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Respiratory Syncytial Virus Decreases p53 Protein to Prolong Survival of Airway Epithelial Cells

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Respiratory syncytial virus (RSV) is a clinically important pathogen. It preferentially infects airway epithelial cells causing bronchiolitis in infants, exacerbations in patients with obstructive lung disease, and life-threatening pneumonia in immunosuppressed patients. In addition, RSV infection early in life has been associated with the subsequent development of asthma (1–8). RSV is a member of the Paramyxoviridae family and consists of a negative strand RNA genome in a nucleocapsid surrounded by an envelope (9). Entry into the host respiratory epithelium is by cell surface fusion, and infection leads to viral replication and subsequent host inflammatory responses (10–15).

The host suppressor protein, p53, is a potent inhibitor of cell proliferation (16, 17) and the most frequently inactivated gene in human cancers (18, 19). The p53 protein is a transcription factor that is usually short-lived and expressed at very low levels in normal cells. When activated by cellular stresses such as DNA damage, p53 induces the expression of gene products that promote apoptotic cell death or permanent cell cycle withdrawal (19–22).

Notably, Mdm2 is a transcriptional target of p53; thus, the interdependent activities of p53 and Mdm2 comprise a negative auto-regulatory feedback loop (28, 29).

Recent studies have investigated the effect of viral infection on p53. Inflammatory cells in influenza pneumonia activate p53 directly, leading to apoptosis (32). A subsequent paper examined the effect of influenza infection on p53 in respiratory epithelial cells and found that the mechanism of increased p53 in influenza infection is increased transcription (33). One study investigated the effects of multiple viruses on HT1080 and HepG2 cells and found that the amount of p53 was decreased in encephalomyocarditis virus and human parainfluenza virus type 3 infection due to protein kinase R-mediated inhibition of translation (34). Another study concluded that p53 actually enhances the ability of human CMV to...
replicate in fibroblasts (35). Finally, a recent study suggested that poliovirus induces p53 degradation in a proteasome, promyelocytic leukemia-dependent and in an Mdm2-dependent manner in human glioblastoma astrocytoma cells (36). These conflicting results suggest that the effect of viral infection on the amount of p53 may be virus and/or cell type specific.

In contrast, recent studies have investigated the effect of p53 on viral replication. HT1080 cells were treated with p53 small interfering RNA, and vesicular stomatitis virus replication increased (34). Similarly, poliovirus replication increased when U2OS cells were treated with p53 small interfering RNA (36). In contrast, other studies suggested that p53 enhances viral replication. When fibroblast p53 null cells were treated with human CMV, viral titers were attenuated (35), and cells expressing functional p53 allowed for more adenoviral replication than cell lines deficient in functional p53 (37).

The link between p53 and inflammation is less clear. A study of non-small cell lung carcinoma surgical specimens showed a correlation between IL-8 mRNA expression and p53 mutations (38). Another study of psoriasis also showed an inverse relationship between IL-8 and p53: a decrease in p53 expression and an increase in IL-8 expression was observed in psoriatic skin lesions (39). One study demonstrated that p53 has suppressive activity on the inflammatory pathways leading to activation of AP-1 and NF-kB, and this activity is mediated by phosphatase and tensin homolog (40). Suppression of inflammatory pathways might be one other mechanism by which p53 further potentiates cell death.

We have previously shown that RSV activates both proapoptotic and antiapoptotic pathways in airway epithelial cells, and the antiapoptotic effects of RSV during the first few hours of infection are mediated through PI3K and the downstream mediator, Akt (41, 42). Recent work in our lab also indicated that epidermal growth factor receptor and subsequent ERK activation lead to an alteration in the Bcl-2 protein balance, favoring survival and delayed apoptosis (42). One key regulator of cell death is p53, which may be regulated by Akt. We hypothesized that RSV delays cellular apoptosis and prolongs cell survival by activating Akt, which phosphorylates Mdm2 and leads to p53 proteasome degradation. This decrease in p53 and delay in apoptosis paradoxically does not augment viral replication and allows for an enhanced host cell inflammatory response.

