The interferon (IFN)-inducible antiviral state is mediated in part by the Type I interferons (IFNs) such as IFN-β. Type I IFNs, primarily IFN-β, produce 2′,5′-oligoadenylate (2-5A) via RNase L (Rnasel) and OAS (Oas1a, Oas2, Oas3) enzymes. This activates RNase L, which degrades RNA, leading to apoptosis through the JNK and PKR pathways. However, the effects of RNase L and OAS on IFN induction vary in different cell types. In mouse embryonic fibroblasts (MEFs), RNase L gene knockout decreased IFN induction. In contrast, in macrophages, RNase L deletion increased IFN induction, likely due to differences in basal levels of OAS/RNase L. The OAS/RNase L pathway is essential for antiviral innate immunity, and its activity can either enhance or suppress IFN production, depending on the cell type. Basal levels of OAS/RNase L in macrophages reduce, rather than increase, virus induction of IFN-β.

IMPORTANCE Type I IFNs are crucial for animal and human health. Previous studies have shown that the OAS/RNase L pathway amplifies antiviral innate immunity by enhancing IFN-β production in mouse embryonic fibroblasts and in virus-infected mice. Here, we report that high basal levels of OAS/RNase L in macrophages reduce, rather than increase, virus induction of IFN-β, RNA damage and apoptosis caused by RNase L were the likely reasons for the decreased IFN-β production in virus-infected macrophages. Our studies suggest that during viral infections, the OAS/RNase L pathway can either enhance or suppress IFN production, depending on the cell type. IFN regulation by RNase L is suggested to contribute to tissue protection and survival during viral infections.

OBSErvATION

Cell-Type-Specific Effects of RNase L on Viral Induction of Beta Interferon

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ABSTRACT

The interferon (IFN)-inducible antiviral state is mediated in part by the 2′,5′-oligoadenylate (2-5A) synthetase (OAS)/RNase L system. 2-5A, produced from ATP by OAS proteins in response to viral double-stranded RNA, binds to and activates RNase L. RNase L restricts viral infections by degrading viral and cellular RNA, inducing autophagy and apoptosis, and producing RNA degradation products that amplify production of type I interferons (IFNs) through RIG-I-like receptors. However, the effects of the OAS/RNase L pathway on IFN induction in different cell types that vary in basal levels of these proteins have not been previously reported. Here we report higher basal expression of both RNase L and OAS in mouse macrophages in comparison to mouse embryonic fibroblasts (MEFs). In MEFs, RNase L gene knockout decreased induction of IFN-β by encephalomyocarditis virus infection or poly(rI):poly(rC) (pIC) transfection. In contrast, in macrophages, RNase L deletion increased (rather than decreased) induction of IFN-β by virus or pIC. RNA damage from RNase L in virus-infected macrophages is likely responsible for reducing IFN-β production. Similarly, direct activation of RNase L by transfection with 2-5A induced IFN-β in MEFs but not in macrophages. Also, viral infection or pIC transfection caused RNase L-dependent apoptosis of macrophages but not of MEFs. Our results suggest that cell-type-specific differences in basal levels of OAS and RNase L are determinants of IFN-β induction that could affect tissue protection and survival during viral infections.
less IFN-β, as measured in serum, after infection with encephalomyocarditis virus (EMCV) (intraperitoneally) or Sendai virus (intranasally) than do wild-type (WT) mice (11). The small RNA cleavage products produced by RNase L that enhance IFN-β production can be either cellular (self) or viral (nonself) and typically have some double-strand regions, a 5′-hydroxyl, and a phosphate at the 2′, 3′-cyclic terminus (11, 12). Previously we showed in human hepatoma HuH7 cells that RNase L released a small RNA product from the NS5B region of hepatitis C virus (HCV) genomic RNA that bound to RIG-I, causing displacement of its repressor domain, stimulating its ATPase function, and propagating signaling through the mitochondrial antiviral signaling protein (MARTS) to the IFN-β gene (12). The small RNA from HCV RNA was also shown to induce a hepatic innate immune response when injected into mice. Similarly, an mRNA of paramyxovirus virus 5 activated type I IFN production, independently of the encoded protein, by a pathway involving RNase L and MDA5 (13).

