ERK-1/2 activity is required for efficient RSV infection

Xiaoyuan Kong[a], Homero San Juan[a,b,c], Aruna Behera[a], Mark E. Peeples[d], Jerry Wu[e], Richard F. Lockey[a], Shyam S. Mohapatra[a,b,*]

[a] *The Joy McCann Culverhouse Airways Disease Research Center, Division of Allergy and Immunology, Department of Internal Medicine, MDC-19, 12901 Bruce B. Downs Blvd, Tampa, FL 33612, USA*
[b] *Department of Medical Microbiology and Immunology, University of South Florida College of Medicine and VA Hospital, 12901 Bruce B. Downs Blvd, Tampa, FL 33612, USA*
[c] *Universidad del Norte, Barranquilla, Colombia*
[d] *Rush Medical College, Chicago, IL, USA*
[e] *H. Lee Moffitt Cancer Center, Tampa, FL, USA*

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Abstract Respiratory syncytial virus (RSV) infection up-regulates the expression of genes encoding proinflammatory mediators in bronchial epithelial cells. However, the specific signaling events immediately following RSV exposure are poorly understood. Herein, we report that RSV attachment to A549 cells activates both ERK-1 and ERK-2 pathways within 5 min. Inhibition of ERK pathways significantly decreases RSV infection of these cells compared to controls. These results demonstrate that the activation of the ERK-1/2 is required in RSV-induced early gene expression.

Key words: Respiratory syncytial virus; Signal transducer and activator of transcription-1α; Extracellular signal-regulated kinase; Bronchial epithelial cell

1. Introduction

The development of novel therapies against infection by respiratory syncytial virus (RSV) is a global priority. A typically short incubation period of 1–2 days and the need to induce protective immunity in infants within the first 2 months of life is a major impediment to the development of effective vaccines against RSV infection [1–3]. Developing an antiviral prophylaxis requires a comprehensive molecular understanding of the early events of infection. In cultured epithelial cells and in the murine model, RSV infection up-regulates the expression of several cytokines and chemokines such as interleukin (IL)-1β, IL-6, IL-8, tumor necrosis factor-α, macrophage inflammatory protein-1α, RANTES, interferon-β, and intercellular adhesion molecule-1, which contribute to the acute inflammation [4–6]. Activation of transcription factors such as the nuclear factor-κB (NF-κB) and signal transducers and activators of transcription (STATs) is required for the expression of these inflammatory molecules in RSV-infected cells [7–14].

Based on the transfac promoter analysis of RSV-induced early genes identified in microarray studies, we postulated that RSV activates multiple signaling pathways in epithelial cells [15,16]. This analysis revealed binding sites for activator protein-1 suggesting that extracellular signal-regulated kinases (ERKs) may also be involved in early gene expression. ERK-1 and ERK-2 mediate specific responses to diverse stimuli, including viruses, cytokines, and growth factors and hormones [17–21]. RSV-induced production of IL-8 and RANTES is dependent on activated ERK-2 [22,23]. However, the role of ERKs in early signaling responses in RSV infection remains poorly understood. Also, whether interrupting ERK pathways can alter the course of viral infection is not known.

In this study, the results demonstrate that both ERK-1 and ERK-2 are rapidly activated in A549 cells upon RSV exposure and that ERK-1/2 activation is required for a successful RSV replication.

2. Materials and methods

2.1. Virus strains and cell culture

RSV A2 strain was obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA). In some experiments, rgRSV (an engineered RSV expressing enhanced green fluorescent protein) was used. A549 cell line, representing type II alveolar epithelial cells, was obtained from the ATCC. The cells were grown at 37°C in 5% CO_2 in RPMI medium, supplemented with 10% fetal bovine serum. Normal human bronchial epithelial cells (NHBE) were obtained from Cambrex (Walkersville, MD, USA) and grown in serum-free bronchial epithelium growth medium supplemented as indicated by the manufacturer. NHBE at passage 2 and 3 was used for the experiments.

2.2. Reagents

Heparin and heparinase were obtained from Sigma-Aldrich (St. Louis, MO, USA). AG490 (Janus kinase (JAK) inhibitor), PD98059 (MAPK/ERK kinase-1 (MEK-1) inhibitor), were obtained from Calbiochem (San Diego, CA, USA). Polyclonal anti-phospho antibodies or antibodies to STAT-1, ERK, or IκBα were obtained from Cell Signaling (Beverly, MA, USA). Fluorescein isothiocyanate (FITC)-labeled anti-RSV N mouse monoclonal antibody was obtained from Chemicon (Temecula, CA, USA).

