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A systems approach to understanding human rhinovirus and influenza virus infection

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ABSTRACT

Human rhinovirus and influenza virus infections of the upper airway lead to colds and the flu and can trigger exacerbations of lower airway diseases including asthma and chronic obstructive pulmonary disease. Novel diagnostic and therapeutic targets are still needed to differentiate between the cold and the flu, since the clinical course of influenza can be severe while that of rhinovirus is usually more mild. In our investigation of influenza and rhinovirus infection of human respiratory epithelial cells, we used a systems approach to identify the temporally changing patterns of host gene expression from these viruses. After infection of human bronchial epithelial cells (BEAS-2B) with rhinovirus, influenza virus or co-infection with both viruses, we studied the time-course of host gene expression changes over three days. We modeled host responses to these viral infections with time and documented the qualitative and quantitative differences in innate immune activation and regulation.

Introduction

Rhinovirus and influenza virus are among the leading causes of infections of the upper respiratory tract and epithelial cells are usually the initial targets of these viral infections (Eccles, 2005; Nichols et al., 2008). The patterns of host responses evoked by each virus are beginning to emerge. Recent in vitro studies have identified overall changes as well as key host factors during influenza virus infection (Watanabe et al., 2010; Shapira et al., 2009). In vivo studies on subjects who were experimentally infected with rhinovirus (Proud et al., 2008) or influenza (Woods et al., 2013) have identified host-derived changes in gene expression and biological processes, and some of the findings may have diagnostic potential (Zaas et al., 2013). Smith et al. (2012) described 67 host biological pathways that were up-regulated in common by seven different respiratory viruses including rhinovirus and influenza by reviewing published data from a number of laboratories. Zaas et al. (2009) developed gene expression signatures from peripheral blood cells that distinguished individuals experimentally infected with either rhinovirus, influenza virus, or respiratory syncytial virus and more recently this group described a RT-PCR based gene expression signature from blood cells that could detect and discriminate between two types of influenza virus in experimentally infected subjects (Zaas et al., 2013).

While classifiers of single virus infections would be clinically valuable, especially if they are derived from multiple cell types and different stages of infection, a given individual may be infected with more than one virus. Indeed, Greer et al. (2009) and Casalegno et al. (2010) reported that detection of rhinovirus was associated with a reduced probability of detecting influenza virus in clinical samples. In another co-infection study, 30 samples (13%) from individuals infected with the influenza virus were positive by a PCR assay for 31 viral co-pathogens of which the most prominent was rhinovirus (61%) (Esper et al., 2011). While co-infection may be common, more recent studies found that disease severity and clinical course were essentially similar in patients with co-infections compared to patients with single infections (Choi et al., 2015; Asner et al., 2014; Blyth et al., 2013; Navarro-Mari et al., 2012).

Several groups have studied gene expression changes in epithelial cells after infection in vitro with rhinovirus [see Gene Expression Omnibus (accession GSE55271, Schuler et al., 2014; accession GSE28904, Naim et al. unpublished; accession GSE27973, Proud et al., 2012) or influenza virus (Li et al., 2011; Mitchell et al., 2013; Josset et al., 2014; see also accession GSE48466, Gerlach et al., 2013)], but co-infection with both viruses in a well-controlled in vitro system

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has not been explored nor has an extensive time-course of host cell transcriptional changes following viral infection of a respiratory cell been investigated. Thus in order to better understand the host respiratory cellular transcriptional response to either rhinovirus, influenza virus and co-infection with both viruses, we performed a detailed time-course analysis of transcriptional changes following infection of the human bronchial epithelial cell line (BEAS-2B). From this analysis, we detail the changes in host cell biological processes and transcription that result from infection, including changes that occur with co-infection of influenza and rhinovirus.

Results and discussion

Virus infections and derivation of differentially expressed genes

We evaluated the productivity, timing, and specificity of virus infection by measuring two mRNAs specific for influenza virus, the influenza A matrix protein 2 (M2) and non-structural protein 1 (NS; accession Z21498), and an amplicon that was specific for the rhinovirus 16 genome (accession EU096003). As shown in Fig. 1A, viral specific gene expression was only observed in the appropriate samples and changes in the expression of the viral specific genes demonstrated that productive infections occurred. To confirm the expected host cellular response to rhinovirus and influenza virus infection, we evaluated the temporal transcriptional profiles of 3 genes that were previously found to be up-regulated during infection of epithelial cells by these viruses: ICAM1, the receptor for rhinovirus 16 (Papi and Johnson, 1999); CXCL10, a chemokine for monocytes and macrophages (Spurrell et al., 2005); and TLR3, an intracellular receptor for double-stranded viral RNA (Hewson et al., 2005; Guillot et al., 2005). Steady-state levels of these mRNAs were strongly induced by viral infection as expected, but with several virus specific differences as shown in Fig. 2. CXCL10 and TLR3 mRNA levels peaked at 24 h, earlier after influenza infection compared to rhinovirus infected cells, while ICAM1 mRNA levels were higher after rhinovirus infection, raising the possibility that subtle viral infection specific differences in regulation of these genes exist. As a final evaluation of the robustness of viral infection and the host cell response to viral infection, we compared the mRNA and protein changes for three cytokines that were previously shown to change during respiratory virus infection in vitro as well as in vivo. As shown in Supplementary File 3 steady-state mRNA levels for IL-6, CXCL10 and CCL5 peaked at 48–60 h post-infection (depending on the virus infection) while the levels of the corresponding proteins secreted into the medium peaked 12–48 h later as expected for simple transcriptional regulation. Finally, as a general validation of the microarray results we reconfirmed the expression of three host mRNAs that increased during the infection time-course (DDX60, IFI27, SCD) and three mRNAs that decreased during the time-course (CBX5, FBN2, EPCAM). Messenger RNA levels measured by RT-PCR corresponded closely with the microarray results for each of these mRNAs Supplementary File 4.