Also RNase L was shown to be a factor in type I IFN induction in response to herpes simplex virus 2 infection (14). Recently, we reported large differences (up to 100-fold) in basal expression of different OAS isoforms between different types of primary mouse cells (15). Notably, bone marrow-derived macrophages (BMMs), as well as primary microglia, brain resident macrophages, expressed the highest levels of different OAS isoforms compared with several other primary cell types. In a separate study, peritoneal macrophages (p-Macs) were observed to have high basal expression of OAS (16). In addition, we previously compared basal levels of RNase L in 9 rodent and 11 human cell lines (17). The mouse and human macrophage-like cell lines RAW264.7 and U937, respectively, were among the cell lines expressing the highest levels of RNase L protein. In the same study, different mouse fibroblast cell lines (NIH 3T3, SVT2, and L929) were shown to express relatively low levels of RNase L protein. However, the effect of RNase L on IFN induction might vary, depending on where the IFN is measured, the subtype of IFN being measured, the type of virus and its route of infection, and the type of cells that are infected. Here we have investigated the last of these variables, namely cell-type-specific effects of RNase L on IFN induction.

Levels of mRNAs for Rnasel and different Oas genes were monitored by quantitative reverse transcription-PCR (qRT-PCR) in MEF cell lines, BMMs, peritoneal macrophages (p-Macs), and freshly isolated splenic macrophages (Fig. 1A). Compared to MEFs, BMMs had elevated levels of mRNAs for Rnasel (200-fold), Oasl (9-fold), Oas2 (34-fold), Oas3 (7-fold), and Oas1 (32-fold) (Fig. 1A). Oasl, Oas2, and Oas3 encode OAS isoforms that are enzymatically active, whereas Oasl encodes a protein that lacks the ability to synthesize 2-5A and is a negative regulator of type I IFN synthesis (18). Similarly, compared to MEFs, p-Macs had increased levels of mRNAs for Rnasel (260-fold), Oasl (7-fold), Oas2 (19-fold), Oas3 (3-fold), and Oas1 (8-fold). To control for possible activation of macrophages in vitro, splenic macrophages were isolated by fluorescence-activated cell sorter (FACS) and immediately processed for RNA isolation followed by qRT-PCR. Splenic macrophages also had increased basal levels of mRNAs for Rnasel (142-fold), Oasl (4.6-fold), Oas2 (2.2-fold), and Oas3 (3.5-fold); however, Oas1 mRNA levels were unchanged. These findings show enhanced basal mRNA expression of Rnasel and most enzymatically active isoforms of OAS in macrophages compared with MEFs. Western blot assays were done to monitor differences in RNase L protein levels. Whereas RNase L was observed in wild-type BMMs, p-MACs, and splenic macrophages, RNase L was not observed in wild-type MEFs (Fig. 1B). However, in WT MEFs, low levels of Rnasel mRNA were detected by qRT-PCR, and low levels of RNase L protein were apparent by monitoring characteristic, discrete RNA cleavage products during poly(rI):poly(rC) transfection or encephalomyocarditis virus (EMCV) infection (Fig. 1A and C, lanes 2 and 3, respectively). The RNA fragments were, however, produced at much higher levels in p-Macs and BMMs than in MEFs (Fig. 1C, lanes 5, 6, 8, and 9). Consistent with our prior results (11), 2-5A transfection induced IFN-β in WT MEFs but not in Rnasel−/− MEFs, as determined by enzyme-linked immunosorbent assay (ELISA) (Fig. 1D). In contrast, 2-5A transfection failed to induce IFN-β in either WT or Rnasel−/− BMMs (Fig. 1D). We verified uptake of 2-5A and activation of RNase L in BMMs with rRNA cleavage assays (data not shown).

