Adenosine deaminases acting on RNA (ADARs) catalyze the C-6 deamination of adenosine (A) to produce inosine (I), which behaves as guanine (G), thereby altering base pairing in RNAs with double-stranded character. Two genes, *adar1* and *adar2*, are known to encode enzymatically active ADARs in mammalian cells. Furthermore, two size forms of ADAR1 are expressed by alternative promoter usage, a short (p110) nuclear form that is constitutively made and a long (p150) form that is interferon inducible and present in both the cytoplasm and nucleus. ADAR2 is also a constitutively expressed nuclear protein. Extensive A-to-G substitution has been described in mouse polyomavirus (PyV) RNA isolated late times after infection, suggesting modification by ADAR. To test the role of ADAR in PyV infection, we used genetically null mouse embryonic fibroblast cells deficient in either ADAR1 or ADAR2. The single-cycle yields and growth kinetics of PyV were comparable between *adar1*<sup>−/−</sup> and *adar2*<sup>−/−</sup> genetic null fibroblast cells. While large T antigen was expressed to higher levels in *adar1*<sup>−/−</sup> cells than *adar2*<sup>−/−</sup> cells, less difference was seen in VP1 protein expression levels between the two knockout MEFs. However, virus-induced cell killing was greatly enhanced in PyV-infected *adar1*<sup>−/−</sup> cells compared to that of *adar2*<sup>−/−</sup> cells. Complementation with p110 protected cells from PyV-induced cytotoxicity. UV-irradiated PyV did not display any enhanced cytopathic effect in *adar1*<sup>−/−</sup> cells. Reovirus and vesicular stomatitis virus single-cycle yields were comparable between *adar1*<sup>−/−</sup> and *adar2*<sup>−/−</sup> cells, and neither reovirus nor VSV showed enhanced cytopathicity in *adar1*<sup>−/−</sup>-infected cells. These results suggest that ADAR1 plays a virus-selective role in the host response to infection.
editing of the highly structured HDV antigenic site that leads to the conversion of an amber stop codon to a tryptophan codon, allowing the synthesis of large delta antigen (18, 53).

In contrast to the highly selective A-to-I editing seen with GluR-B, 5HTT-2CR, and HDV RNAs, nonspecific and multiple-site adenosine deamination of viral and cellular RNAs has been observed when RNA substrates possesses extensive duplex character (10, 17). Two examples of the hyperediting of viral RNAs during lytic and persistent infection include measles virus, where biased A-to-I (G) hypermutations were first described (6), and mouse polyomavirus (22). With human measles virus, an acute infection can lead to a persistent infection in the brain and a very rare but often fatal disease, subacute sclerosing panencephalitis (SSPE). The characterization of viral RNA from SSPE autopsies reveals clustered A-to-I (G) mutations in the M gene and less frequently in other measles virus genes (6, 35). The identity of the ADAR enzyme responsible for editing measles virus RNA in human infection is unknown, although ADAR1 does suppress measles virus-induced apoptosis and activation of PKR in cell culture infection (47).

Mouse polyomavirus (PyV) is a small DNA virus, possessing an ~5-kb double-stranded circular DNA genome within naked virions whose capsid is formed by three proteins, VP1, VP2, and VP3 (3, 21). In mouse cells permissive for productive PyV infection, following attachment to ganglioside receptors and endocytosis, trafficking to the endoplasmic reticulum, and then translocation to the cytosol, virion disassembly occurs. The viral minichromosome then is transported through nuclear pores to the nucleus at late times after infection possess extensive A-strands of the genome; spliced early transcripts encode the T antigens, including large T (important for DNA replication), small T and Ags, late transcripts encode the capsid structural proteins. Late antigens, including large T (important for DNA replication), and VP3 (3, 21). In mouse cells permissive for productive PyV infection, following attachment to ganglioside receptors and endocytosis, trafficking to the endoplasmic reticulum, and then translocation to the cytosol, virion disassembly occurs. The viral minichromosome then is transported through nuclear pores to the nucleus, where viral transcription, DNA replication, and subsequent progeny virion assembly occur. Early and late promoters drive viral transcription from opposite DNA strands of the genome; spliced early transcripts encode the T antigens, including large T (important for DNA replication), and late transcripts encode the capsid structural proteins. Late in PyV infection, early mRNA is downregulated by nuclear antisense late-strand RNA (28). Early-strand RNAs present in the nucleus at late times after infection possess extensive A-to-G (I) changes (22), which is consistent with hyperdeamination by an ADAR activity (1, 33, 46). However, it is not known whether ADAR1 or ADAR2 affects PyV replication or the host response to PyV infection.

