cells (BHK-21) for plaque assay were grown in RPMI 1640 medium containing 5% FBS. The human lung epithelial carcinoma cell line A549 was maintained in F-12 medium (Invitrogen) supplemented with 10% FBS. The tetracycline-regulated expression human embryonic kidney 293 cell line (Invitrogen) was grown in DMEM (Sigma-Aldrich) containing 10% FBS and 3 µg/ml blasticidin (Invivogen). Roferon-A, a recombinant IFN-α2a, was obtained from Roche. Hygromycin was purchased from Invivogen. Doxycycline (Dox) and puromycin were obtained from Clontech and Sigma, respectively. Mouse monoclonal anti-dsRNA Ab (J2 mAb; English & Scientific Consulting), rabbit monoclonal anti-hemagglutinin (HA) Ab (Upstate Biotechnology), mouse monoclonal anti-HA Ab (Covance), and mouse monoclonal anti-RNase L Ab (Novus Biologicals) were used.

Plasmid construction and lentivirus generation

The cDNAs encoding human OAS (p42, p44, p46, p48, p52, p69, p71, and p100) were amplified by PCR using the specific primers listed in Table I. The cDNA fragments were cloned into the TA vector pCR3.1 (Invitrogen) and subcloned to a HA-tagged pcDNA3 expression vector (pcDNA3/HA) with an in-frame fusion of HA-tag at the N-termini. All of the cDNAs were sequenced, and their sequences were the same as those reported in GenBank (Table I). The cDNA fragments were cloned into the self-inactivating lentiviral vector (pSIN), in which the expression of various OAS proteins. For wild-type RNase L and the R667A mutant of RNase L, an R667A mutated RNase L, was generated by single-primer mutagenesis as previously described (27), using the primer annealing to nt 2143–2191 of RNase L mRNA, with the mutated sequences underlined (5’-TCGGTGTATCT GCTAAAAGTCATCAGGAAATTTGGAGAACACATTGATGAA-3’).

The self-inactivating lentiviral vector (pSIN), in which the expression of an inserted gene is under the control of a constitutive spleen focus-forming virus promoter (28), was used in this study for the expression of various OAS proteins. For wild-type RNase L and the R667A mutant of RNase L, the pLenti6.3/V5-TOPO expression vector and TOPO TA cloning kit (Invitrogen) were used. A lentivirus-based, inducible expression construct (pLKO-shRNA) targeting firefly luciferase was used in the expression of OAS and RNase L. An R667A mutated RNase L, was generated by single-primer mutagenesis as previously described (27), using the primer annealing to nt 2143–2191 of RNase L mRNA, with the mutated sequences underlined (5’-TCGGTGTATCT GCTAAAAGTCATCAGGAAATTTGGAGAACACATTGATGAA-3’).

The self-inactivating lentiviral vector (pSIN), in which the expression of an inserted gene is under the control of a constitutive spleen focus-forming virus promoter (28), was used in this study for the expression of various OAS proteins. For wild-type RNase L and the R667A mutant of RNase L, the pLenti6.3/V5-TOPO expression vector and TOPO TA cloning kit (Invitrogen) were used. A lentivirus-based, inducible expression construct (pLKO-shRNA) targeting firefly luciferase was used in the expression of OAS and RNase L. An R667A mutated RNase L, was generated by single-primer mutagenesis as previously described (27), using the primer annealing to nt 2143–2191 of RNase L mRNA, with the mutated sequences underlined (5’-TCGGTGTATCT GCTAAAAGTCATCAGGAAATTTGGAGAACACATTGATGAA-3’).

The self-inactivating lentiviral vector (pSIN), in which the expression of an inserted gene is under the control of a constitutive spleen focus-forming virus promoter (28), was used in this study for the expression of various OAS proteins. For wild-type RNase L and the R667A mutant of RNase L, the pLenti6.3/V5-TOPO expression vector and TOPO TA cloning kit (Invitrogen) were used. A lentivirus-based, inducible expression construct (pLKO-shRNA) targeting firefly luciferase was used in the expression of OAS and RNase L. An R667A mutated RNase L, was generated by single-primer mutagenesis as previously described (27), using the primer annealing to nt 2143–2191 of RNase L mRNA, with the mutated sequences underlined (5’-TCGGTGTATCT GCTAAAAGTCATCAGGAAATTTGGAGAACACATTGATGAA-3’).