To determine effects of RNase L on IFN-β induction by dsRNA, cells were transfected with different concentration of pIC (Fig. 1E). Similar to our prior study (11), RNase L enhanced IFN-β induction to a much greater extent in WT MEFs than in Rnasel−/− MEFs (Fig. 1E). Remarkably, however, the effect of RNase L on IFN induction was reversed in both BMMs and p-Macs. IFN-β accumulated to higher levels in Rnasel−/− BMMs (6.5-fold) and p-Macs (4.5-fold) than in WT p-Macs and BMMs (in response to 2 μg per ml of pIC) (Fig. 1E). Without transfection reagent, however, there was no effect of RNase L deletion on pIC induction of IFN-β in BMMs and p-Macs, suggesting that Toll-like receptor 3 (TLR3)-mediated induction of IFN was unaffected by RNase L (19) (data not shown).

To extend these studies to viral induction of IFN-β, cells were infected with EMCV. Viral infection induced IFN-β in WT MEFs but not in Rnasel−/− MEFs (Fig. 1F). Again, there was a reversal of the effect of RNase L on IFN-β induction in the myeloid cell types. Deletion of RNase L increased IFN-β production in response to EMCV infection by about 2-fold in p-Macs and BMMs (Fig. 1F). To determine if the differences in viral induction of IFN-β were related to the antiviral effect of RNase L, after one cycle of virus growth (8 h at a multiplicity of infection [MOI] of 0.1), viral yields were measured by plaque assays on type I IFN receptor (Ifnar)−/− MEF indicator cells. Viral yields were increased 85-, 8-, and 5-fold in Rnasel−/− MEFs, p-Macs, and BMMs, respectively, compared to the corresponding WT cells (Fig. 1G). Therefore, RNase L deficiency resulted in higher viral yields in MEFs than in p-Macs and BMMs. These results are consistent with the observation that RNase L gene knockout increased IFN-β production in p-Macs and BMMs but decreased IFN-β production in MEFs. In the Rnasel−/− MEFs, the decrease in IFN-β production in combination with a loss of nuclease activity likely combined to cause an increase in EMCV titers. In Rnasel−/− BMMs, the absence of nuclease activity normally provided by RNase L overrides the increase in IFN levels, allowing virus to replicate to higher titers in Rnasel−/− than in WT macrophages.