Using MEF cell lines genetically null for either ADAR1 or ADAR2 protein, the known active A-to-I RNA editing enzymes, we tested the role of ADARs in the growth of PyV and the host response to PyV infection. We found that the kinetics of growth and yield of infectious PyV were comparable in adar1 null and adar2 null MEFs and were more efficient and higher than in wild-type MEF cells. However, in the absence of ADAR1, PyV infection induced a pronounced cytopathic effect that was not seen in the absence of ADAR2 or in wild-type MEFs. The ADAR1 p110 isoform was sufficient to protect against the PyV-induced cell death seen in adar1 null cells, which was not observed with UV-inactivated PyV. These results suggest that the singular absence of either ADAR1 or ADAR2 does not negatively affect PyV replication, although the absence of ADAR1 enhances PyV-induced cell death.

**Materials and Methods**

**Cells, maintenance, and interferon treatment.** Mouse embryo fibroblast (MEF) cells homozygous null in adar1 p110 (50) or adar2 p150 (44) and wild-type MEF cells were generously provided by Kazuko Nishikura (The Wistar Institute, Philadelphia, PA); adar1 p150+/− MEFs homozygous null for the p150 isoform of ADAR1 were as described previously (51). Wild-type and stat1−/− MEF cells (31) were provided by Robert Schreiber (Washington University, St. Louis, MO). Cells were maintained in Dulbecco’s modified essential medium (DMEM) containing 10% (vol/vol) fetal bovine serum (HyClone), 1 mM sodium pyruvate, 100 μg/ml penicillin, and 100 U/ml streptomycin (Invitrogen). UC1B mouse fibroblast cells were obtained from Thomas Benjamin (Harvard Medical School, Boston, MA) and were maintained in DMEM containing 5% (vol/vol) fetal bovine serum and 3% sodium bicarbonate. Mouse L cells were grown as described previously (39). IFN treatment was with 1,000 U/ml of recombinant IFN-α/β (PBL, Biochemical Laboratories) for 24 h (39). Parallel cultures were left untreated as controls.

**Viruses and infections.** Mouse polyomavirus (PyV) RA strain was generously provided by T. Benjamin (Boston). Virus infections were carried out at a multiplicity of infection (MOI) of 5 PFU/cell in phosphate-buffered saline containing 2% (vol/vol) fetal bovine serum, 0.8 mM CaCl2, 0.4 mM MgCl2, 100 μg/ml penicillin, and 100 U/ml streptomycin. Virus yields were determined by plaque assay using UC1B mouse fibroblasts. Plaques developed under agarose overlay within 6 to 8 days postinfection and were detected by staining with neutral red. Alternatively, the plaque assay for PyV also was carried out using adar1−/− cells; in these cases, plaques developed within 3 to 4 days postinfection.

**Cell viability assay.** The MTT colorimetric assay was carried out essentially as described by Mosmann (32). At the indicated time after infection, 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyltetrazolium bromide (MTT reagent; Invitrogen) prepared as a 5-mg/ml stock solution in water was added to medium in each well to give a final MTT concentration of 0.5 mg/ml. Cells were incubated for 30 min at 37°C, the medium was removed, and the MTT dye then was released from attached cells with dimethyl sulfoxide using a volume equal to the reverse transcription-PCR (RT-PCR) (7).