2.3. Treatment with inhibitors

A549 cells were incubated with AG490 (50 μM) or PD98059 (80 μM) for 4 h at 37°C and then infected with RSV. At 30 min, 60 min, and 240 min following infection, the cells were washed with phosphate-buffered saline (PBS) and the total protein extracted (see Section 2.7). In some experiments, subconfluent NHBE cells were exposed to AG490 (50 μM) and PD98059 (80 μM) for 2 h at 37°C prior to rgRSV infection.

*Corresponding author. Fax: (1)-813-974 8907.
E-mail address: smohapat@hsc.usf.edu (S.S. Mohapatra).

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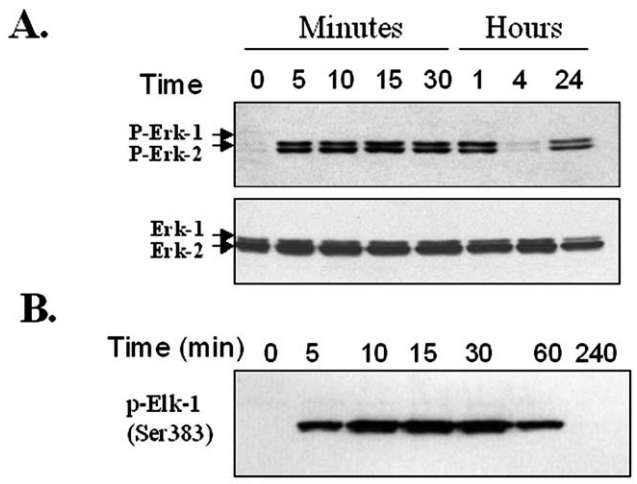

Fig. 1. Exposure to RSV activates ERK-1 and ERK-2 in A549 cells. A: RSV exposure induces rapid phosphorylation of ERK-1/2 in A549 cells. Western blots of total proteins extracted from uninfected cells and RSV-infected cells for 5, 10, 15 or 30 min and for 1, 4, or 24 h probed with anti-phospho ERK-1/2, stripped, and then re-probed with anti-ERK-1/2. B: Activity assay of ERK-1/2 immunoprecipitated from 200 μg of total proteins extracted from uninfected cells and sucrose-purified RSV-infected cells.

2.4. Heparin and heparinase treatment

Either A549 cells or RSV was incubated with 1000 U/ml heparin at 37°C for 2 h before proceeding to infection for 30 min at 37°C. After treatment, the cells were washed with PBS and total protein was extracted. In some experiments, heparinase (10 U/ml) was used following the same protocol explained above.

2.5. RSV purification and plaque assay

RSV was PEG-precipitated and purified on two successive sucrose density gradients as described [16]. Plaque assay was used to determine infectious viral titers of purified fractions, as described [14,16]. Because of the possible contamination of ATCC RSV with human adenovirus type 1, we tested our purified preparations for the presence of adenovirus by polymerase chain reaction using specific primers that amplify a product of 213 bp and previously reported by Cameron et al. [24] and found that our preparations were not contaminated with adenovirus.

2.6. Immunofluorescence

Acetone-fixed cells were stained with FITC-labeled anti-RSV monoclonal antibodies, as described [16]. RSV positive cells (green fluorescence) were counted randomly from 15 different spots and from two or three different slides for each treatment group and the percentages of infected cells were plotted for wild type (WT) and dominant negative (DN) mutant cells.

2.7. Immunoblotting

Whole cell protein extracts were prepared by lysing cells in 50 mM HEPES, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 10% glycerol, 0.5% NP-40, 0.1 mM phenylmethylsulfonyl fluoride, 2.5 μg/ml leupeptin, 0.5 mM NaF, and 0.1 mM sodium vanadate. 50 μg whole cell protein was subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) in 10% polyacrylamide and then transferred onto a nitrocellulose membrane, which was processed following the (insert) manufacturers' instructions. The antibody reactions were detected by enhanced chemiluminescence using Lumiglo (Cell Signaling Technology).