The self-inactivating lentiviral vector (pSIN), in which the expression of an inserted gene is under the control of a constitutive spleen focus-forming virus promoter (28), was used in this study for the expression of various OAS proteins. For wild-type RNase L and the R667A mutant of RNase L, the pLenti6.3/V5-TOPO expression vector and TOPO TA cloning kit (Invitrogen) were used. A lentivirus-based, inducible expression construct (pLKO-shRNA) targeting firefly luciferase was used in the expression of OAS and RNase L. An R667A mutated RNase L, was generated by single-primer mutagenesis as previously described (27), using the primer annealing to nt 2143–2191 of RNase L mRNA, with the mutated sequences underlined (5’-TCGGTGTATCT GCTAAAAGTCATCAGGAAATTTGGAGAACACATTGATGAA-3’).
the two helper plasmids, pMD.G and pCMVΔR8.91, with Lipofectamine 2000 reagent (Invitrogen). Transfected cells were incubated at 37°C for 4–5 h and then refed with fresh medium. Cell supernatants containing the viral particles were harvested 24–60 h after transfection and stored at −80°C. Lentivirus titers were determined with the Quick-Titers lentivirus titer kit, an HIV p24-based ELISA (Cell Biolabs).

Establishment of stable cell lines

T-REx-293 cells were transfected with pcDNA5/TO encoding HA-tagged wild-type RNase L, a nuclease-dead mutant of RNase L (R667A), OAS p42, or OAS3 p100 and selected with hygromycin (250 μg/ml) and blasticidin (5 μg/ml) for 8 days. Individual colonies were picked and expanded. To generate human RNase L knockdown cells, the cells were transduced with the lentiviral-based shRNA vector targeting RNase L (shRNase L) or negative control luciferase (shLuc). To verify the knockdown effect, cell lysates were harvested for Western blotting with an anti-RNase L Ab. Cells with or without RNase L knockdown were infected with DEN-2 (MOI = 0.1 for 72 h and MOI = 5 for 48 h) and then the virus titers (FFU/ml) were determined by plaque-forming assays on BHK-21 cells. Results are averages and SD of two independent experiments. The viral titers of indicated groups were compared by two-tailed Student’s t tests.

Immunofluorescent assay

A549 cells were transduced with the lentiviral vectors expressing the various HA-tagged OAS proteins for 72 h and then infected with DEN-2 (multiplicity of infection (MOI) = 20) for 24 h. The cells were fixed with 4% formaldehyde and permeabilized in PBS (pH 7.4, 0.1% Tween 20) and subsequently incubated with the primary Ab overnight. The blots were then treated with a HRP-conjugated secondary Ab and developed with an ECL system (Amersham/GE Healthcare). For reblotting, the membrane was washed with 1× ReBlot plus strong Ab stripping solution (Chemicon) for 15 min at room temperature. The membrane was then blocked twice with 5% skim milk in PBS-T for 5 min followed by reprobing with the primary Ab.

Immunofluorescent assay

A549 cells were transduced with the lentiviral vectors expressing the various HA-tagged OAS proteins for 72 h and then infected with DEN-2 (multiplicity of infection (MOI) = 20) for 24 h. The cells were fixed with 4% formaldehyde and permeabilized in PBS (pH 7.4, 0.1% Tween 20). DEN protein expression was detected using a mouse anti-DEN-2 NS3 Ab and Alexa Fluor 488 goat anti-mouse Ab (Molecular Probes). The expression of HA-tagged OAS was detected with a rabbit anti-HA Ab, biotin-conjugated secondary anti-rabbit Ab (Jackson ImmunoResearch), and streptavidin-conjugated Cy3 (Jackson ImmunoResearch Laboratories). The nuclei were stained with DAPI (Molecular Probes).