**siRNA-mediated transient knockdown of ADAR1.** The pCDNA 3.1/neo vector constructs that express the constitutive p110 form of human ADAR1, either the wild type or the H910Q, E912A mutant lacking deaminase activity (26), were as previously described (25). DNA plasmid transfections were carried out using Lipofectamine 2000 (Invitrogen) by following the manufacturer’s recommendations. DNA and Lipofectamine 2000 were diluted and mixed in Opti-MEM (Invitrogen) at a ratio of 1.3 before being applied to monolayer cultures in six-well dishes. Stable pools of adar1−/− MEF cells expressing hADAR1 p110, or with empty vector as a control, were generated following transfection by selection for G418 resistance (300 μg/ml). The expression of human ADAR1 in MEFs was verified by reverse transcription-PCR (RT-PCR) (7).

**Western immunoblot analysis.** Whole-cell extracts were prepared using high-salt lysis buffer containing 0.5% NP-40, 400 mM NaCl, 10 mM KCl, 1 mM EDTA, and 20 mM HEPES, pH 7.9; 1 mM dithiothreitol (DTT), 1 mM phenylmethylsulfonyl fluoride, and 1% (vol/vol) protease inhibitor cocktail (Sigma) were added to the lysis buffer just prior to use. Proteins were fractionated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, transferred to nitrocellulose membranes which were blocked using phosphate-buffered saline containing 5% (wt/vol) nonfat milk, and subsequently probed with primary antibody as indicated. Rabbit polyclonal antibody was used to detect PyV capsid protein VP1; guinea pig polyclonal antibody was used to detect mouse ADAR1 (6), and mouse monoclonal antibodies were used to detect PyV large T antigen and...
RESULTS

Multiplication of polyomavirus is comparable in adar1−/− and adar2−/− mouse cells. We first examined the adar1−/− and adar2−/− MEF cell lines in the absence and presence of IFN treatment for the expression of ADAR1 and ADAR2, both by PCR and by Western immunoblot assay. As previously reported, ADAR1 expression appeared unchanged in adar2−/− cells (16), and in adar1−/− cells where ADAR2 function is not detectably changed (12, 50), neither exon 1A-containing transcripts that encode p150 nor exon 1B-containing transcripts that encode the p110 size isoform of ADAR1 were observed even following IFN-α/IFN-γ treatment (Fig. 1A). However, the p150 isoform of ADAR1 was induced by IFN treatment in both adar2−/− and adar+/+ MEF cells measured by both PCR (Fig. 1A) and immunoblot assay (Fig. 1B).

We next examined the effect of ADAR1 or ADAR2 deficiency on the growth of polyomavirus in MEF cells. As shown in Fig. 2, the growth kinetics and infectious yields of PyV were similar in adar1−/− and adar2−/− MEF lines. The comparable and efficient growth of PyV in both adar1−/− and adar2−/− cells was unexpected based on the report that early RNA isolated at late times after PyV infection shows extensive base modification consistent with ADAR-mediated A-to-I editing (22). Because the yield of PyV was similar in the adar1−/− and adar2−/− MEFs (Fig. 2) and also higher than that observed with two different wild-type adar+/+ MEF cell lines (Fig. 2; data not shown), the growth of two additional viruses, reovirus and vesicular stomatitis virus, was measured in these cell lines. The single-cycle yields of reovirus, and also vesicular stomatitis virus, were comparable in the adar1−/−, adar2−/−, and adar+/+ MEF lines (Table 1).

ADAR1 deficiency leads to enhanced polyomavirus-induced cytotoxicity. When determining the growth of PyV in ADAR-sufficient and -deficient MEF cells, we observed a greater cytopathic effect following the infection of adar1−/− cells compared to the infection of either adar2−/− or adar+/+ cells, as illustrated by the phase-contrast images shown in Fig. 3. The cytopathic effect seen in adar1−/− MEFs was characterized by a rounding of the cells followed by their detachment and death seen at 48 h after infection.

To quantify the PyV-induced cytotoxicity, the colorimetric...
Materials and Methods.