2.8. Mitogen-activated protein (MAP) kinase assay

A549 cells were infected with purified RSV and harvested at various times post infection, as specified. ERK-1/2 were immunoprecipitated and their kinase activity was tested using an Elk-1 fusion protein (Cell Signaling Technology, Beverly, MA, USA). Elk-1 phosphorylation at Ser383 was determined by Western blot.

2.9. DNA transfection

WT and DN mutant MEK-1 has been described previously [25]. In addition, plasmid DNA of WT and mutant STAT-1α was also used in other experiments. A549 cells grown in a 100 mm tissue culture plate to 70–80% confluence were transfected with 12 μg of DNA and 35 μl of Lipofectin (Life Technology) for 15 h at 37°C following the guidelines of the instruction manual. Cells were then infected with RSV for 30 min at 37°C before cell protein was extracted.

2.10. Statistical analysis

Statistical significance was analyzed using Student's *t*-test for paired observations. A $P < 0.05$ level of significance (two-sided) was utilized throughout.

3. Results

3.1. RSV exposure induces ERK-1 and ERK-2 phosphorylation in A549 epithelial cells

RSV infection induced the increased phosphorylation of ERK-1 and ERK-2 in A549 cells, as demonstrated by Western blotting using corresponding antibodies and phospho-antibodies (Fig. 1A). The phosphorylation of the ERK-1 and ERK-2 was induced at 5 min post RSV exposure and continued until 2 h post RSV infection. At 4 h following exposure to RSV, ERK-1 and ERK-2 phosphorylation was not detected; however, ERK-1 and ERK-2 phosphorylation reappeared at 24 h after infection. Assays of A549 cells infected with sucrose-purified RSV at a multiplicity of infection of 1 confirmed that the rapid activation of ERK-1/2 is due to RSV per se and not to cytokines or growth factors (Fig. 1B). The rapid activation of ERK-1/2 suggested that RSV attachment is responsible for triggering the signaling. In fact, heparin, a known inhibitor of RSV attachment [26,27], abolished the RSV-induced activation of ERK-1/2 (Fig. 2).

3.2. ERK-1/2 phosphorylation is linked with activation of STAT-1α and of IκB

A549 cells exhibit activation of STAT-1α and STAT-3 and NF-κB following exposure to RSV [16]. Specific inhibitors to STAT-1α and ERK-1/2 pathways were used to determine if blocking one pathway affects the phosphorylation of both of them and IκBα (Fig. 3A,B). A decrease in RSV-induced STAT-1α phosphorylation and in IκBα phosphorylation in cells pre-incubated with AG490 suggests a possible interaction between these two pathways. Although AG490 failed to inhibit ERK activation, PD98059 significantly affected the

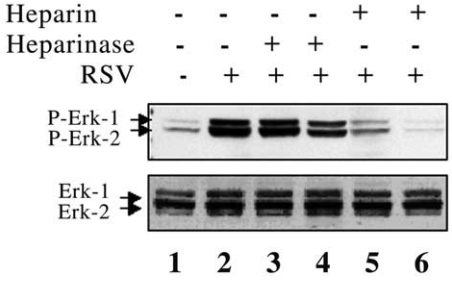

Fig. 2. Effect of heparin and heparinase on RSV-induced ERK-1/2 activation. Proteins were isolated from untreated cells (lane 1) or cells infected with RSV for 30 min (lanes 2–6) after pretreatment of cells for 30 min with heparinase (lane 3) or heparin (lane 5), or infected with virus pretreated with heparinase (lane 4) or heparin (lane 6) prior to addition to the cells. Proteins were separated by SDS–PAGE, blotted and probed with anti-phospho ERK-1/2 and then reprobed with anti-ERK-1/2.

