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Mitochondrial reactive oxygen species modulate innate immune response to influenza A virus in human nasal epithelium

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ABSTRACT

The innate immune system of the nasal epithelium serves as a first line of defense against invading respiratory viruses including influenza A virus (IAV). Recently, it was verified that interferon (IFN)-related immune responses play a critical role in local antiviral innate immunity. Reactive oxygen species (ROS) generation by exogenous pathogens has also been demonstrated in respiratory epithelial cells and modulation of ROS has been reported to be important for respiratory virus-induced innate immune mechanisms. Passage-2 normal human nasal epithelial (NHNE) cells were inoculated with IAV (WS/33, H1N1) to assess the sources of IAV-induced ROS and the relationship between ROS and IFN-related innate immune responses. Both STAT1 and STAT2 phosphorylation and the mRNA levels of IFN-stimulated genes, including *Mx1*, *2,5-OAS1*, *IFIT1*, and *CXCL10*, were induced after IAV infection up to three days post infection. Similarly, we observed that mitochondrial ROS generation increased maximally at 2 days after IAV infection. After suppression of mitochondrial ROS generation, IAV-induced phosphorylation of STAT and mRNA levels of IFN-stimulated genes were attenuated and actually, viral titers of IAV were significantly higher in cases with scavenging ROS. Our findings suggest that mitochondrial ROS might be responsible for controlling IAV infection and may be potential sources of ROS generation, which is required to initiate an innate immune response in NHNE cells.

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Recently, it was confirmed that interferon (IFN) is primarily responsible for protection against viral invaders in the respiratory tract and IFN-related immune responses play an important role in local antiviral innate immunity (Hansel et al., 2013). Type I and III IFNs trigger strikingly similar responses in target cells resulting from phosphorylation of transcription factors STAT1 and STAT2, which facilitates intracellular induction of IFN-stimulated genes (ISGs) (Schneider et al., 2014). These ISGs encode proteins such as Mx1, OAS or IFIT and, reportedly, rapid production of ISGs could actually be associated with controlling viral infections and degradation of viral particles (Schneider et al., 2014).

Reactive oxygen species (ROS) are believed to be inevitable toxic by-products that cause cellular damage or stress (Mittal et al., 2014). However, mounting evidence suggests the generation of ROS to be an important component of the host's arsenal against invading microorganisms (Kotsias et al., 2013; Kim et al., 2011). Mitochondria are dynamic double-membrane-bound organelles that are involved in a wide range of cellular processes and. mitochondria are a significant source of ROS that modulate several signaling pathways (Cloonan and Choi, 2012; West et al., 2011). Until now, it has been assumed that ROS are primarily produced by NADPH oxidase systems in non-phagocytic cells, such as respiratory epithelial cells (Kim et al., 2011). However, it has also been suggested that mitochondria are involved in oxidative phosphorylation and ROS generation in respiratory inflammatory diseases (West et al., 2011). Although mitochondrial oxidative metabolism also seems to be a critical source of cellular ROS in many eukaryotic cells, it is not clear whether mitochondrial ROS facilitate immune signaling; additional research is needed to delineate the mechanisms of mitochondrial ROS in the nasal epithelium.