Virus yields were measured as the Dearing strain of reovirus serotype 3 or the Indiana serotype of vesicular stomatitis virus (VSV) in mouse embryo fibroblast cell lines homozygous for the ADAR1 null mutation (adar1−/−) or ADAR2 null mutation (adar2−/−) or in wild-type MEF cells (adar+/+) as described in Materials and Methods.

Efficient expression of polyomavirus early and late proteins in adar1−/− MEF cells. We next tested whether genetic deficiency in either ADAR1 or ADAR2 affected viral protein expression. We carried out Western immunoblot analyses at increasing times after infection using antibodies against PyV large T antigen and capsid protein VP1 as beacons for early and late protein expression. Efficient early and late PyV protein production was found in both adar1−/− and adar2−/− MEF lines and higher than that of adar1+/+ cells (Fig. 5B). Large T antigen accumulated to higher levels in adar1−/− cells than in adar2−/− or adar1+/+ cells (Fig. 5A), whereas the level of VP1 expression was more comparable between the adar1−/− and adar2−/− MEF lines and higher than that of adar1+/+ cells (Fig. 5B).

Knockdown of ADAR1 expression in adar2−/− MEFs does not affect virus growth but causes enhanced virus-induced cytotoxicity. As an approach to test the effect of the double deficiency of ADAR1 and ADAR2 proteins on PyV growth and virus-induced cytotoxicity, we transiently knocked down ADAR1 in the background of the adar2−/− MEFs that genetically lack ADAR2. As shown in Fig. 6A, the transient knockdown of ADAR1 in the adar2−/− cells led to reduced cell viability following PyV infection compared to that of adar2−/− MEFs either not transfected or transfected with Dharmacon control siRNA. However, the reduction of ADAR1 by siRNA knockdown in the adar2−/− MEFs did not significantly affect PyV growth (Fig. 6B). The decrease in ADAR1 steady-state protein levels in adar2−/− MEFs was verified by Western blot analysis (Fig. 6C).

Expression of human ADAR1 in adar1−/− MEFs impairs polyomavirus-induced cytotoxicity and suppresses virus growth. Because the enhanced PyV growth and virus-induced cytotoxicity phenotypes of adar1−/− MEF cells were observed...
in the absence of IFN treatment, we asked whether the expression of the p110 constitutive form of ADAR1 was sufficient to reverse the aforementioned phenotypic effects (Fig. 3). adar1<sup>−/−</sup> cells were transfected with either the expression construct encoding human ADAR1 p110 or with empty vector, and then cell populations were selected for G418 resistance. As shown both by microscopy (Fig. 7A) and by the MTT assay (Fig. 7B), adar1<sup>−/−</sup> MEF cells stably transfected to express the p110 form of human ADAR1 acquired the phenotype of ADAR1-sufficient cells and were protected from the severe cytopathic effect induced by PyV infection. To further test whether the p110 isoform of ADAR1 was sufficient to protect against PyV-induced cytopathic effects, adar1<sup>p150</sup><sup>−/−</sup> MEF cells with the selective genetic disruption of p150 expression while leaving p110 expression intact (51) were examined. The adar1<sup>p150</sup><sup>−/−</sup> MEF cells did not display enhanced PyV-induced cytoxicity (Fig. 7B).

As an approach to begin to assess whether ADAR1 deaminase activity is required to confer protection against PyV-induced cytotoxicity or whether the ADAR1 p110 protein without catalytic activity is sufficient, we also expressed the H910Q, E912A catalytic-deficient mutant p110 protein (26) in adar1<sup>−/−</sup> MEFs and then infected them with PyV. As shown in Fig. 7B, the mutant ADAR1 p110 protected against cytotoxicity as measured by the MTT assay. Finally, adar1<sup>−/−</sup> cells that were transfected with the empty vector and selected for G418 antibiotic resistance were as susceptible to the cytopathic effect of PyV as the adar1<sup>−/−</sup> parental cells (Fig. 7A). As shown in Table 2, the expression of p110 human ADAR1 in the adar1<sup>−/−</sup> MEFs significantly reduced the single-cycle yield of PyV to a level similar to that seen in adar1<sup>+/+</sup> MEFs. The high steady-state level of large T antigen observed in the absence of ADAR1 (Fig. 5B) also was reduced by ectopic complementation with p110 ADAR1 in the adar1<sup>−/−</sup> MEFs (data not shown).

