Age-Dependent Poliovirus Replication in the Mouse Central Nervous System Is Determined by Internal Ribosome Entry Site-Mediated Translation

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Mouse cells are not permissive for the replication of human rhinovirus type 2 (HRV2). To determine the role of the HRV2 internal ribosome entry site (IRES) in determining species specificity, a recombinant poliovirus (P1/HRV2) was constructed by substituting the poliovirus IRES with the IRES from HRV2. This recombinant virus replicated in all human and murine cell lines examined, demonstrating that the HRV2 IRES does not limit viral replication in transformed murine cells. P1/HRV2 replicated in the brain and spinal cord in neonatal but not adult mice transgenic for the poliovirus receptor, CD155. Passage of P1/HRV2 in mice led to selection of a virus that caused paralysis in neonatal mice. To determine the relationship between HRV2 IRES-mediated translation and replication of P1/HRV2 in mice, recombinant human adenoviruses were used to express bicistronic mRNAs in murine organs. The results demonstrate that the HRV2 IRES mediates translation in organs of neonatal but not adult mice. These findings show that HRV2 IRES-mediated translation is a determinant of virus replication in the murine brain and spinal cord and suggest that the IRES determines the species specificity of HRV2 infection.

Human rhinoviruses (HRVs) are nonenveloped, positive-stranded RNA viruses of the family Picornaviridae (8). Approximately 100 HRV serotypes have been identified and divided into two groups based on receptor usage. The receptor for major group HRVs is human intracellular adhesion molecule 1 (hICAM-1), and the receptor for minor group HRVs is human low-density lipoprotein receptor (8). HRVs replicate in the epithelium of the human respiratory tract (1, 2, 11, 41) and are responsible for the majority of common cold infections of humans (36). Only humans develop clinical symptoms after HRV infection; experimental asymptomatic infections have been documented in chimpanzees (10) and gibbons (35). A rodent model for infection with wild-type HRV has not been identified (22).

A small-animal model of HRV infection would be useful for elucidating the mechanisms of HRV-induced pathogenesis and to develop therapeutic interventions. The limited host range of most HRV serotypes (8) has hindered establishment of such a model. Mouse cells are not permissive for the replication of minor group serotypes, despite the ability of these viruses to enter these cells after binding the murine homolog of human low-density lipoprotein receptor. Two exceptions are HRV1A (37) and a variant of HRV2 (HRV2/L) selected by passage in mouse cells (43). The changes required for growth of HRV2/L in mouse cells have not been identified (43). HRV2/L produces altered P2 proteins in infected cells, suggesting that the block to HRV2 replication in murine cells could be due to a defect in RNA replication (29). This conclusion is supported by the finding that HRV2/L is less sensitive than HRV2 to chemical inhibitors of RNA replication (43). It is believed that mouse cell lines are neither permissive nor susceptible to infection with most major group HRVs. While the block to replication of HRV14 and HRV16 in mouse L cells is relieved upon synthesis of hICAM-1, HRV39 fails to replicate (17). Passage of HRV39 in mouse cells producing hICAM-1 led to the identification of a virus, HRV39/L, that can replicate in these cells (16). Amino acid changes in viral proteins 2B and 3A mediate HRV39 growth in mouse cells.

HRV host range may be determined in part by translation of the viral mRNA. The 5' ends of picornaviral mRNAs are not linked to a 7-methylguanosine cap structure and cannot be translated by 5' end-dependent initiation as are most cellular mRNAs (6, 24, 25). Instead, initiation on picornaviral RNA is mediated by the viral internal ribosome entry site (IRES), a cis-acting RNA sequence that binds ribosomes in the absence of an mRNA cap or free 5' end (3, 4, 7, 32, 33). It has been suggested that the limited tropism of poliovirus, a member of the same virus family as HRV, is determined by organ-specific differences in IRES-mediated translation (5, 14, 42). This hypothesis has been disproved by the finding that the IRES of poliovirus and other picornaviruses mediates translation in organs that are not permissive for virus replication (9, 20, 39).