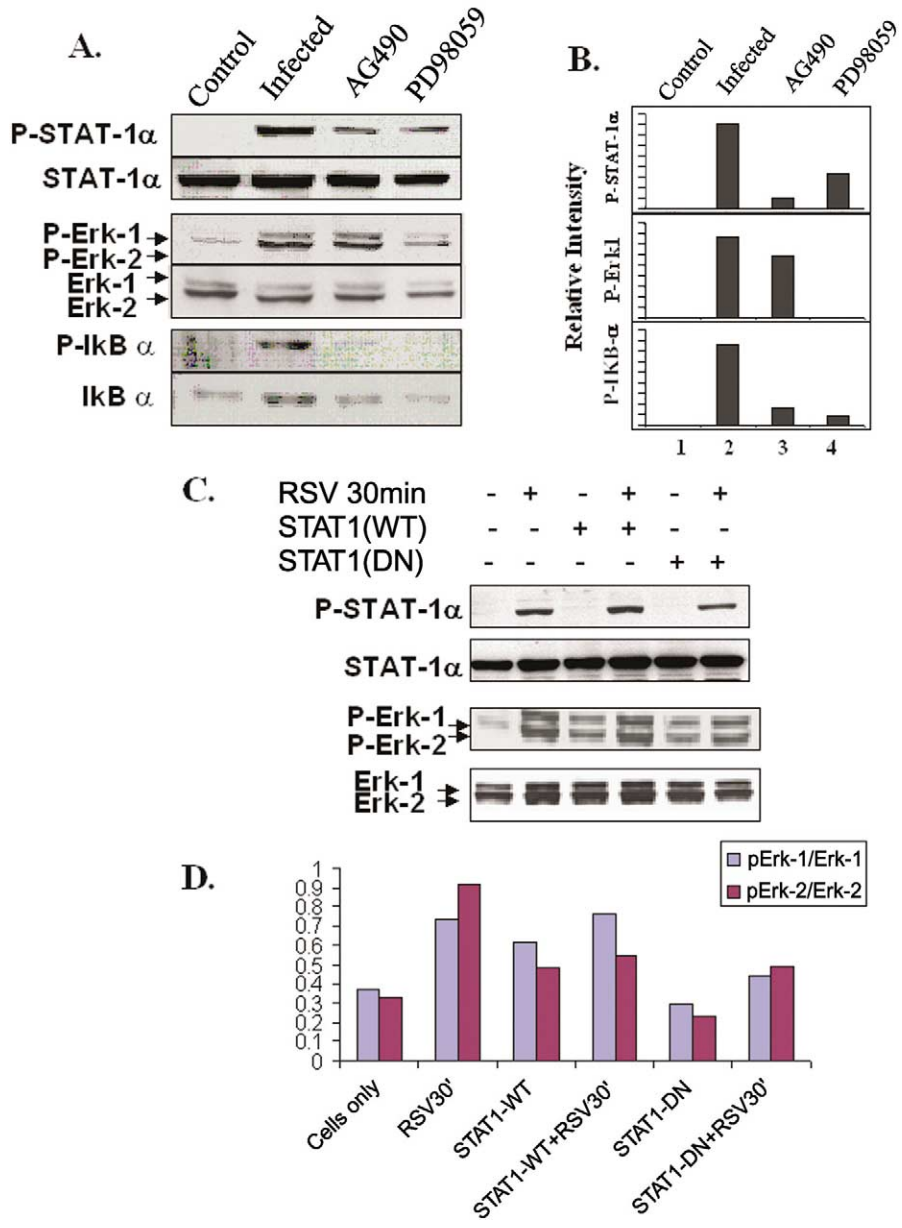

Fig. 3. Effects of inhibitors of phosphorylation of STAT-1α and ERK-1/2. A: AG490- or PD98059-treated A549 cells were infected with RSV for 15 min. Total proteins were Western-blotted using specific anti-phospho antibodies to STAT-1α, ERK-1/2 or IκBα. Lane 1: mock-infected, lanes 2–4: RSV infected for 30 min, lane 3: cells treated with 50 μM of AG490 prior to RSV infection, lane 4: cells treated with 25 μM of PD98059 for 4 h prior RSV infection. B: The bands were quantified by densitometry and the relative intensity of phosphoSTAT-1α, phospho-ERK and phospho-IκBα with respect to uninfected control (lane 1) was determined. The experiment was repeated twice and the result of a representative experiment is shown. C: Effect of DN STAT-1α on ERK activation. A549 cells were co-transfected with RL-TK plasmid and either the WT (pRc/CMV-STAT-1α) (lanes 3 and 4) or DN mutant (pRc/CMV-STAT-1α) (Y701F) (lanes 5 and 6) and were infected with RSV for 30 min after 24 h of transfection. Total proteins were extracted and Western-blotted using antibodies to phospho-STAT-1α, phospho-ERK-1/2 and reprobed with antibodies to STAT-1α or ERK-1/2. D: The bands were quantified by densitometry and the relative intensity of phospho-ERK and ERK was determined in each band. The experiment was repeated twice and the result of a representative experiment is shown.