**Materials and Methods**

**Chemicals**

Chemicals were obtained from Sigma-Aldrich and Calbiochem. Protease inhibitors were obtained from Roche Applied Science. Nutlin-3 (catalog no. 444143) and LY294002 (catalog no. 440202) were purchased from Santa Cruz Biotechnology (sc-126), and rabbit polyclonal IgG (FL-393) Ab to p53 was obtained from Santa Cruz Biotechnology (sc-6243). Rabbit polyclonal Ab to phospho-p53 (Ser15) was purchased from Cell Signaling Technology (no. 9284). Rabbit polyclonal Ab to phospho-Mdm2 (Ser15) was obtained from Cell Signaling Technology (no. 3521). Rabbit polyclonal IgG Ab to Mdm2 (N-20) was obtained from Santa Cruz Biotechnology (sc-813). Rabbit polyclonal Ab to phospho-Akt (Ser473) (no. 9271), cleaved caspase 7 (Asp20) (no. 9491), and cleaved poly(ADP-ribose) polymerase (Asp214) (no. 9541) were obtained from Cell Signaling Technology. Rabbit mAb to cleaved caspase 3 (Asp175) (no. 9664) was obtained from Sigma-Aldrich.

**Human tracheobronchial epithelial (HTBE) cells**

Human TBE cells were obtained under a protocol approved by the University of Iowa Institutional Review Board. Epithelial cells were isolated from tracheal and bronchial mucosa by enzymatic dissociation and cultured in Laboratory of Human Carcinogenesis LHC-8e medium on plates coated with collagen/albumin for study up to passage 10 as previously described (43). For infection, cells at 80% confluence were treated with human RSV strain A-2 at a multiplicity of infection (MOI) of 2. Viral stocks were obtained from Advanced Biotechnologies. The initial stock (1 × 10^7 TCID_{50}) was aliquoted and kept frozen at −153°C, and a fresh aliquot was thawed for each experiment. The virus was never refrozen. UV light-inactivated RSV was prepared by exposure of a 1/30 dilution of the live virus with PBS to 18 J of UV light at 4°C.

**Vero cells**

Vero cells were cultured in MEM (Invitrogen Life Technologies) supplemented with 10% FBS (IRH Biosciences), penicillin-streptomycin, L-glutamine, non-essential amino acids, and sodium pyruvate (all from Invitrogen Life Technologies).

**Cell protein isolation**

Whole cell protein was prepared by lysing the cells on ice for 20 min in 30 μl of lysis buffer (0.05 M Tris (pH 7.4), 0.15 M NaCl, 1% Nonidet P-40, with added protease and phosphatase inhibitors: 1 protease minitab/10 ml (Roche Applied Science) and 100 μl of 100x phosphatase inhibitor mixture/10 ml (no. 524625; Calbiochem). Lysates were sonicated on ice continuously for 20 s at a 70% duty cycle with a microtip limit of 2, using a Tekmar Sonic Disruptor. Lysates were kept at 4°C for 30 min and spun at 15,000 × g for 10 min to remove insoluble debris, and the supernatant was saved. Protein concentrations were determined using a commercial Protein Assay kit from Bio-Rad. Cell lysates were stored at −70°C until use.

**Western blot analysis**

Protein (40 μg) was mixed 1:1 with 2× sample buffer (20% glycerol, 4% SDS, 10% 2-ME, 0.05% bromphenol blue, and 1.25 M Tris (pH 6.8)) and separated using SDS-PAGE. Cell proteins were transferred to polyvinylidene difluoride membranes (Bio-Rad). Equal loading of proteins was evaluated using Ponceau S dye staining (Sigma-Aldrich). The polyvinylidene difluoride membrane was saturated with methanol, washed, and then incubated with primary Ab. Blots were washed four times and incubated with HRP-conjugated anti-IgG Ab (1/5000 to 1/40000). Immunoreactive bands were developed using a chemiluminescent substrate, ECL, and ECL Plus (Amersham Biosciences) and detected by autoradiography. Protein levels were quantified using densitometry via a FluorS scanner and Quantity One software for analysis (Bio-Rad). Densitometry is expressed as the fold increase of experimental value per control value.