Results obtained with a transgenic mouse model for poliomyelitis have implicated the HRV2 IRES as a determinant of HRV2 host range (14, 15). Poliovirus infection of mice transgenic for the human poliovirus receptor gene, CD155 (TgPVR mice) leads to virus replication in neurons of the brain and spinal cord and flaccid paralysis (23, 38). Unlike wild-type poliovirus, a recombinant poliovirus strain with the IRES of HRV2 does not replicate or cause disease in CD155 transgenic...
mice. These results were interpreted to indicate that HRV2 IRES-mediated translation is specifically blocked in the central nervous system (CNS) and that, by extension, organ-specific differences in IRES-mediated translation can determine viral organ tropism. An alternative explanation is that activity of the HRV2 IRES is blocked in all murine organs. These hypotheses cannot be reconciled solely by the infection of mice with recombinant poliovirus strains, because poliovirus replication in organs other than the CNS is blocked by the alpha/beta interferon response (19).

In this study, we examined the role of the HRV2 IRES in viral host range by analyzing HRV2 IRES-mediated translation in the context of both a bicistronic mRNA and P1/HRV2, a recombinant poliovirus strain that is dependent on the HRV2 IRES. Direct measurement of HRV2 IRES-mediated translation in murine organs was achieved by quantitation of the reporter proteins encoded by a bicistronic mRNA. HRV2 IRES-mediated translation was observed in the brain and spinal cord in neonatal mice, and P1/HRV2 was found to replicate in these organs in neonatal CD155 transgenic mice. In contrast, HRV2 IRES-mediated translation was not observed in the brain and spinal cord in adult mice, consistent with the absence of P1/HRV2 replication in these organs. The lack of HRV2 IRES-mediated translation in adult mice may explain the block to HRV2 replication in this host. Neonates are generally more susceptible to infection with neurotropic viruses (13, 26–28), and our findings suggest that IRES-mediated translation may be a novel determinant of this trait.

FIG. 1. IRES-mediated translation in cultured cells. (A) Schematic of bicistronic reporter DNA encoded by reporter plasmids and recombinant adenoviruses. The arrow indicates the transcription initiation site of the murine cytomegalovirus immediate early promoter. Firefly luciferase and Renilla luciferase have independent translation initiation and termination codons. SV40 An, simian virus 40 polyadenylation signal. The bicistronic reporter DNA is included in bicistronic plasmids used for DNA transformation of cultured cells and in recombinant adenoviruses used for assay of IRES-mediated initiation in mouse organs. (B) Translation mediated by the IRES of poliovirus (filled bars) or HRV2 (open bars) in the indicated cell lines transformed with a plasmid encoding the bicistronic mRNA. To control for transformation efficiency, Renilla luciferase expression was normalized to firefly luciferase expression. IRES activity (y axis) is the relative increase (n-fold) in Renilla luciferase compared with results obtained using a plasmid that does not have an IRES. Data points are the means of three transformations, and error bars indicate standard deviations. (C) Genome structure of poliovirus type 1 strain Mahoney (P1/M) and recombinant strain P1/HRV2. Poliovirus polyprotein (open box), predicted AUG initiation codons, and IRES are indicated. HRV2 sequence is shaded. (D) Single-cycle replication of poliovirus type 1 strain Mahoney (filled symbols) and P1/HRV2 (open symbols) in HeLa (square), Vero (circle), SH-SY5Y (triangle), and L20B (diamond) cell lines. Data points are the means of two infections.