phosphorylation of both STAT-1α and IκB (Fig. 3B). To determine if phospho-STAT-1α is required for the increase in phospho-ERK-1/2 seen in RSV infection, cells were transfected with plasmids encoding either WT- or DN-STAT-1α. Both STAT-1α and ERK-1/2 phosphorylation decreased in cells transfected with DN STAT-1α compared to WT STAT-1α in response to RSV infection (Fig. 3C). Together, these results suggest that both STAT-1α and ERK-1/2 participate in the phosphorylation of each other as well as in phosphorylation of IκBα in the context of RSV infection.

3.3. Inhibition of ERKs attenuates RSV infection

To examine whether ERKs play a specific role in RSV infection, A549 cells were treated with PD98059 and then infected with RSV. After 24 h of RSV infection, there was a decrease in the percentage of infected cells treated with the inhibitor compared to those treated with control, suggesting that the ERK pathway plays a role during RSV infection (Fig. 4A). Moreover, the number of infected cells in DN MEK-1-transfected A549 cells was reduced in $>60\%$ compared to those transfected with a WT construct (Fig. 4B). These results

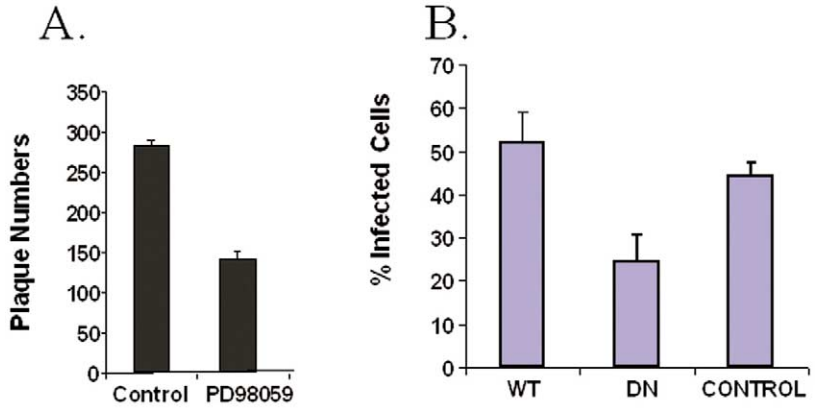

Fig. 4. Inhibition of ERK-1/2 activation decreases RSV infection of A549 cells. A: PD98059- or DMSO-treated A549 cells were infected with RSV. Supernatants were collected from control and treated cells at 72 h. The RSV titers were determined using a HEP-2 cell-based plaque assay as described. B: Single cell immunofluorescent assays of A549 cells co-transfected with RL-TK plasmid and either the WT (pCMV-HA MEK) or DN mutant (pCMV-HA MEKm). Twenty-four hours after transfection, A549 cells were infected with RSV for 90 min and 24 h after the % of infected cells was determined with FITC-RSV antibody.

indicate that ERK-1/2 pathway plays a role during the infection.

3.4. *Requirement of STAT and ERK-1/2 activation for successful RSV infection of primary NHBE cells*

To determine if both JAK-STAT-1α and ERK pathways are required for a successful RSV infection in primary NHBE cells, they were exposed to either AG490 or PD98059 before being infected with rgRSV. Exposure of NHBE to AG490 or PD98059 caused a significant reduction in the number of infected cells (Fig. 5). These results strongly suggest that JAK-STAT-1α and ERK-1/2 are required for a successful RSV infection in bronchial epithelial cells.