**FIGURE 1.** The anti-DEN-2 effect of human RNase L. A, A549 cells were transduced with the lentiviral vector expressing the wild-type RNase L or the R667A mutant of RNase L for 72 h, and then cells were infected with DEN-2 (MOI = 5). Twenty-four hours after infection, cells were fixed and permeabilized for an immunofluorescent assay. HA-tagged RNase L (red), dengue viral protein NS3 (green), and 4’,6-diamidino-2-phenylindole (DAPI; blue)-stained cells were photographed using a fluorescent microscope. Arrows indicate the cells expressing HA-tagged wild-type RNase L or R667A mutant of RNase L. The Greek letter α represents the prefix “anti-.” B, T-REx-293 cells transduced with lentiviruses inducibly expressing control vector (−), HA-RNase L, or HA-RNase L R667A mutant were cultured in the absence (−) or presence (+) of Dox (1 μg/ml) for 16 h and then infected with DEN-2 (MOI = 0.1). At 48 h post infection, the cell lysates were harvested for western blotting with Abs against DEN-2 NS3, HA-tagged RNase L, and actin as indicated. C, A549 cells were transduced with the lentiviral-based shRNA vector targeting RNase L (shRNase L) or negative control luciferase (shLuc). To verify the knockdown effect, cell lysates were harvested for Western blotting with an anti-RNase L Ab. D, Cells with or without RNase L knockdown were infected with DEN-2 (MOI = 0.1 for 72 h and MOI = 5 for 48 h) and then the virus titers (FFU/ml) were determined by plaque-forming assays on BHK-21 cells. Results are averages and SD of two independent experiments. The viral titers of indicated groups were compared by two-tailed Student’s t tests.
Confocal imaging

A549 cells were seeded on coverslips in 12-well plates for 16 h and were then transduced with a lentiviral vector expressing the indicated HA-tagged OAS. Seventy-two hours after transduction, cells were infected with DEN-2 (MOI = 20) for 6 h. The cells were rinsed twice with PBS and fixed with 4% formaldehyde for 30 min followed by permeabilization with 0.5% Triton X-100 for 10 min. Subsequently, human OAS proteins were stained with a rabbit monoclonal anti-HA Ab and probed with a biotin-conjugated secondary anti-rabbit Ab and streptavidin-conjugated Cy3. Viral dsRNA was detected using a mouse anti-dsRNA Ab and Alexa Fluor 488 goat anti-mouse Ab. Images were taken with an LSM 510 META confocal system (Carl Zeiss) using a Plan-Apochromat ×100 oil-immersion objective lens.

Assay of RNase L activity

A549 cells transduced with the indicated lentivirus (MOI = 2) for 72 h were infected with DEN-2 (MOI = 5) for 24 h. Total cellular RNA was extracted by using an RNeasy total RNA kit (Qiagen) and quantified by measuring UV absorbance at 260 nm. Cellular RNA (250 ng) was separated with an RNA 6000 Nano Chip and analyzed with a 2100 Bioanalyzer (Agilent Technologies) to determine the integrity of 28S and 18S rRNA.

Results

RNase L plays an antiviral role against DEN-2 infection in human cells

To assess the role of the human OAS/RNase L system in DEN-2 replication, we used a lentivirus expression system to overexpress RNase L in human A549 cells. Overexpression of wild-type RNase L, but not of a nuclease-dead mutant of RNase L (R667A) (29), rendered the cells resistant to DEN-2 infection (Fig. 1A).

FIGURE 2. Protein expression patterns of human OAS isoforms. 293 cells were transfected with pcDNA3 plasmids encoding various N-terminally HA-tagged OAS1 (p42, p44, p46, p48, and p52), OAS2 (p69 and p71), OAS3 (p100), and OASL (p59 and p30) proteins for 24 h, and cell lysates were then harvested for Western blotting with an anti-HA Ab.