MATERIALS AND METHODS

Cells, plasmids, and viruses. S3 HeLa, A549, and L20B (31) cells were grown in Dulbecco’s modified Eagle medium (Invitrogen, Carlsbad, Calif.), 10% bovine calf serum (HyClone, Logan, Utah), and 1% penicillin-streptomycin (Invitrogen). Neuro-2A and SH-SY5Y cells were grown in the same medium except that 10% fetal bovine serum (Invitrogen, Carlsbad, Calif.) was used. For plaque assays HeLa cells were grown in Dulbecco’s modified Eagle medium (Specially Media, Phillipsburg, N.J.), 0.2% NaHCO3, 5% bovine calf serum, 1% penicillin-streptomycin, and 0.9% Bacto-agar (Difco, Franklin Lakes, N.J.).

Plasmid pP1/HRV2, an infectious poliovirus DNA clone in which nucleotides 108 to 745 are replaced with HRV2 sequence, was created as follows. Nucleotides 108 to 610 of the HRV2 genome were amplified by PCR from a plasmid encoding a bicistronic mRNA with the HRV2 IRES flanked by the firefly and Renilla luciferase coding regions (Fig. 1A) was created as follows. The bicistronic reporter DNA includes the promoter and coding regions has also been described previously (20). The creation of a plasmid encoding a bicistronic mRNA with the poliovirus IRES flanked by the firefly and Renilla luciferase coding regions has also been described previously (20).

Recombinant human adenoviruses encoding bicistronic reporter mRNA were produced using the Admax system (Microbiix, Toronto, Ontario, Canada). Viruses encoding bicistronic mRNAs were created by recombination in 293 cells between the calcium phosphate-transfected adenovirus genome plasmid pBHGfrtDE13FLP and the pDC516-based plasmids described above that encode bicistronic mRNA. Recovered virus was subjected to two rounds of plaque purification as described by the manufacturer (Microbiix, Toronto, Ontario, Can-
IRES activity. To control for IRES-independent Renilla luciferase (Chemicon, Temecula, Calif.).

To produce poliovirus strains P1/M and P1/HRV2, viral DNA clones p7M and pPIHRV2 were linearized by restriction enzyme cleavage and used as templates for runoff transcription by T7 RNA polymerase (Promega, Madison, Wis.). RNA was transfected into HeLa cells using DEAE-Dextran, and after 3 days intracellular virus was released by three freeze-thaw cycles and used once in HeLa cells. Virus was subjected to two rounds of plaque purification, and virus stocks were produced in HeLa cells.

Poliovirus replication in cultured cells. Monolayers of adherent cells were infected at a multiplicity of 10 PFU per cell. At different times after infection, cells were scraped into tubes and subjected to three freeze-thaw cycles to release intracellular virus. The virus titer in each sample was determined by plaque assay of HeLa cell monolayers.

Assay for IRES-dependent translation in continuous cell lines. Cells at 70% confluence in 35-mm dishes were transfected with 3 μg of plasmids encoding bicistronic mRNAs using Lipofectamine Plus (Invitrogen, Carlsbad, Calif.). Growth medium was replaced after 3 h. After 24 h, cells were washed with 1 ml of phosphate-buffered saline (PBS) and lysed in 1 ml of passive lysis buffer (Promega, Madison, Wis.). A dual lucerase assay (Promega, Madison, Wis.) and a Lumat LB9507 luminometer (EG&G Bertold, Oak Ridge, Tenn.) were used to determine firefly lucerase and Renilla lucerase activity levels. To control for variations in transcription or translation, the ratio of firefly lucerase activity to Renilla lucerase activity was determined and defined as IRES activity. To control for IRES-independent Renilla lucerase translation, the activity of each IRES was normalized to the ratio determined in lysates from cells transfected with a plasmid lacking an IRES. The concentration of lucerase protein was calculated with reference to a standard curve generated by using known concentrations of recombinant firefly lucerase (Fisher Scientific, Springfield, N.J.) and Renilla lucerase (Chemicon, Temecula, Calif.).