4. Discussion

This report provides evidence in support of a rapid and transient activation of ERK-1/2 in RSV-infected A549 cells. Such activation appears to be dependent on the presence of phospho-STAT-1α and is required for both STAT-1α and IκBα phosphorylation. Results of studies using DN mutants and specific inhibitors indicate that ERK-1/2 activation is im-

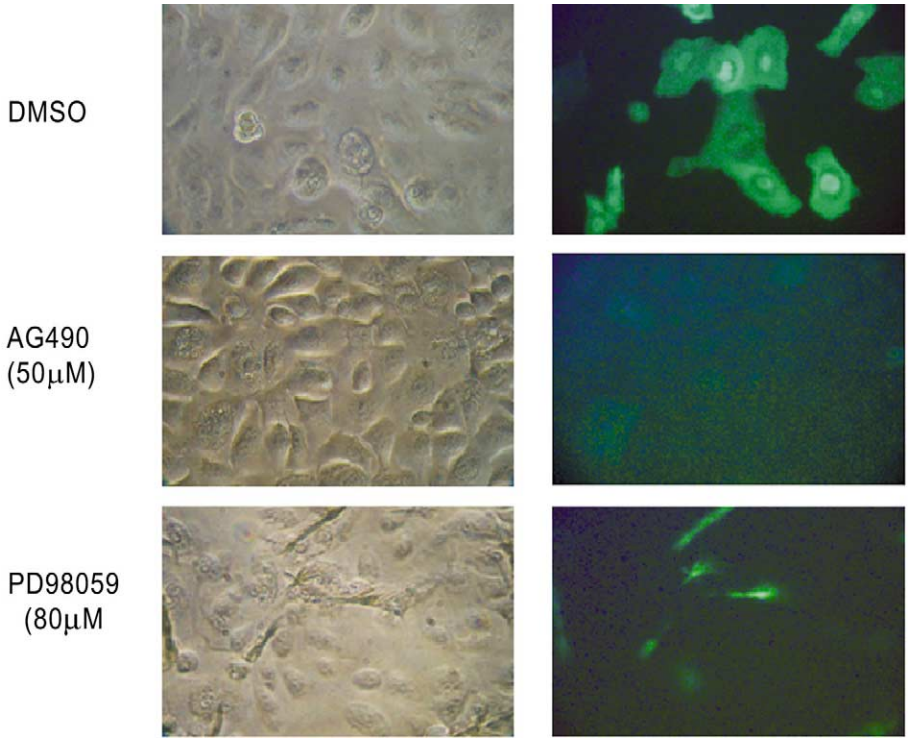

Fig. 5. Effects of (insert) STAT and ERK-1/2 inhibitors on RSV infection of NHBE cells. NHBE cells were treated with either AG490 (50μM) or PD98059 (80μM) for 2 h. DMSO was used as a mock control. After inhibitor removal, cells were infected with rgRSV for 2 h. Then, rgRSV was removed and growth medium with inhibitors was added to the cells for 16 h before fluorescent images as well as light transmission images were taken.

portant for successful RSV infection. Also, ERK-1 and ERK-2 activation constitutes an integral pathway of early signaling cascades involved in RSV infection.

RSV was reported to activate ERK-2 at the early stages of infection (10 min after RSV exposure) and that such activation was required for IL-8 production [22]. However, given that RSV supernatant contains a number of cytokines and chemokines which may activate ERK-2, the significance of this report was unclear. In this study we confirm this report by testing ERK activity in A549 cells exposed to sucrose-purified RSV, and present evidence of ERK-1 activation. The rapid activation of ERK-1 and ERK-2 is consistent with our previous finding that a number of genes are activated within 30 min of RSV infection [16].

The rapid activation of ERK-1/2 after RSV infection led to the hypothesis that RSV attachment causes ERK signaling, which is supported by the fact that the heparin- or heparinase-treated RSV did not induce ERK-1/2 phosphorylation. Both RSV-G and -F proteins were reported to bind to heparan sulfate on the cell surface [28–30]. Interestingly, however, the treatment of A549 cells with heparinase, which abrogated STAT-1α phosphorylation, did not affect the phosphorylation of ERK-1/2. The reason for the segregation of signaling in heparinase-treated A549 cells after RSV exposure is unclear.

The fact that PD98059 and DN STAT-1α inhibited the phosphorylation of STAT and ERK pathways, respectively, suggests the existence of cross-talk between these pathways during RSV infection. Previously, Stancato et al. have reported that STAT-1 may scaffold signaling components required for activation of the Raf/MEK/ERK signaling cascade [31].

Notably, STAT-1α activation is also required for NF-κB activation in infected cells evidenced by AG490 effect upon IκBα phosphorylation. This agrees with a previous report in which the JAK inhibition prevented the degradation of IκBα and blocked the translocation of NF-κB p65 into the nucleus [32].