FIGURE 3. Antiviral assays of human OAS family members against DEN-2 infection. A549 cells were transduced with lentiviral vectors expressing the HA-tagged OAS1 isoforms (p42, p44, p46, p48, and p52) (A–E), OAS2 isoforms (p69 and p71) (F and G), OAS3 (p100) (H), and OASL isoforms (OASL p59 and OASL p30) (I and J). Seventy-two hours after transduction, cells were infected with DEN-2 (MOI = 20) for 24 h. Cells were then fixed and permeabilized for an immunofluorescent assay. HA-tagged OAS (red)-, dengue viral protein NS3 (green)-, and 4′,6-diamidino-2-phenylindole (DAPI; blue)-stained cells were photographed by using a fluorescent microscope. The Greek letter α represents the prefix “anti-.”
DEN-2 NS3 protein expression as measured by Western blotting (Fig. 1B). Furthermore, A549 cells deprived of RNase L expression by transduction with a lentivirus-based shRNA targeting human RNase L (shRNase L) (Fig. 1C) resulted in higher levels of DEN-2 production as seen by 14- and 39-fold increases in viral production for low and high MOI of infection, respectively (Fig. 1D). These results demonstrate that RNase L is involved in controlling DEN-2 replication in human cells.

Anti-DEN-2 activity of human OAS family members

To identify the antiviral potential of human OAS gene family members, we cloned and constructed plasmids that individually expressed the N-terminally HA-tagged OAS1 isoforms (p42, p44, p46, p48, and p52), OAS2 isoforms (p69 and p71), OAS3 (p100), and OASL isoforms (p30 and p59) (Table I). These constructs expressed the proteins of the expected molecular sizes as detected by Western blotting with anti-HA Ab (Fig. 2). A549 cells transduced with lentivirussexpressing the individual OAS proteins were then infected with DEN-2, and immunofluorescent assays were conducted to determine the antiviral potential of each human OAS family member. The DEN-2 viral protein NS3 was not detected in cells expressing human OAS1 p42 (Fig. 3A) or OAS1 p46 (Fig. 3C), or OAS3 p100 (Fig. 3H), whereas DEN-2 NS3 was readily detected in cells expressing the other members of human OAS and OASL isoforms. These data indicate that OAS1 p42, OAS1 p46, and OAS3 p100 possess antiviral activity against DEN-2 infection. We then used an inducible system to overexpress OAS1 p42 and OAS3 p100 in T-REx-293, a human embryonic kidney 293 cell line stably expressing the tetracycline repressor protein, with or without RNase L knockdown to assess whether the antiviral mechanism was mediated by RNase L. The levels of OAS1 p42 and OAS3 p100 induction by Dox and RNase L knockdown were verified by Western blotting (Fig. 4, A and C). The cells were then infected with DEN-2 at high and low MOIs (MOI = 5 and 0.1), and viral titrations were conducted by plaque assay. Like the results for A549 cells (Fig. 1C), higher viral production was noted in T-REx-293 cells with RNase L knockdown compared with the parental T-REx-293 cells at high or low MOIs of DEN-2 infection and regardless of Dox treatment (Fig. 4, B and D). Furthermore, the induction of human OAS1 p42 resulted in 4.3- and 14-fold decreases in viral yield at high and low MOIs, respectively (Fig. 4B). Similarly, 7- and 11-fold viral reductions were noted in cells inducibly expressing OAS3 p100 at high and low MOIs of DEN-2 infection, respectively (Fig. 4D). Furthermore, the anti-DEN-2 activity of p42 and p100 depended on RNase L, because the antiviral effect was lost in cells deprived of RNase L expression (Fig. 4, B and D). Western blot detection of viral NS3, a nonstructural protein expressed in virus-replicating cells (Fig. 4, A and C), corroborated the results of infectious viral titration (Fig. 4, B and D). Thus, our results indicate that human OAS1 p42/p46 and OAS3 p100 block DEN-2 replication through an RNase L-mediated mechanism.