Assay for IRES-dependent translation in murine organs. C57BL/6J mice (The Jackson Laboratory, Bar Harbor, Maine) were inoculated (at 4 weeks or 1 to 2 days old) as follows: intraperitoneally with 10^9 PFU of recombinant human adenovirus for assays of heart, lung, liver, kidney, and ileum; intramuscularly with 5 x 10^9 PFU for assays of brain and spinal cord. The volume of the inoculation was 50 μl for 4-week-old mice or 15 μl for 1- to 2-day-old mice. Sixteen to 24 h after infection, mice were sacrificed, and organs were removed and homogenized with a PowerGen 125 homogenizer (Fisher Scientific, Springfield, N.J.) in 0.5 ml of passive lysis buffer. Crude protein extracts were prepared, and the dual lucerase assay and the Lumat LB9507 luminometer were used to determine firefly lucerase and Renilla lucerase activities in the extracts. To control for variation in adenovirus infection or transcription, the ratio of firefly lucerase activity to Renilla lucerase activity was determined and defined as IRES activity. To control for IRES-independent Renilla lucerase translation, the activity of each IRES was normalized to the ratio determined in organs from mice infected with an adenovirus lacking an IRES. All experimental mouse protocols adhered to Institutional Animal Care and Use Committee guidelines and were approved by the Institutional Animal Care and Use Committee of Columbia University Medical Center, New York, N.Y.

Infection of TgPVR mice with poliovirus. TgPVR mice transgenic for the human poliovirus receptor gene (38) were genotyped to ensure that they carried the human poliovirus receptor gene. Mice lacking the CD155 gene were used as negative controls. Tail fragments were incubated overnight at 55°C in 0.2 ml of PBS, and the resulting RNA was used to determine firefly lucerase and Renilla lucerase activities in the extracts. To control for variation in adenovirus infection or transcription, the ratio of firefly lucerase activity to Renilla lucerase activity was determined and defined as IRES activity. To control for IRES-independent Renilla lucerase translation, the activity of each IRES was normalized to the ratio determined in organs from mice infected with an adenovirus lacking an IRES. All experimental mouse protocols adhered to Institutional Animal Care and Use Committee guidelines and were approved by the Institutional Animal Care and Use Committee of Columbia University Medical Center, New York, N.Y.

To assay viral replication, the following inoculations were performed. TgPVR mice (either 4 weeks old or 1 to 2 days old) were inoculated intraperitoneally with 10^9 PFU or 10^8 PFU of poliovirus type 1 Mahoney strain in a volume of 50 μl (4-week-old mice) or 15 μl (1- to 2-day-old mice). TgPVR mice (either 4 weeks old or 1 to 2 days old) were inoculated intraperitoneally with 2 x 10^5 PFU or 5 x 10^5 PFU of poliovirus strain P1/HRV2 in a volume of 50 μl (4-week-old mice) or 15 μl (1- to 2-day-old mice). At different times after infection, mice were sacrificed, and organs were removed and homogenized in PBS with 0.2% bovine calf serum with a PowerGen 125 homogenizer. Intracellular virus was released by three freeze-thaw cycles, and cellular debris was removed by centrifugation at 16,100 x g for 15 min at 4°C. The titer of infectious virus in the supernatant of each sample was determined by plaque assay of HeLa cell monolayers.

Generation of virulent P1/HRV2. TgPVR mice were genotyped as described previously. Neonatal TgPVR mice were inoculated intracerebrally with 5 x 10^8 PFU of P1/HRV2. Mice were sacrificed after 3 days, and brains were homogenized in 1 ml of PBS with 0.2% bovine calf serum with a PowerGen 125 homogenizer in 0.5 ml of passive lysis buffer. To control for variations in infection or transcription, the ratio of firefly lucerase activity to Renilla lucerase activity was determined and defined as IRES activity. To control for IRES-independent Renilla lucerase translation, the activity of each IRES was normalized to the ratio determined in organs from mice infected with a recombinant adenovirus lacking an IRES. All experimental mouse protocols adhered to Institutional Animal Care and Use Committee guidelines and were approved by the Institutional Animal Care and Use Committee of Columbia University Medical Center, New York, N.Y.