**DISCUSSION**

The objective of our study was to assess the role of ADAR1 and ADAR2 in the growth of mouse polyomavirus in culture and in the accompanying cellular response to PyV infection. We found, using genetically null MEF cell lines, that PyV replicated efficiently and to high yield in both adar1<sup>−/−</sup> MEFs and adar2<sup>−/−</sup> MEFs. Most strikingly, however, the PyV infection of adar1<sup>−/−</sup> MEFs induced a strong cytopathic response that was not observed following the infection of either adar2<sup>−/−</sup>, adar1<sup>p150</sup><sup>−/−</sup>, or wild-type MEFs. Furthermore, the complementation of adar1<sup>−/−</sup> MEFs with ADAR1 p110 was sufficient to abrogate the PyV-induced cytototoxic response. Our studies were undertaken because of the extensive base modification seen in PyV early-strand RNA transcripts at late

![FIG. 4. UV treatment of polyomavirus eliminates the enhanced cytotoxicity observed in polyomavirus-infected adar1<sup>−/−</sup> MEF cells. Results of the colorimetric MTT assay to measure the fraction of remaining viable adar1<sup>−/−</sup>, adar2<sup>−/−</sup>, or adar1<sup>+/+</sup> MEF cells following PyV infection were compared to those for uninfected parallel cultures. The MTT assay was carried out at 72 h after infection as described in Materials and Methods. (A) Infection with PyV (Virus). (B) Infection with an amount of virus equivalent to that for panel A, except the virus was UV treated prior to infection (uv-Virus).](http://jvi.asm.org/content/jvi/71/3/8342/F4)

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**TABLE 2. Human p110 ADAR1 isoform suppresses polyomavirus single-cycle yield when expressed in MEF cells homozygous null for adar1<sup>−/−</sup>**

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Polyoma virus yield (PFU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>adar1&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>1 × 10&lt;sup&gt;6&lt;/sup&gt;</td>
</tr>
<tr>
<td>adar1&lt;sup&gt;−/−&lt;/sup&gt; [h-p110]</td>
<td>1 × 10&lt;sup&gt;5&lt;/sup&gt;</td>
</tr>
<tr>
<td>adar&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>8 × 10&lt;sup&gt;7&lt;/sup&gt;</td>
</tr>
<tr>
<td>adar&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>2 × 10&lt;sup&gt;5&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*<sup>a</sup> Virus yields were measured using the RA strain of wild-type mouse polyomavirus in mouse embryo fibroblast cell lines homozygous for ADAR1 null mutation (adar1<sup>−/−</sup>) or ADAR2 null mutation (adar2<sup>−/−</sup>), in wild-type MEF cells (adar<sup>−/+</sup>), and in an adar1<sup>−/−</sup> cell population selected to stably express human p110 ADAR1 (adar1<sup>−/−</sup> [h-p110]) as described in Materials and Methods.*
times during infection, a modification characteristic of A-to-I editing by an ADAR activity (22). We anticipated that if the antisense-induced ADAR editing of double-stranded structures resulting from overlapping transcripts was a critical component of the early-to-late switch in PyV gene expression, then the genetic disruption of either adar1<sup>−/−</sup> or adar2<sup>−/−</sup> might alter virus growth. This was not observed. PyV yields were comparably high in adar1<sup>−/−</sup> MEFs and adar2<sup>−/−</sup> MEFs. It has been convincingly demonstrated that overlap between early and late transcripts and polyadenylation signals leads to A-to-G substitutions by sequence analysis (11, 22). Our results are most consistent with the version of the model by Gu et al. (11), in which the formation of the double-stranded RNAs in the region of the overlapping polyadenylation signals, rather than the editing per se, is the critical determinant for the regulation of expression, because the genetic deficiency of either ADAR1 or ADAR2 singularly did not reduce virus growth in MEFs (Fig. 2). Likewise, the transient knockdown of ADAR1 with siRNA in the background of the ADAR2 genetic deficiency did not affect PyV growth in MEFs (Fig. 6B). In contrast to our findings, after reducing ADAR1 about 4-fold in NIH 3T3 cells by siRNA transfection, a defect in early-to-late switching was described with a strong inhibition of virus growth as measured by reduced viral RNA (11). An endonuclease activity has been described that specifically cleaves hyperedited I-containing dsRNA, but this enzyme reportedly is cytoplasmic (41). It seems unlikely that this nuclease plays a role in modulating PyV transcript levels, as the described PyV early-strand-edited transcripts reportedly are not unstable and accumulate in the nucleus (22). Whether the difference between our findings, obtained with genetically null adar1 cells, and the results of Gu et al. (11), obtained with ADAR1 knockdown cells, is due to the different cells used or the siRNA knockdown transfection strategy compared to that of genetic knockouts is unclear. However, the transient siRNA-mediated knockdown of ADAR1 in adar2<sup>−/−</sup> MEFs caused enhanced PyV-induced cytotoxicity even though virus yield was not significantly affected. These results provide an additional line of evidence that ADAR1, and not ADAR2, is the deaminase responsible for protection against virus-induced cytopathic effects, and that ADAR deficiency does not adversely affect PyV growth, in agreement with observations obtained with adar1<sup>−/−</sup> MEFs.