Apoptosis is known to occur in cells that sustain high levels of RNA damage due to RNase L activity (5, 6, 8). Therefore, cell viability and apoptosis were monitored in cell types expressing low and high basal levels of OAS and RNase L. There was no effect on cell viability of pIC transfection in WT MEFs or Rnasel−/− MEFs as determined by 3-(4,5-dimethyl-2-thiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) colorimetric assay (Fig. 2A). In contrast, pIC transfection reduced...
FIG 1 Basal levels of OAS and RNase L regulate induction of IFN-β by virus or pIC. (A) Basal levels of Rnasel and Oas1a, Oas2, Oas3, and Oasl1 mRNAs were determined by qRT-PCR with total RNA isolated with mirVANA RNA kits (LifeTechnology) with Oas primers described previously (15) and with Rnasel forward primer: 5′-TAGGGAAACATCATGAGGA-3′ and reverse primer 5′-CTGCCTCTGGAACGCTGAG-3′. RNA expression relative to GAPDH (glyceraldehyde-3-phosphate dehydrogenase) mRNA was expressed as 2^(-ΔΔCT), where ΔΔCT represents the threshold cycle (Ct) of the gene of interest – the Ct of GAPDH. The open bar represents the baseline determined with RNA from Rnasel/Δ/Δ MEFs. The data are shown as the means ± standard deviation (SD) calculated from a minimum of 3 (up to 6) biological replicates. MEFs immortalized with simian virus 40 (SV40) T antigen were cultured in RPMI 1640 with 10% fetal bovine serum (FBS) (5). Isolation and culture conditions for BMMs and thioglycolate-elicited p-Macs were as described previously (24, 25). Splenic macrophages were isolated by (Continued)
viability of WT p-Macs or BMMs by ~2-fold, whereas pIC transfection had no effect on viability of RNasel−/−/H11002/ p-Macs or BMMs. Similarly, no apoptosis was detected in pIC-transfected WT or RNasel−/−/H11002 MEFS as determined by lack of caspase 3/7 activation (Fig. 2B). However, caspase activation assays showed that pIC transfection induced apoptosis of WT p-Macs and BMMs, but not in RNasel−/−/ p-MACs and BMMs (Fig. 2B). These results were confirmed by monitoring poly(ADP-ribose) polymerase (PARP) cleavage in Western blots (Fig. 2C). Cleaved PARP was observed after pIC transfection of WT BMMs and p-Macs but not in identically treated WT MEFS. EMCV infection resulted in death of both WT and RNasel−/− MEFS (Fig. 2D). However, while EMCV reduced viability of WT BMMs by about 2.5-fold, a smaller (1.2-fold) reduction of viability was observed in EMCV-infected RNasel−/− BMMs (Fig. 2D). EMCV appears to have caused necrotic death of MEFS, as there was no measurable apoptosis as determined by lack of caspase 3/7 activity (Fig. 2E). In contrast, EMCV infection caused apoptosis of WT BMMs but not of RNasel−/− BMMs, as determined by caspase 3/7 activity assays (Fig. 2E). In addition, PARP cleavage assays showed viral induction of apoptosis in WT BMMs and WT p-Macs but not in WT MEFS (Fig. 2F). These findings show that RNase L contributes to apoptosis of BMMs and p-Macs in response to either pIC transfection or EMCV infection. However, low levels of OAS isofoms and RNase
levels of OAS and RNase L in macrophages also contribute to prevent spread of the virus into the liver parenchyma and the in liver sinusoidal resident macrophages, Kupffer cells, seems to murine coronavirus mouse model, we found that RNase L activity and RNase L result in lower levels of type I IFNs, at least in re-

signaling to the IFN-

response to pIC transfection or EMCV infection. However, despite high basal levels of OAS and RNase L. In cell types, such as macrophages, that have high basal levels of OAS (15) and RNase L (Fig. 1A), there is a different outcome—reduced rather than increased production of IFN-β following viral infection. RNase L is known to cleave both viral and cellular RNA molecules, including both mRNA and rRNA in intact ribosomes (7, 20). The damage to cellular RNA by RNase L, resulting in inhibition of protein synthesis (6, 21) and apoptosis (5, 6, 8), is the likely cause of reduced IFN synthesis in macrophages. The RNase L-mediated suppression of IFN production in macrophages occurs despite high expression of the IFN-stimulated gene products RIG-I and MDA5 (15) that function in signaling to the IFN-β gene. Furthermore, Mda5 expression as determined by qRT-PCR was 35-fold higher in BMMs than in MEFs (data not shown). We previously reported that induction of IFN-β in response to 2-5A transfection was partially reduced in Mda5−/− or Rig-i−/− MEFs compared with WT MEFs (11). These findings highlight the robustness of RNase L activity in myeloid cells that result in the degradation of mRNA and rRNA, thereby reducing IFN induction despite high levels of MDA5, a protein that senses both pIC and EMCV (22).

We propose that viral infections of cell types that mediate tissue integrity (such as fibroblasts) produce higher levels of type I IFNs as a result of RNase L and the small RNAs that it generates. The amplification of IFN production contributes to tissue protection (11). In virus-infected macrophages, high basal levels of OAS and RNase L result in lower levels of type I IFNs, at least in response to pIC transfection or EMCV infection. However, despite different patterns of the type I IFN response, both MEFs and macrophages contribute to host survival. In a prior study, using the murine coronavirus mouse model, we found that RNase L activity in liver sinusoidal resident macrophages, Kupffer cells, seems to prevent spread of the virus into the liver parenchyma and the consequent development of hepatitis (23). In this manner, high levels of OAS and RNase L in macrophages also contribute to tissue protection.

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