Infection of TgPVR mice with poliovirus. TgPVR mice transgenic for the human poliovirus receptor gene (38) were genotyped to ensure that they carried the human poliovirus receptor gene. Mice lacking the CD155 gene were used as negative controls. Tail fragments were incubated overnight at 55°C in 0.2 ml of PBS, and the resulting RNA was used to determine firefly lucerase and Renilla lucerase activities in the extracts. To control for variation in adenovirus infection or transcription, the ratio of firefly lucerase activity to Renilla lucerase activity was determined and defined as IRES activity. To control for IRES-independent Renilla lucerase translation, the activity of each IRES was normalized to the ratio determined in organs from mice infected with an adenovirus lacking an IRES. All experimental mouse protocols adhered to Institutional Animal Care and Use Committee guidelines and were approved by the Institutional Animal Care and Use Committee of Columbia University Medical Center, New York, N.Y.

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Results

HRV2 IRES-mediated translation in cultured cells. To determine whether HRV2 IRES-directed translation might regulate viral replication in cultured cells, internal initiation me-
mediated by the HRV2 IRES was quantitated in human and murine cell lines. Human HeLa and A549 cells and murine L20B (L cells that produce CD155) (31) and neuroblastoma N2A cells were transformed with plasmids that produce bicistronic mRNAs encoding firefly and Renilla luciferases separated by the HRV2 IRES (Fig. 1A). The same cell lines were also transformed with plasmids encoding bicistronic mRNAs possessing the poliovirus IRES. As previously demonstrated (4), the HRV2 IRES mediates translation of the second open reading frame of the bicistronic mRNA. In the human cell lines tested, the HRV2 IRES confers a greater than 10-fold increase in relative Renilla luciferase expression over a control plasmid that lacks an IRES (Fig. 1B). In the murine cell lines, activity of the HRV2 IRES is significant but less pronounced. In L20B and N2A cells, presence of the IRES results in a five- and twofold increase, respectively, in relative Renilla luciferase expression compared with a control plasmid that lacks an IRES (Fig. 1B). In all cell lines tested, the activity of the poliovirus IRES is greater than that of the HRV2 IRES (Fig. 1B).

The recombinant poliovirus strain P1/HRV2 was created to study HRV2 IRES-mediated translation in the context of viral replication. In the genome of this virus, the poliovirus IRES is replaced with the cognate sequence from the HRV2 genome (Fig. 1C). As previously demonstrated (14), the HRV2 IRES can functionally replace the poliovirus IRES. Transfection of HeLa cells with P1/HRV2 RNA results in generation of infectious virus that produces plaques on HeLa cell monolayers (data not shown). Single-step growth analysis of P1/HRV2 in cell lines of primate and murine origin reveals a defect in replication compared with a control plasmid that lacks an IRES (Fig. 1D). In murine L20B cells, the delay in virus production persists at least 4 h longer than in primate cells (Fig. 1D).

Age-dependent replication of P1/HRV2 in murine CNS. P1/HRV2 replication is defective in the murine L20B cell line (Fig. 1D). To determine if dependence on the HRV2 IRES causes a similar replication defect in vivo, infections were carried out in TgPVR mice, which are transgenic for the human poliovirus receptor gene CD155 (38). After intraperitoneal inoculation, no significant rise in the titer of P1/HRV2 was observed in the brain and spinal cord in adult TgPVR mice (Fig. 2B). In contrast, poliovirus type 1 replicates to high titers in the brain and spinal cord in adult TgPVR mice (Fig. 2A). After inoculation of neonatal TgPVR mice, titers of P1/HRV2 increased 1,300-fold and 110-fold, respectively, in the brain and spinal cord (Fig. 2D). Titers of poliovirus type 1 increased 10,000-fold and 1,000-fold, respectively, in the brain and spinal cord in neonatal TgPVR mice (Fig. 2C). P1/HRV2 is cleared from the brain and spinal cord in nontransgenic neonates (Fig. 2D). The human poliovirus receptor is required for increases in P1/HRV2 titer in the neonatal brain and spinal cord, which indicates that P1/HRV2 replicates in these organs.