Our results reveal that the absence of either ADAR1 (in adar1<sup>−/−</sup> MEFs) or ADAR2 (in adar2<sup>−/−</sup> MEFs), or the reduction of both ADAR1 and ADAR2 (siRNA knockdown of ADAR1 in adar2<sup>−/−</sup> MEFs), increased virus growth. However, when both ADAR1 and ADAR2 are present, viral gene expression and yield are significantly reduced. Furthermore, complementation studies indicate that the catalytic activity of ADAR1 p110 is not required to establish the condition of reduced viral expression that appears to be dependent upon the presence of both ADAR proteins. Conceivably, both ADAR1 and ADAR2 are obligatory components of a cellular complex that, when present, downregulates viral gene expression. Inosine-containing dsRNA is reported to bind a stress-granule-like complex and downregulate gene expression in trans (40), but the possible occurrence of such downregulation in PyV-infected cells mediated by edited viral RNA species is not yet known. Both ADAR1 and ADAR2 in mammalian cells are active enzymes and generate I-containing RNA (10, 38); however, possibly the catalytic activity of only one of the two proteins is required, with the other ADAR protein being necessary but having a catalytically independent role.

It is conceivable that the modulation of noncoding RNA by
an ADAR contributes to the determination of the outcome of the virus-host interaction. Examples of cellular microRNAs are known whose production or targeting is altered by the action of ADARs (14, 19, 33). Interestingly, mouse polyoma-virus has been shown to encode two microRNAs that direct the cleavage of early PyV mRNAs (44). The inhibition of the PyV microRNAs increases early protein levels, but interestingly the PyV microRNAs appear to be dispensable for the virus infection of cultured cells and intact mice, as mutant virus that fails to make the microRNAs replicates and transforms well (44).