For our analysis of the microarray data, differentially expressed genes from the BEAS-2B cells infected with rhinovirus (RV), influenza virus (IV) or both viruses (RV+IV) were derived by comparison with mock-infected cells. Hierarchical cluster analysis (not shown) revealed that relatively few host cell genes were differentially expressed by 8 h post-infection. But by 24 h, the expression of hundreds of genes was changing. Slightly more genes were differentially expressed after IV infection compared to RV infection at 24–36 h post-infection, but this pattern was reversed by 48 h post-infection and at later times (Fig. 1B and C). There were more up-regulated than down-regulated genes, and down-regulated genes tended to lag up-regulated genes. Fig. 1D compares the total number of unique host genes for each virus infection. For example, 310 genes were up-regulated and 141 were down-regulated in all three virus infections, while RV, IV and RV+IV specific and commonly up- and down-regulated genes were also observed. Supplementary File 5 lists the differentially expressed genes shown in Fig. 1D. These differentially expressed genes were subsequently used for enrichment analysis and for the derivation of pathways and networks.

The changing pattern of host cell responses after infection

Next, using the differentially up-regulated host genes from each infection and at each time point, we derived a time-series map of the changing pattern of biological processes, shown in Fig. 3. This analysis demonstrated that some host cell pathways were common to both viruses, including the RIG-I-like receptor (Entrez id: DDX58) signaling pathway which senses cytoplasmic viral RNA, and other

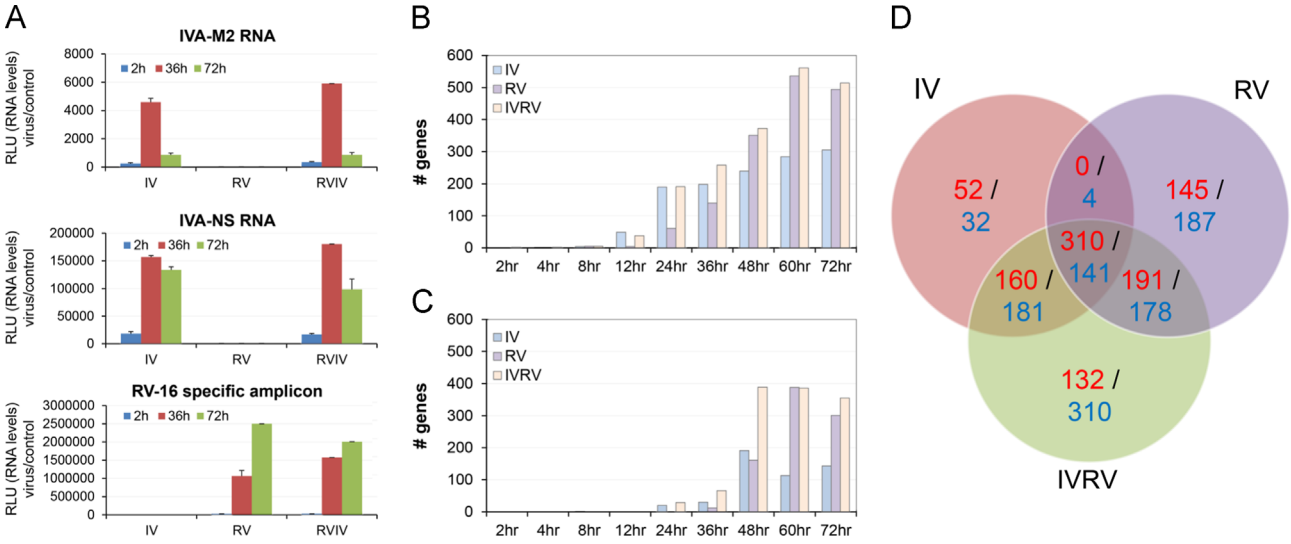

Fig. 1. Differentially expressed host genes (FDR <0.01; log2 $\pm$ 1.5). (A) Assay of influenza virus or rhinovirus amplicons. The levels of influenza-a M2 mRNA, influenza-a NS mRNA, and an amplicon specific for the rhinovirus 16 genome were determined by qRT-PCR. (B) Up-regulated host genes relative to mock-infected cells; (C) down-regulated host genes relative to mock-infected cells; (D) Venn diagram showing how the differentially expressed host genes overlap among the three virus infections. Uniquely expressed up or down regulated genes were derived for each virus infection over all time points and compared. Red labels, up-regulated genes; blue labels, down-regulated genes.