Age-dependent HRV2 IRES-mediated translation in the murine CNS. To determine whether the failure of P1/HRV2 to replicate in the brain and spinal cord in adult TgPVR mice is caused by a block of viral protein synthesis, HRV2 IRES-mediated translation was measured in adult and neonatal mice by infecting mice with recombinant human adenovirus vectors that produce bicistronic mRNAs encoding firefly and Renilla luciferases separated by the HRV2 or poliovirus IRES (20). Poliovirus IRES-mediated translation was observed in the brain and spinal cord in both adult and neonatal mice (Fig. 3). In contrast, translation mediated by the HRV2 IRES is highly age dependent. In adult mice, HRV2 IRES-mediated translation in the brain or spinal cord was not detected (Fig. 3). In newborn mice, HRV2 IRES-mediated translation was observed in both the brain and spinal cord (Fig. 3). HRV2 IRES-mediated translation in these neonatal organs is approximately
10- and 7-fold greater, respectively, than in adults. Poliovirus IRES activity in neonatal brain and spinal cord is also higher than in the same adult organs (six- and fivefold greater, respectively).

Isolation of a P1/HRV2 variant that causes disease in neonatal mice. In the murine model of poliomyelitis, poliovirus infection is followed by virus replication in the CNS and the development of flaccid paralysis (23, 38). Infection of neonatal mice with P1/HRV2, a recombinant virus dependent on the IRES of HRV2, is followed by increased virus titer in the CNS (Fig. 2D), but paralysis is not observed. To determine if virulence of P1/HRV2 is blocked absolutely, viral variants that replicate more efficiently in the CNS were selected by the serial infection of newborn mice with P1/HRV2. Passage of P1/HRV2 in neonatal mice led to the isolation of viruses with increased virulence. The initial stock of P1/HRV2 did not cause paralysis in TgPVR neonates (Fig. 4A). Virus recovered from the CNS of one of these mouse caused flaccid paralysis in 19% of inoculated TgPVR neonates (Fig. 4A). After a second passage in neonatal mice, the virus caused flaccid paralysis in 64% of inoculated TgPVR neonates (Fig. 4A). The virulence of this virus is attenuated: the dose that causes paralysis in 64% of neonates is approximately 25,000 times the 50% lethal dose of a virulent poliovirus strain in neonates. Furthermore, this virus does not cause disease in adult TgPVR mice (data not shown). The virulent virus recovered from the third round of infection replicated to similar titers in the neonatal CNS as P1/HRV2 (Fig. 2D and 4B). Nucleotide sequence analysis of the genome of the third-passage virus did not reveal changes from the P1/HRV2 sequence (data not shown).

Age-dependent HRV2 IRES-mediated translation in extra-neural murine organs. To determine whether the age-dependent block to HRV2 IRES-mediated translation is specific for the CNS, translation was assayed in extra-neural organs by infecting mice with adenovirus vectors that encode bicistronic mRNAs. The poliovirus IRES mediates high levels of translation in the liver and kidney in both adult and neonatal mice (Fig. 5). HRV2 IRES-mediated translation was observed in neonatal but not adult liver or kidney (Fig. 5). HRV2 IRES-mediated translation in neonatal liver and kidney is approximately 14- and 16-fold greater, respectively, than in adults, while poliovirus IRES-dependent translation in neonatal liver and kidney is sixfold greater than in adults (Fig. 5). These results demonstrate that HRV2 IRES-mediated translation in the mouse is age dependent and is detected in the neonatal but not adult brain, spinal cord, liver, and kidney.

DISCUSSION

The experiments reported here were designed to determine whether the block to HRV2 replication in mice and in mouse cell cultures is at the level of IRES-mediated initiation. Using a bicistronic reporter plasmid, we observed HRV2 IRES-dependent translation in cultured mouse L cells. Furthermore, the recombinant poliovirus P1/HRV2, which is dependent on the HRV2 IRES, replicates in L cells. The block to HRV2 replication in L cells must therefore be at a stage after viral translation, such as RNA replication or virion assembly. The study of HRV2 strains selected for growth in L cells should identify the nature of the block to replication.