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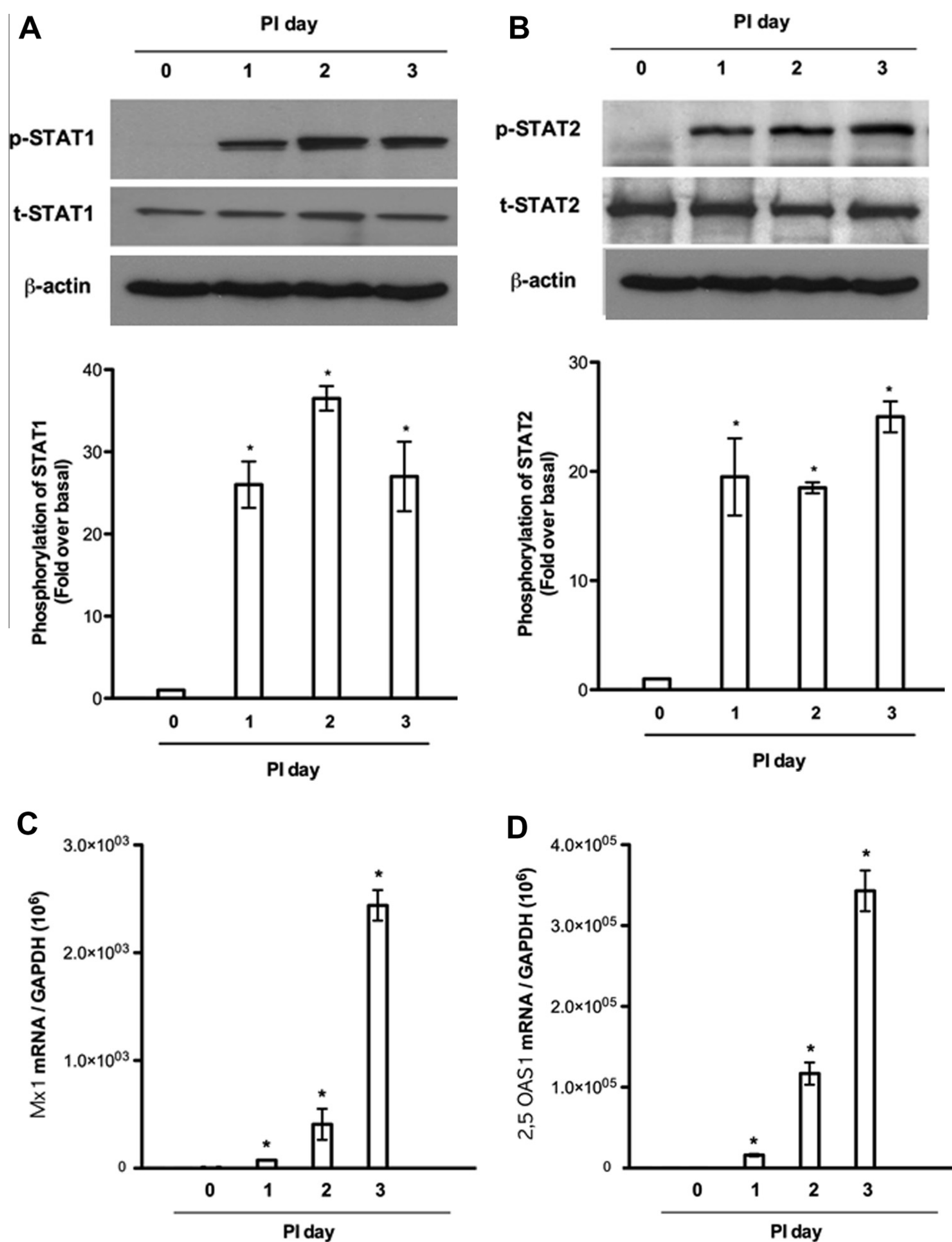

Fig. 1. IFN-related immune response is induced after IAV infection in NHNE cells. NHNE cells from five healthy volunteers were inoculated with WS/33 (H1N1) for 1, 2, and 3 days at MOI 1. Western blot analysis and densitometry revealed that phosphorylation of STAT1 (A) and STAT2 (B) is enhanced from until 3 dpi. Cell lysates were assayed using real-time PCR. The results show that Mx1 (C), 2,5-OAS1 (D), IFIT1(E) and CXCL10 (F) mRNAs are induced after IAV infection from 1 dpi and are significantly higher until 3 dpi. The protein levels of Mx1, 2,5-OAS1, IFIT1 (G) and secreted CXCL10 (H) were induced from 1 day after IAV infection. The Western blot results presented here are representative of five independent experiments. PCR and ELISA results are presented here as the mean ± standard deviation (SD) from five independent experiments ($^*p < 0.05$ when compared to mRNA levels of mock-infected cells).

In this study, we investigated whether mitochondrial ROS induce antiviral immune response in the nasal epithelium and described the specific roles of mitochondria in IFN-related innate immune system, subsequent ROS generation against influenza A virus (IAV) infection.

Previously, we performed gene expression analysis using the Affymetrix GeneChip Human Gene 1.0 ST Array (Santa Clara, CA, USA) with total RNA from NHNE cells after 2 days of IAV infection. The analysis indicated that 11 genes are active in IFN-mediated immune responses, including four ISGs, *MX1*, *OAS1*, *IFIT1* and *CXCL10*, and their transcription factors STAT1 and STAT2 (Kim et al., 2013). To analyze this in greater detail, we obtained NHNE cells from five healthy subjects and infected cells with WS/33 (H1N1) at MOI 1. The cell lysates were harvested one, two, and three days post infection (dpi). We then measured the phosphorylation of STAT1 and STAT2 using Western blot analysis, and the results showed that both STAT1 and STAT2 phosphorylation increased significantly from 1 dpi (STAT1: 26.3-fold over PI 0 day, STAT2: 19.5-fold over PI 0 day), and was maintained for up to 3 dpi (STAT1: 27.6-fold over PI 0 day, STAT2: 25.0-fold over PI 0 day, Fig. 1A and B).

Next, we determined the levels of transcription for ISGs, including *Mx1*, *2,5 OAS1*, *IFIT1*, and *CXCL10* after 1, 2, and 3 days post IAV infection using real-time PCR. Increased Mx1, 2,5 OAS1, IFIT1, and CXCL10 gene expression was observed from 1 dpi and peaked at 3 dpi (Mx1: 2440.1 ± 141.4, 2,5 OAS1: 343024.35 ± 25196.1, IFIT1: 286054.3 ± 27535.9, CXCL10: 1,760,637 ± 280324.5, Fig. 1C–F). We then measured the protein expression of Mx1, 2,5

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