The evidence that inhibiting ERK significantly decreases RSV infection in A549 and NHBE cells suggests that this pathway may be important in turning on genes for virus replication and/or morphogenesis. To rule out the possibility that this effect on viral replication is due to diminished cell proliferation, viable cells were enumerated at 12, 24, 48, and 72 h after treatment with inhibitors. The results showed no significant difference in cell numbers between treated and untreated cells (data not shown).

Involvement of ERKs in virus infection is not unprecedented. ERK pathway activation is required at different levels during HIV-1 infection. The association of ERK-2 with different HIV-1 strains derived from T cells and promonocytic cells has been reported [33]. ERK phosphorylates several HIV-1 proteins important for viral replication, such as Vif, Rev, Tat, p17(Gag), and Nef [34]. HIV-1 infectivity is enhanced when cells are treated with ERK stimulators or when cells are transfected with activated forms of Ras, Raf, and MEK molecules [34]. In addition, specific inhibitors of the ERK pathway such as PD98059 reduced the infectivity of HIV-1 virions [34]. Finally, HIV-1 infection of brain microvascular endothelia is dependent on the activation of the ERK pathway and inhibition of this pathway repressed virus entry [35].

In the same line, the ERK activation pathway is also required during the early stages of influenza infection. Inhibiting the normal process that conveys the activation of ERK impairs influenza virus replication because it affects the early stage of nuclear export of viral ribonucleoprotein, probably as a result of impaired activity of the viral nuclear export protein [36,37].

In addition to impairing viral infection, there are other instances in which virus propagation could be affected by blocking the function of the ERK pathway. In the case of Borna disease virus, the ERK pathway seems to be required for the virus to spread to neighboring cells [38]. Regarding viral pathogenesis, ERK activation has been implicated in the development of Visna virus infection-associated encephalitis, and if this pathway is inhibited, viral replication is abolished secondary to a defect in Rev function [39]. Present evidence also indicates that hepatitis virus B, C, and E activate ERK through different means and take advantage of such activation as a strategy for their own survival [40]. In the early stages of infection, vaccinia virus triggers the activation of ERK through an unknown mechanism. Interestingly, when cells are exposed to inhibitors of the ERK pathway, the viral multiplication is impaired [41].

Taken together, all of these previous reports highlight the role the ERK pathway in the efficient infection and replication of certain virus species as well as how evolution has endowed these viruses to develop mechanisms that allowed them to sequester host cell signaling pathways. This report establishes for the first time that STAT-1α, and ERK-1/2 are required for successful RSV infection. The demonstration that RSV infection is inhibited in primary NHBE cells confirms the generality of the signaling requirement seen in A549 cells.

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References

[1] Simoes, E.A. (1999) Lancet 354, 847–852.
[2] Hussell, T. and Openshaw, P. (1999) Curr. Opin. Microbiol. 2, 410–414.
[3] Coffin, S.E. and Offit, P.A. (1997) Adv. Pediatr. Infect. Dis. 13, 333–348.
[4] Becker, S., Koren, H.S. and Henke, D.C. (1993) Am. J. Respir. Cell Mol. Biol. 8, 20–27.
[5] Elias, J.A., Zheng, T., Einarsson, O., Landry, M., Trow, T., Rebert, N. and Panuska, J. (1994) J. Biol. Chem. 269, 22261–22268.
[6] Garofalo, R., Mei, F., Espejo, R., Ye, G., Haeberle, H., Baron, S., Ogra, P.L. and Reyes, V.E. (1996) J. Immunol. 157, 2506–2513.
[7] Bitko, V., Velazquez, A., Yang, L., Yang, Y.C. and Barik, S. (1997) Virology 232, 369–378.
[8] Garofalo, R., Sabry, M., Jamaluddin, M., Yu, R.K., Casola, A., Ogra, P.L. and Brasier, A.R. (1996) J. Virol. 70, 8773–8781.
[9] Fiedler, M.A., Wernke-Dollries, K. and Stark, J.M. (1996) J. Virol. 70, 9079–9082.
[10] Jamaluddin, M., Casola, A., Garofalo, R.P., Han, Y., Elliott, T., Ogra, P.L. and Brasier, A.R. (1998) J. Virol. 72, 4849–4857.
[11] Bitko, V. and Barik, S. (1998) J. Virol. 72, 5610–5618.
[12] Chini, B.A., Fiedler, M.A., Milligan, L., Hopkins, T. and Stark, J.M. (1998) J. Virol. 72, 1623–1626.
[13] Casola, A., Garofalo, R.P., Jamaluddin, M., Vlahopoulos, S. and Brasier, A.R. (2000) J. Immunol. 164, 5944–5951.
[14] Behera, A.K., Matsuse, H., Kumar, M., Kong, X., Lockey, R.F. and Mohapatra, S.S. (2001) Biochem. Biophys. Res. Commun. 280, 188–195.