To determine whether the block to HRV2 replication in mice is a consequence of restricted IRES-dependent translation, we made use of a transgenic mouse model for poliovirus pathogenesis (38). Poliovirus type 1 replicates to high titers and causes paralytic disease in TgPVR mice. When the poliovirus IRES is replaced with the cognate sequence from HRV2, the recombinant virus does not replicate or cause disease in adult TgPVR mice. However, P1/HRV2 does replicate in the brain and spinal cord in neonatal TgPVR mice, although paralysis does not occur. The block to replication of P1/HRV2 in the brain and spinal cord in adult mice is associated with a defect in HRV2 IRES-mediated translation that is relieved in the brain and spinal cord in neonatal mice.

Our findings are compatible with the conclusions of a previous study, which demonstrated that a poliovirus recombinant dependent on the HRV2 IRES does not replicate or cause disease in adult CD155 transgenic mice (14). The authors of that study asserted that attenuation of viral virulence was due to a block to HRV2 IRES-mediated translation that is specific...
for the CNS and concluded that organ-specific differences in IRES activity can determine viral tropism. By directly measuring translation, we have shown that HRV2 IRES-mediated translation is defective not only in the CNS but also in kidney and liver. This result is not surprising, for direct studies of viral IRES-mediated translation in mice have not identified an IRES that determines viral organ tropism or is active only in certain organs (9, 20, 39).

Neonatal mice are more susceptible than adult mice to viral infection, a property that has been attributed to differences in their immune and apoptotic responses (13, 26–28). Our results indicate that IRES activity may also be a determinant of increased susceptibility of neonates to viral infection. The results of previous studies have shown that IRES-mediated translation is greater in neonatal mice (9, 39), but no biological consequences were attributed to this pattern. Our results indicate that HRV2 IRES-mediated translation is higher in organs of neonatal mice than in adults, and this activity is associated with the increased susceptibility of neonates to viral infection. IRES-mediated translation in neonates may be facilitated by greater expression of IRES trans-acting factors (ITAFs), cellular proteins necessary for IRES function (3). For example, the translation initiation by the IRES of foot-and-mouth disease virus requires ITAF45, a protein that is produced in proliferating, nondifferentiated cells (34). The HRV2 IRES may similarly depend on an ITAF that is synthesized during neonatal development. The translation deficiency was also observed in liver and kidney in adult mice, suggesting that mouse tissues are generally unable to support HRV2 IRES-dependent initiation. P1/HRV2 did not replicate in nonneural organs in neonatal TgPVR mice (data not shown), most likely as a consequence of the alpha/beta interferon response that limits poliovirus replication to the brain and spinal cord in mice (19).

In many animal models of picornavirus pathogenesis, disease is closely associated with virus replication (18, 21, 30, 40). However, replication of unpassaged P1/HRV2 in the neonatal brain does not lead to disease, indicating that other determinants may modulate picornavirus virulence. The absence of disease is not likely a consequence of a general defect in virus replication, because in cultured cells the growth of P1/HRV2, although decreased, is similar to that of the neurovirulent strain P1/HCV (20). Serial passage of P1/HRV2 in neonatal mice led to the isolation of a virus that causes paralytic disease; however, attempts to identify the mutations responsible for acquisition of virulence were unsuccessful. Such mutations may be selected against in HeLa cells and might be lost during the amplification of recovered virus in HeLa cells. Alternatively, the population of virulent virus recovered after two serial infections in mice may be relatively small. Mutations responsible for virulence may be identified after further enrichment for virulent strains by additional rounds of serial infection. The identification of such mutations should provide information on the mechanisms by which P1/HRV2 induces paralytic disease in mice.

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