**Quantitative RT-PCR**

Total RNA was isolated using the Absolutely RNA RT-PCR Miniprep kit (Stratagene) following the manufacturer’s instructions. RNA was quantified using RiboGreen kit (Invitrogen Life Technologies). Total RNA (1 μg) was reverse-transcribed to cDNA using iScript cDNA Synthesis kit (Bio-Rad) according to the manufacturer’s instructions. PCR was performed using 2 μl of cDNA and 48 μl of master mix containing iQ SYBR Green Supermix (Bio-Rad), 15 pmol of forward primer and 15 pmol of reverse primer, in a MyiQ Single-Color Real-Time PCR Detection System as follows: 3 min at 95°C followed by 45 cycles of 10 s at 95°C and 20 s at 72°C. The fluorescence signal generated with SYBR Green I DNA dye was measured during annealing steps. Specificity of the amplification was confirmed using melting curve analysis. Data were collected and recorded by MyiQ Optical System Software version 2.0 (Bio-Rad) and expressed as a function of threshold cycle (Ct). Relative gene expression was normalized to HPRT or GAPDH mRNA using ΔCt method as previously described (44). Specific primer sets used are as the following: HPRT (forward) 5′-TGGTAAAGGCTTTGTATTTCC-3′ (reverse) 5′-TCC CCTGTTGACTGGTTCAT-3′; p53 (forward) 5′-CACATGACGAGGT GTGAG-3′ (reverse) 5′-CCACTGTTGATGTTAG-3′; and RSV N-gene (forward) 5′-GCTCCTTGAGAAGTCAAGTTGAATGA-3′ (reverse) 5′- TGTCGGTGGTTGTTACAGTT-3′. Gene-specific primers were custom-synthesized and purchased from Integrated DNA Technologies based on design using gene-specific nucleotide sequences from the National Center for Biotechnology Information sequence databases and PrimerQuest Web interface (Integrated DNA Technologies) or Primer3 Web interface (45).

**Cell death assay**

Human TBE cells were plated at 50% confluence. The cells were pretreated with either 10 μM Nutlin-3 or control medium followed by RSV (MOI of 2) for 0, 24, 48, or 72 h. Nonadherent cells were collected to 1.5-ml tubes, and adherent cells were detached by incubation with trypsin followed by neutralization with complete medium (MEM, 10% FBS, and gentamicin). The detached cells were pooled with the corresponding nonadherent cells and stained with Guava Technologies ViaCount reagent, which contains...
propidium iodide and a cell-permeable dye for nucleated cells LDS-751, following the manufacturer’s protocol. Personal Cell Analysis flow cytometer (Guava Technologies) was used to identify nucleated cells (total cells) and propidium iodide stained nucleated cells (dead cells) to determine the percentage of cell death.

Plaque assay

Viral titters of HTBE RSV-infected cells were measured by standard plaque assay using 90% confluent Vero cells. Briefly, HTBE cells were exposed to RSV (MOI of 2) or Nutlin-3 and RSV (MOI of 2) for 72 h. Supernatants were collected, and frozen at −70°C to be assayed later by plaque assay. Vero cells were treated with serial 10-fold dilutions of the HTBE supernatant/lysed cell mixture. The cell cultures were incubated at 37°C, 5% CO₂ for 90 min, with gentle rocking of the plates every 15 min. Overlay, consisting of EMEM (Cambrex), 10% FBS (JRH Biosciences), L-glutamine (Invitrogen Life Technologies), penicillin-streptomycin (Invitrogen Life Technologies), and 1% SeaKem ME Agarose (Cambrex) was prepared, and 4 ml of cooled overlay was added to each sample. Samples were gently swirled to mix and then allowed to cool in a laminar flow hood for 15 min. The agar solidified, plates were incubated for 5 days at 37°C, 5% CO₂. Cells were stained by adding 2 ml of neutral red (Fisher Scientific) overlay and the concentration of virus calculated.