[15] Quandt, K., Frech, K., Karas, H., Wingender, E. and Werner, T. (1995) Nucleic Acids Res. 23, 4878–4884.
[16] Kong, X. et al. (2003) Biochem. Biophys. Res. Commun. 306, 616–622.
[17] Li, J.D., Feng, W., Gallup, M., Kim, J.H., Gum, J., Kim, Y. and Basbaum, C. (1998) Proc. Natl. Acad. Sci. USA 95, 5718–5723.
[18] Bruder, J.T. and Kovesdi, I. (1997) J. Virol. 71, 398–404.
[19] Jahnke, A. and Johnson, J.P. (1994) FEBS Lett. 354, 220–226.
[20] Garcia, R. et al. (1997) Cell Growth Differ. 8, 1267–1276.
[21] Improta, T. and Pine, R. (1997) Cytokine 9, 383–393.
[22] Chen, W., Monick, M.M., Carter, A.B. and Hunninghake, G.W. (2000) Exp. Lung Res. 26, 13–26.
[23] Pazdrak, K., Olszewska-Pazdrak, B., Liu, T., Takizawa, R., Brasier, A.R., Garofalo, R.P. and Casola, A. (2002) Am. J. Physiol. Lung Cell Mol. Physiol. 283, L364–L372.
[24] Cameron, R., Buck, C., Merrill, D. and Luttick, A. (2003) Virus Res. 92, 151–156.
[25] Catling, A.D., Schaeffer, H.J., Reuter, C.W., Reddy, G.R. and Weber, M.J. (1995) Mol. Cell. Biol. 15, 5214–5225.
[26] Feldman, S.A., Hendry, R.M. and Beeler, J.A. (1999) J. Virol. 73, 6610–6617.
[27] Bourgeois, C., Bour, J.B., Lidholt, K., Gauthray, C. and Pothier, P. (1998) J. Virol. 72, 7221–7227.
[28] Karger, A., Schmidt, U. and Buchholz, U.J. (2001) J. Gen. Virol. 82, 631–640.
[29] Feldman, S.A., Crim, R.L., Audet, S.A. and Beeler, J.A. (2001) Arch. Virol. 146, 2369–2383.
[30] Techaarpornkul, S., Collins, P.L. and Peeples, M.E. (2002) Virology 294, 296–304.
[31] Stancato, L.F., Yu, C.R., Petricoin, E.F. and Larner 3rd, A.C. (1998) J. Biol. Chem. 273, 18701–18704.
[32] Cruz, M.T., Duarte, C.B., Goncalo, M., Carvalho, A.P. and Lopes, M.C. (2001) Nitric Oxide 5, 53–61.
[33] Cartier, C. et al. (1997) J. Virol. 71, 4832–4837.
[34] Yang, X. and Gabuzda, D. (1999) J. Virol. 73, 3460–3466.
[35] Liu, N.Q. et al. (2002) J. Virol. 76, 6689–6700.
[36] Pleschka, S., Wolff, T., Ehrhardt, C., Hobom, G., Planz, O., Rapp, U.R. and Ludwig, S. (2001) Nat. Cell Biol. 3, 301–305.
[37] Ludwig, S., Planz, O., Pleschka, S. and Wolff, T. (2003) Trends Mol. Med. 9, 46–52.
[38] Planz, O., Pleschka, S. and Ludwig, S. (2001) J. Virol. 75, 4871–4877.
[39] Barber, S.A., Bruett, L., Douglass, B.R., Herbst, D.S., Zink, M.C. and Clements, J.E. (2002) J. Virol. 76, 817–828.
[40] Panteva, M., Korkaya, H. and Jameel, S. (2003) Virus Res. 92, 131–140.
[41] de Magalhaes, J.C. et al. (2001) J. Biol. Chem. 276, 38353–38360.