Our results suggest that ADAR1 impairs cell-destructive processes following PyV infection. The protective effect of ADAR1 is not limited to PyV and mouse cells. ADAR1 protects against measles virus-induced cytopathology in human cells stably knocked down for ADAR1 using a short hairpin RNAi (shRNAi) strategy (47) and mouse cells stably expressing CD150 (51). ADAR1 also protects against virus-induced cytopathic effect with myxoviruses in addition to measles virus, but not lymphocytic choriomeningitis virus, vesicular stomatitis virus, or reovirus (23, 51). The cytotoxicity seen in adar1−/− MEF cultures following PyV infection was not simply the consequence of the production of high levels of infectious virus; rather, infectious virus yields correlated best with the level of capsid protein expression. Both adar1−/− MEFs and adar2−/− MEFs were permissive for PyV multiplication; both null MEFs gave similarly high yields of progeny virus with comparable kinetics, and both MEF lines showed the efficient expression of VP1. However, the strong cytopathic effect seen beginning at 48 h after PyV infection was observed only in adar1−/− MEFs, not in adar2−/− or wild-type MEFs. However, adar2−/− MEFs transiently knocked down for ADAR1 displayed enhanced PyV-induced cytoxicity. The mechanistic basis of this cell destruction observed in the absence of ADAR1 is not yet known, nor is it clearly established whether the cytotoxicity results primarily from necrotic or apoptotic processes. The broad-spectrum caspase inhibitor z-VAD-fmk impairs virus-induced apoptotic responses in, for example, the cases of vaccinia virus-infected (54) and measles virus-infected (47) cells in culture. However, treatment with this pharmacologic inhibitor of caspase activity had no discernible effect on the PyV-induced cytotoxicity in adar1−/− MEF cells (unpublished observations).

The finding that PyV virus growth occurred to a significantly higher titer in both adar1−/− and adar2−/− MEFs than in adar+/+ MEFs possessing both ADAR1 and ADAR2 was unexpected. In contrast, VSV and reovirus yields were comparable for adar+/+, adar1−/−, and adar2−/− cells, which is consistent with prior observations for adar1 p150−/− and adar1+/+

![Figure 6](http://jvi.asm.org/)
MEF cells (51) and also human cells stably knocked down for both p110 and p150 (23). The complementation of the adar1−/− MEFs with ADAR1 p110 reduced PyV growth to levels comparable to that seen in wild-type adar+/+. The expression of ADAR1 p110 in the adar1−/− MEFs also impaired the virus-induced cytotoxic response observed with PyV-infected adar1−/−, but not adar2−/−, MEFs. Conversely, the genetic disruption of ADAR1 p150 expression while maintaining normal p110 expression in MEFs did not lead to enhanced PyV-induced cytotoxic effects. These results suggest that the presence of both ADAR1 and ADAR2 leads to the suppression of PyV growth in MEFs, whereas the absence of ADAR1, most likely the absence of the p110 isoform of ADAR1, but not the absence of ADAR2 results in the enhanced cytotoxic response seen following PyV infection.

ADAR1 is known to play a role in cell survival under a variety of physiologic conditions, including embryogenesis and virus infection (12, 13, 47, 50, 51). The genetic disruption of adar1, either both p110 and p150 (12, 13, 50) or p150 alone (51), leads to embryonic death between days 11.5 and 12.5. Widespread apoptosis is seen in adar1−/− embryos in many tissues at E11.5 (50). MEF cells derived from adar1−/− embryos are prone to apoptosis induced by serum deprivation (50), and the loss of ADAR1 in hematopoietic stem cells leads to rapid apoptosis (13). Measles virus-induced apoptosis is enhanced in human cells made stably deficient in both p110 and p150 ADAR1, and this cytotoxicity occurs in a manner that correlates with the absence of the ADAR1-mediated suppression of the activation of proapoptotic activities as exemplified by PKR and IRF-3 (23, 47). Consistently with these observations, synthetic I:U-dsRNA has been shown to suppress the induction of apoptosis and activation of IRF3 (48).

The finding that the catalytic activity of ADAR1 was not required for the phenotypic rescue of PyV-infected adar1−/− cells is intriguing. adar1−/− MEFs expressing either the H910Q, E912A mutant p110 protein that is devoid of catalytic activity (26) or the wild-type p110 protein with deaminase activity were less susceptible to PyV-induced cell destruction and produced less PyV than vector control adar1−/− MEFs. This observation suggests a role for p110 ADAR1 other than, or in addition to, acting as an RNA deaminase, a role by which the p110 protein is able to modulate a cellular process that affects the host response to PyV infection. It is now of the utmost importance to identify the process affected. An editing-independent function for ADARs is not without precedent. For example, ADAR1 and ADAR2 have been reported to

![Figure 7](http://jvi.asm.org/)
modulate microRNA and siRNA pathways independently of their catalytic RNA editing activity (14).

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