Longer Life Foundation Final Report

Title: Identification of human genetic variants for high risk of severe influenza disease
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Abstract

Personalized and precision medicine is been made possible by the advent of genomic medicine. Cancer treatment in particular has made significant progress towards the improvement of patient care, increased survival and reduced costs, but progress has lagged in other diseases. Genomic medicine can also impact the treatment or prevention of other life-threatening diseases such as influenza. Influenza virus kills nearly 500,000 individuals each year and this number can increase several-fold during an influenza pandemic. Epidemiologic studies have identified rare genomic polymorphisms in the antiviral interferon pathway that are linked to severe influenza infections and hospitalizations. Interferons are secreted proteins that induce cell-intrinsic antiviral immunity in virus-infected and uninfected neighboring cells. The effect of common polymorphisms in genes in this pathway on influenza susceptibility has not been evaluated. Here, we compared virus replication and antiviral gene expression in primary human airway epithelial cells from different individuals after influenza virus infection. We observed large differences in the host response and virus production between cells from different donors. However, these differences were independent of two polymorphisms in genes involved in the antiviral immune response. Findings in this study provide a basis for a more detailed analysis on the role of host genetic variation on influenza virus infection and associated cellular responses in humans.

Lay Summary

Genetic variation affects all aspects of life, including infectious diseases. The impact of this variation on the susceptibility to influenza virus and associated disease is unknown. To address this, we established cultures of airway epithelial cells, the primary target cell of influenza virus, from different individuals and quantified the amount of virus produced from these cells. We also measured the cellular antiviral response after virus infection. Large differences in influenza virus replication and antiviral immune response were detected between cells from different donors, albeit the differences did not associate with common polymorphisms in two genes involved in the antiviral immune response. Overall, these data indicate that genetic variation does affect influenza virus infection and replication and warrants further studies into identifying the variation and associated host genes that causes this difference. This information can be used to develop novel antiviral therapies or identify at-risk individuals that require additional vaccination or anti-influenza prophylactic treatment.

Introduction

Influenza virus infections kill 500,000 individuals worldwide and result in nearly 200,000 hospitalizations annually in the U.S. Severe influenza is associated with increased and prolonged virus replication as a result of poor innate and adaptive immune responses. IFN-induced antiviral immunity is essential for controlling early virus replication and we hypothesize that genetic polymorphisms in key genes in this pathway predispose to increased virus replication and susceptibility to influenza disease.

Methods

Culture and influenza infection of primary human airway epithelial cells. Primary airway epithelial cells cultures from 40 different donors were expanded and seeded into 24-well tissue culture plates. Confluent monolayers were inoculated with a low dose (20 $TCID_{50}$) of A/California/04/2009 H1N1 virus (n = 31) or A/New York/55/2004 H3N2 virus (n = 8). Culture supernatant from the infected cells was collected at 24, 48 and 72 hours post-infection and the viral titer in the supernatants was quantified by virus titration assays in Madin-Darby canine kidney cells. Additional wells were inoculated cells with a high dose ($2x10^5$ $TCID_{50}$) of A/California/04/2009 H1N1 virus and used to measure the cellular innate and antiviral immune response after influenza virus infection.

Genotyping of the cells. PCR assays were developed to identify the genotype at two genetic polymorphisms in IRF7 (rs12805435) and IFNL3 (rs12979860) gene. To determine the sex of the cell donor, we developed a separate PCR assay to identify male and female cell lines.

Analyzing the antiviral immune response after influenza virus infection. RNA is extracted from mock-infected and H1N1 virus infected cells eight hours after infection. The RNA is reverse-transcribed into cDNA and used to quantify the amount of messenger RNA (mRNA) encoding for the interferon-beta gene and the Interferon Induced Protein with Tetratricopeptide Repeats 1 (IFIT1) antiviral gene using gene-specific primer-probe sets. The fold increase in mRNA over mock-infected cells is determined using beta-actin as the housekeeping gene.

Results

Primary human airway epithelial cells from 40 different donors were genotyped for a polymorphism (rs12805435) in the IRF7 gene. Of those, 12 were homozygous for the C-allele, 17 were homozygous for the T-allele and 11 were heterozygous at this position. Thirty-two of these were genotyped for a polymorphism (rs12979860) in the IFNL3 gene. Fourteen donors were homozygous for the C-allele, seven were homozygous for the T-allele and 11 were heterozygous. Finally, the cells in our cohort were from 17 females and 23 males.

Primary human airway epithelial cells are very susceptible to infection with low doses of influenza virus and some cell cultures produced more than 10^4 infectious units per mL in 24 hours after infection. Importantly, we observed a 100-1000 fold difference in H1N1 and H3N2 virus titer in the supernatant of cells from different donors. This difference diminished at 48 and 72 hours post-infection when the virus titer in the supernatant reaches a maximum.

The cellular innate immune response to virus infection also varied considerably between cells from different donors. The fold-increase in IFN-beta and IFIT1 mRNA expression was between 3- and 1000-fold, and 1- and 800-fold respectively, after influenza infection, indicating large differences in the cellular immune response to virus infection between cells from different donors.

The antiviral immune response and virus titer between cells harboring the different genotypes of IRF7 and IFNL3 was not statistically different, suggesting that these two polymorphisms have no effect on the influenza virus replication in these cells under these conditions. Also, we observed no difference in the virus titer between cells from male and female donors.

Discussion, including implications and potential long-term extensions

Large differences in virus replication were observed between primary cells from different individuals, suggesting that genetic polymorphisms facilitate or inhibit influenza virus replication. Identifying these polymorphisms and associated host genes will allow us to identify susceptible individuals.

Future plans including planned grant submissions

Future studies will seek a more complete understanding of human genetic polymorphisms on influenza virus replication and disease. The results of this study will be used to write a manuscript reporting the differences in virus replication and innate immune response in primary cells from different donors. The data is also used to apply for NIH funding looking at the role of genetic variation in specific host genes on influenza virus replication and immunity.