Subcellular localization of OAS and viral RNA in DEN-2-infected cells

As different OAS isoforms have been reported to be associated with different subcellular fractions (30–32), we tested whether the different anti-DEN-2 activities triggered by these OAS members (Fig. 3) could be attributed to the colocalization of OAS proteins...
with viral RNA. A549 cells transduced with the indicated HA-tagged human OAS lentiviral expression vectors were infected with DEN-2 for 6 h, at which time point viral RNA was readily detected by an anti-dsRNA Ab (Fig. 5A). The cellular distributions of OAS proteins and viral RNA detected by anti-HA and anti-dsRNA Abs were observed under a confocal microscope. The OAS proteins were mainly localized in the cytoplasm (Fig. 5B), as previously reported (18, 33), and perinuclear localization was also noted for OAS2 p71 (Fig. 5Bg). All of the OAS members colocalized with dsRNA except for OAS2 p69, for which the merged yellow signals were less prominent (Fig. 5Bf). Thus, the discrepancy between anti-DEN-2 activities for the various OAS members cannot simply be explained by different cellular compartmentalization of the OAS family members and viral dsRNA.

**RNase L activity in DEN-2-infected cells overexpressing human OAS isoforms**

It is known that 2-5A synthesized by activated OAS binds to and activates RNase L, which then degrades viral and cellular RNA. We next determined RNase L activity triggered by the various OAS members by detecting the characteristic rRNA cleavage using RNA chips, as previously described (12). A549 cells transduced with lentiviruses expressing the indicated human OAS proteins were infected with DEN-2 for 24 h, and cellular RNA was separated on RNA chips. Cleavage products of 28S and 18S rRNAs were apparent in cells expressing human OAS1 p42/p46 and OAS3 p100 (Fig. 6, lanes 6, 10, and 20). To a lesser extent, rRNA cleavage products were also detected in cells expressing OAS1 p44/p48 and OAS2 p69 (Fig. 6, lanes 8, 12, and 16). The RNA integrity number (RIN), developed to estimate RNA integrity (34), was also determined using the Agilent 2100 Expert software (Agilent Technologies). Interestingly, three of the OAS isoforms with anti-DEN-2 activity, OAS1 p42/p46 and OAS3 p100, had the lowest RIN values among the tested samples, 9.2, 9.3, and 8.6, respectively (Fig. 6). Therefore, these results indicate that human OAS1 p42/p46 and OAS3 p100 trigger higher RNase L activity in DEN-2-infected cells, resulting in stronger antiviral activity.

**The OAS3-mediated rRNA cleavage depends on RNase L**

Because OAS3 preferentially synthesizes 2-5A dimers, which have a lower binding affinity and do not activate RNase L (18, 32, 35, 36), we further assessed whether RNase L is indeed involved in the
rRNA cleavage triggered by OAS3 p100. We overexpressed OAS3 p100 in A549 cells with or without RNase L knockdown (Fig. 7A) and tested for rRNA cleavage patterns in these cells after DEN-2 infection. As shown in Fig. 7B, the rRNA cleavage induced by DEN-2 infection in these OAS3-expressing cells (lanes 7 and 11) was no longer seen in cells devoid of RNase L expression (lanes 8 and 12). Thus, our results indicate that OAS3 p100 activates RNase L and mediates an antiviral effect in DEN-2-infected human cells.

Discussion
DEN causes human diseases ranging from mild acute DF to severe DHF and DSS. The severity and outcome of DEN infection is determined by complex factors such as amplitude of DEN replication, expression of cytokines, activation and proliferation of immune cells, host genetic factors, etc (23, 37). Patients with DHF/DSS tend to have higher viremia titers than patients with DF; hence, DEN levels appear to correlate with the severity of DEN diseases (38, 39). Thus, virus clearance in the early stages of infection may play an important role in diminishing the progression of severe DEN-related diseases. In mice, the Oas1b gene has been identified as a flavivirus resistance gene (6–10); however, the role of human OAS family members in flavivirus infection has not yet been clarified. In this study, we found that three of the 10 human OAS family members, OAS1 p42/p46 and OAS3 p100, mediate a potent anti-DEN-2 activity through an RNase L-dependent mechanism and may contribute to host defense against DEN infection.