Cytokine measurements

Primary HTBE cells were plated at ~80% confluence and exposed to control medium of 10 µM Nutlin-3, RSV (MOI of 2), or RSV and Nutlin-3. Supernatants were collected and frozen at −70°C. Human IL-6 and IL-8 concentrations in cell culture supernatants were determined using DuoSet ELISA kits from R&D Systems.

Statistical analysis

Statistical analysis was performed on densitometry, the cell death assay, real-time PCR, and ELISA data. Significant differences between two groups were determined by Student’s t test with (GraphPad statistical analysis software). Significant differences for over two groups were confirmed by one-way ANOVA with a Bonferroni’s test for multiple comparisons (Graphpad statistical analysis software).

Results

RSV decreases p53 protein

In previous studies, we showed that RSV triggers proapoptotic events late in the course of infection (41, 42). One key regulator of cell death is p53. Recent studies investigated the effect of viral infection on p53 levels, and the findings varied depending on the virus and cell-type studied. We began by looking at how RSV infection affects the level of p53 protein. As shown in Fig. 1A, RSV reduces the amount of p53 and activated phospho-p53 protein in primary HTBE cells, first apparent 6 h after RSV infection but more significantly after 16 and 24 h of RSV infection. The amount of p53 mRNA does not change with RSV infection as measured by quantitative real-time PCR (Fig. 1B), suggesting that regulation is at a posttranscriptional level.

Inhibition of the proteosome preserves p53 protein in RSV infection

Because the change in p53 did not appear to be due to a change in transcription, we investigated whether the mechanism for reduction in p53 protein was increased degradation. We exposed HTBE cells to RSV infection for 24 h with and without the proteosome inhibitor, MG132. As shown in Fig. 2A, the amount of p53 protein was again reduced after 24 h of RSV infection, but this reduction
was prevented with the use of the proteosome inhibitor. In both RSV-infected cells and control cells, p53 protein increased when the proteosome was inhibited, confirming that the mechanism for reduction of p53 both in RSV infection and under normal conditions is proteosome degradation.

RSV activates Mdm2

A negative regulator of p53 is the E3 ubiquitin ligase, Mdm2, which is activated by phosphorylation at Ser166 to ubiquitinate p53 (26). Ubiquitination targets p53 for proteasome degradation. Western blot shows that although RSV does not alter the amount of total Mdm2, it does increase the amount of active phospho-Mdm2 (Ser166) (Fig. 2B). This increase at 1–6 h after RSV infection temporally precedes the reduction in p53 at 16 h. These data demonstrate that RSV infection decreases the level of p53 protein, perhaps by Mdm2-mediated degradation.

Inhibition of the PI3K/Akt pathway decreases phospho-Mdm2 and protects p53 from degradation

We have previously shown that RSV phosphorylates Akt (Ser473) and that this activation of Akt is inhibited by the PI3K chemical inhibitor, LY294002, in both control and RSV-infected cells (41). We further investigated this mechanism by examining how the PI3K/Akt activity affects Mdm2 and p53 in RSV infection. We exposed HTBE cells to RSV in the presence or absence of the PI3K/Akt pathway inhibition, LY294002. As shown in Fig. 3, the amount of phospho-Mdm2 (Ser166) increases after 6 h of RSV infection, but in the presence of the PI3K inhibitor, LY294002, phospho-Mdm2 is significantly decreased in cells exposed to RSV. We selected this time point of 6 h because a significant activation of Mdm2 occurs in RSV infection from 1 to 6 h (Fig. 2), and our previous studies have shown that the PI3K/Akt pathway is an important prosurvival pathway early in RSV infection (41). We also investigated the effect of PI3K/Akt inhibition on the amount of p53. As also shown in Fig. 3, RSV infection results in a decrease