It is well known that the OAS/RNase L system, an IFN-induced antiviral pathway, plays a critical role in innate immunity, controlling the outcome of virus production. RNase L may mediate antiviral actions by cleavage of viral RNA, by eliminating virus-infected cells through apoptosis, and by enhancing IFN-β production through the MDA5/RIG-I/IPS-1 cascade (40–42). The role of RNase L in controlling WNV replication has been demonstrated, with higher WNV production occurring in cells deprived of RNase L activity by using a dominant negative mutant or genetic knockdown (11, 12). The role of RNase L in DEN infection is more obscure, and the antiviral effect of IFN in murine embryonic
fibroblasts is thought to be independent of RNase L (43). As DEN-2 replication was reduced by RNase L overexpression (Fig. 1, A and B) and enhanced by RNase L knockdown in human A549 and T-REC–293 cells (Fig. 1, D and Fig. 4), we provide evidence showing that RNase L plays a protective role in host defense against DEN-2 infection in human cells.

The three forms of OAS are characterized by different subcellular locations and enzyme parameters (44, 45). OAS1 and OAS2 synthesize higher oligomers of 2-5A, whereas OAS3 tends to synthesize 2-5A dimers, which do not activate RNase L (18, 32, 35, 36). The notion that OAS3 may not act through RNase L is supported by a recent report that human OAS3 exhibits antiviral activity against alphaviruses, such as the Chikungunya virus, the Sindbis virus, and the Semliki Forest virus, through an RNase L-independent pathway (46). Our results demonstrate that OAS3 is able to trigger RNase L activation characterized by RNA cleavage (Figs. 6 and 7) and that RNase L is required for the anti-DEN-2 activity of OAS3 (Fig. 4). Although OAS3 preferentially synthesizes 2-5A dimeric molecules, a small proportion of its products are 2-5A oligomers (18, 32). Moreover, the requirement of dsRNA concentration for optimal OAS3 activation is ~100 times lower than that for human OAS1 and OAS2 (30, 45), suggesting that OAS3 might be more sensitive in sensing virus RNA. Thus, we speculate that human OAS3 is activated in the early stages of DEN-2 infection by low concentrations of viral RNA to synthesize 2-5A, including the majority of 2-5A dimeric molecules and a minority of higher oligomers of 2-5A, which then activate latent RNase L to control DEN-2 replication. A recent clinical study showing that patients with DSS have lower levels of OAS3 expression (23) supports our notion that human OAS3 might control DEN replication and influence the severity and outcome of DEN diseases.

The human OAS1 gene contains eight exons and through alternative splicing it gives rise to five isoforms, all of which include exons 1–5 but differ in their C-terminal sequences (13, 14). It is known that a “G” sequence at the exon 6 splice-acceptor site allows splicing to generate OAS1 p46; whereas an “A” sequence at this position drives splicing to occur further downstream to generate p48 and p52. Furthermore, the “A” allele has a higher gene frequency in individuals with low basal OAS activity than in individuals with high OAS activity (13). Interestingly, this “A” allele has recently been identified as a risk factor for initial infection with WNV (20). Our results showing that OAS1 p46 but not OAS1 p48/p52 exhibited anti-DEN-2 activity (Fig. 3) suggest that this “A” allele might also be a risk factor for DEN and warrants future study to verify its role in determining the outcome of DEN infection. Because WNV replicated to higher levels in lymphoid tissues from donors with the “A” allele, who does not generate p46, it is likely that WNV is also more frequent in individuals with low basal OAS activity than in individuals with high OAS activity (13). Whether they are candidate human genetic factors for determining human susceptibility to and severity of DEN-related diseases.

Acknowledgments

We thank the National RNAi Core Facility, Taiwan (supported by the National Research Program for Genomic Medicine Grants of National Science Council) for shRNA constructs.

Disclosures

The authors have no financial conflict of interest.

References


Downloaded from http://www.jimmunol.org/ by guest on October 30, 2017