FIGURE 3. Inhibition of AKT decreases phospho-Mdm2 (Ser166) and increases p53 protein. HTBE cells were exposed to medium alone, 20 μM LY294002, RSV (MOI of 2), or 20 μM LY294002 followed by RSV for 6 h. Cells were harvested for cellular protein after 6 h and Western blot analysis was performed for phospho-Mdm2 (Ser166) and p53. Inhibition of the PI3K/Akt pathway with a chemical inhibitor results in less activation of Mdm2 and prevents the RSV-induced reduction of p53. Western blots shown are representative of three experiments.

FIGURE 4. Nutlin-3 maintains p53 protein in airway epithelial cells and leads to earlier cell death. A, HTBE cells were exposed to control buffer, 10 μM Nutlin-3, RSV (MOI of 2), or 10 μM Nutlin-3 followed by RSV. Cells were then harvested for cellular protein after 24, 48, and 72 h, and Western blot analysis was performed for p53. Nutlin-3 leads to stabilization of p53 protein, both in control cells and cells exposed to RSV. Western blots shown are representative of three experiments. Next, hTBE cells exposed to control medium alone, 10 μM Nutlin-3, RSV (MOI of 2), or Nutlin-3 followed by RSV for 24, 48, and 72 h and were mobilized and stained with propidium iodide. Flow cytometry was performed to detect the percentage of cells with <2n DNA content, indicating cell death. One-way ANOVA with a Bonferroni’s test for multiple comparisons shows a statistically significant difference (*, p < 0.05) between cells exposed to RSV and Nutlin-3 as compared with cells only exposed to RSV. Data are representative of three experiments. B, UV light-inactivated RSV does not decrease p53 protein. HTBE cells were exposed to RSV (MOI of 2) or UV light-inactivated RSV for 16 h, with and without Nutlin-3 pretreatment. Whole cell lysates were obtained and Western blot analysis was performed for p53 protein.
increased in RSV-infected cells treated with Nutlin-3. Ribose polymerase. Results indicate that all these markers of apoptosis are formed for cleaved caspase 3, cleaved caspase 7, and cleaved poly(ADP-ribose) polymerase. Results indicate that all these markers of apoptosis are increased in RSV-infected cells treated with Nutlin-3.

Nutlin-3 maintains p53 protein in airway epithelial cells and leads to increased apoptosis

Nutlin-3 (Calbiochem) is a chemical inhibitor of Mdm2 that prevents the Mdm2/p53 association, thus inhibiting Mdm2-mediated degradation of p53. This inhibition increases the level of endogenous p53 protein. As shown in Fig. 4A, adding 10 μM Nutlin-3 to primary HTBE cells increases the amount of p53 protein both in cells only treated with Nutlin-3 and those subsequently exposed to RSV for 24, 48, and 72 h. The RSV-induced reduction in p53 is reversed by Nutlin-3 at all time points. To confirm that this reduction in p53 is the result of viral mechanisms, we compared the effect of live RSV and UV-inactivated RSV on p53 protein. Fig. 4B again shows that following RSV infection, the amount of p53 protein is diminished, but this decrease is not seen when cells are treated with UV-RSV. When control cells, RSV-treated cells, and UV-inactivated RSV-treated cells are pretreated with Nutlin-3, the p53 protein is increased. Next we evaluated the biological effect of increasing p53 protein on cell viability. Primary (HTBE) cells were treated with 10 μM Nutlin-3 with and without RSV exposure. Control and Nutlin-3-treated cells showed no increase in cell death in the absence of RSV infection as measured by propidium iodide staining of whole cells and quantification of cell death with flow cytometry after 0, 24, 48, and 72 h of RSV exposure (Fig. 4A). The fact that Nutlin-3 treatment alone showed no increase in cell death despite marked up-regulation of p53 protein reflects published observations that stressed cells are killed by exposure to Nutlin-3 while unstressed control cells are largely unaffected (46). By comparison, RSV-treated cells show a gradual increase in cell death over time that is markedly enhanced by Nutlin-3 at all time points (Fig. 4A). These data demonstrate that increased endogenous p53 protein accelerates the onset and increases the magnitude of cell death in RSV-infected airway epithelial cells. Preventing RSV-mediated reduction in p53 shortens survival of RSV-infected epithelial cells.

Preventing RSV-mediated degradation of p53 leads to increased apoptosis

We wanted to determine whether preserving endogenous p53 in RSV infection leads to enhanced apoptosis. As shown in Fig. 5, adding 10 μM Nutlin-3 to primary HTBE cells treated with RSV for 24 h increases the amount of multiple markers of apoptosis, including cleaved caspase 3, cleaved caspase 7, and cleaved poly(ADP-ribose) polymerase protein. These results indicate that RSV decreases p53 to delay apoptosis. Preservation of endogenous p53 in RSV infection leads to increased apoptosis as measured by cleaved caspase products.

Increasing p53 enhances viral replication

We next examined the effect of p53 protein on RSV replication. Previous studies investigating the effect of p53 protein on viral replication have yielded disparate results. While p53 promotes adenoviral and CMV replication, it limits poxivirus and vesicular stomatitis virus replication (34–37). Primary HTBE cells were exposed to RSV (MOI of 2) or 10 μM Nutlin-3 followed by RSV and incubated for 72 h. To quantify viral replication, cells were harvested, RNA was isolated, and quantitative real-time PCR was performed. Primers targeted the RSV N-gene. Unpaired Student’s t test indicates a statistically significant difference (*, p < 0.05) between cells exposed to RSV alone and RSV pretreated with Nutlin-3 (n = 3 experiments).

FIGURE 5. Nutlin-3 maintains p53 protein and leads to increased apoptosis. HTBE cells were exposed to control buffer, 10 μM Nutlin-3, RSV (MOI of 2), or 10 μM Nutlin-3 followed by RSV. Cells were then harvested for cellular protein after 24 h, and Western blot analysis was performed for cleaved caspase 3, cleaved caspase 7, and cleaved poly(ADP-ribose) polymerase. Results indicate that all these markers of apoptosis are increased in RSV-infected cells treated with Nutlin-3.

FIGURE 6. p53 protein increases RSV replication. HTBE cells were exposed to RSV (MOI of 2) or 10 μM Nutlin-3 followed by RSV for 72 h. Cells were harvested, RNA isolated, cDNA made, and quantitative real-time PCR performed. Primers targeted the RSV N-gene. Unpaired Student’s t test indicates a statistically significant difference (*, p < 0.05) between cells exposed to RSV alone and RSV pretreated with Nutlin-3 (n = 3 experiments).

FIGURE 7. p53 protein increases RSV replication. HTBE cells were exposed to RSV (MOI of 2) or 10 μM Nutlin-3 followed by RSV. Both cells in the supernatant and adherence cells were collected and sonicated, and 10-fold dilutions were performed. Dilutions were added to 90% confluent Vero cells, overlaid, and incubated for 5 days. Neutral red was added and viral plaques counted. The experiment was performed twice, and photos show representative plaque assays.
cells were exposed to RSV (MOI of 2) or 10 μM Nutlin-3. HTBE cells were exposed to control medium, 10 μM Nutlin-3, RSV (MOI of 2), or Nutlin-3 followed by RSV for the indicated periods of time. Supernatants were collected and analyzed for IL-6 protein by ELISA. One-way ANOVA with Bonferroni’s test for multiple comparisons indicates a statistically significant difference (+, *p < 0.01) between IL-6 protein in cells exposed to RSV and Nutlin-3 as compared with cells only exposed to RSV at all time points. Data are representative of three experiments.

performed with primers specific for the well-conserved RSV N-gene. Fig. 6 shows that the amount of viral RNA is increased ~5-fold when the cells are pretreated with 10 μM Nutlin-3. To confirm these results, a plaque assay was performed. Again, HTBE cells were exposed to RSV (MOI of 2) or 10 μM Nutlin-3 followed by RSV and incubated for 72 h. The supernatant and adherent cells were removed, combined, and sonicated, and a plaque assay was performed. Fig. 7 demonstrates visually and in graphical form that pretreatment with Nutlin-3 decreases the amount of RSV ~3-fold as measured by plaque assay. Increased p53 protein enhances RSV replication.

p53 decreases IL-6 protein

Finally, we wanted to determine the effect of p53 on inflammation in RSV infection, so we investigated whether p53 alters IL-6 protein levels. HTBE cells were exposed to control medium, 10 μM Nutlin-3, RSV (MOI of 2), or 10 μM Nutlin-3 followed by RSV infection for 24, 48, or 72 h. Supernatants were collected, and ELISA was performed to detect IL-6 protein. Fig. 8 shows that increasing the level of p53 attenuates the amount of IL-6 protein in RSV-infected cells at all time points. Similar results were seen when ELISA was performed for IL-8 protein (data not shown).

Discussion

In this study, we demonstrate that RSV decreases the amount of p53 in airway epithelial cells. The biological effect of this decreased p53 is prolonged cell survival (Fig. 9). This study is the first to show that RSV alters the amount of p53 in airway epithelial cells, that the mechanism for this is alteration of p53 via Akt and Mdm2, that RSV alteration of p53 prolongs cell survival, that p53 enhances RSV replication, and that p53 attenuates inflammation in RSV infection.

Other studies have investigated the effect of viral infection on p53. There have been no studies to date that have suggested an alteration in p53 protein in RSV infection. In fact, Marques et al. (34) saw no effect of RSV on p53 protein levels after 16 h of exposure in HepG2 cells. However, the study did find that encephalomyocarditis virus, human parainfluenza virus type 3, Sendai virus, and VVE3L (a varicella virus mutant) induced down-regulation of p53 via inhibition of the protein kinase R and the RNase L pathways. Again, this difference may reflect cell-specific responses to RSV infection or differences in the strain and infectivity of the virus.

Conversely, studies have investigated the effect of p53 on viral infection. p53 protein promotes adenoviral and CMV replication and limits poliovirus and vesicular stomatitis virus replication (34–37). We found that similar to the studies of adenovirus and CMV, p53 protein increases RSV replication. The reason for this finding is unclear and is the subject of current investigation. Multiple studies have demonstrated that RSV induces NF-κB (12, 13, 47), yet our data suggest that when p53 is maintained with Nutlin-3 in RSV infection, IL-6, which is an NF-κB-dependent protein, is reduced. Perhaps increased p53 limits the RSV-induced NF-κB host cell inflammatory response and allows for more viral replication.

The regulation of p53 is complex and controlled by many different factors (19). We have previously shown that RSV increases Akt activity and delays apoptosis (41). Other studies have shown that Akt phosphorylates Mdm2 at Ser166, and this phosphorylated Mdm2 tags p53 for ubiquitination and proteasome degradation (24–27, 30, 31). Other pathways may be involved, but our study indicates that this pathway is key because inhibition of the PI3K/Akt signaling significantly affects activation of Mdm2 and, subsequently, p53 protein levels. Its inhibition virtually eliminates phospho-Mdm2 (Ser166) and preserves p53 in RSV infection.

RSV is a clinically important pathogen, particularly for infants, patients with obstructive lung disease, and the immunosuppressed. Our study demonstrates that RSV prolongs infected cell survival by delaying cell death via posttranslational degradation of p53. The effect of this alteration in p53 protein on viral replication and host cell inflammatory responses will continue to be the subject of future studies. These observations suggest that p53, the PI3K/Akt pathway, and Mdm2 may be important targets for therapy in RSV infection.

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Disclosures
The authors have no financial conflict of interest.

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