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BRIEF REPORT

A Genomic Signature of Influenza Infection Shows Potential for Presymptomatic Detection, Guiding Early Therapy, and Monitoring Clinical Responses

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Early, presymptomatic intervention with oseltamivir (corresponding to the onset of a published host-based genomic signature of influenza infection) resulted in decreased overall influenza symptoms (aggregate symptom scores of 23.5 vs 46.3), more rapid resolution of clinical disease (20 hours earlier), reduced viral shedding (total median tissue culture infectious dose [$TCID_{50}$] 7.4 vs 9.7), and significantly reduced expression of several inflammatory cytokines (interferon-γ, tumor necrosis factor-α, interleukin-6, and others). The host genomic response to influenza infection is robust and may provide the means for early detection, more timely therapeutic interventions, a meaningful reduction in clinical disease, and an effective molecular means to track response to therapy.

Keywords. gene expression; genomic; influenza; oseltamivir.

Host gene expression analyses have been extensively used to describe the pathogenesis of a wide array of acute infections and may also be used to derive signatures with the potential to diagnose various conditions and predict clinical outcomes [1–5]. However, opportunities to use this technology to direct initiation of early therapy and monitor clinical response longitudinally in human subjects over time have been limited. Given its ubiquitous nature, seasonal recurrence, health impacts, and available treatments, influenza infection provides an excellent target for such inquiry. Influenza viruses exhibit ease of communicability, short incubation times, rapid rates of viral mutation, and involve significant morbidity with resultant loss of productivity, severe complicating diseases, and increased risk of death [6]. Treatment of acute influenza with neuraminidase inhibitors such as oseltamivir has variably been shown to decrease symptoms and duration of viral shedding, and it may decrease the incidence of secondary complications such as bacterial lower respiratory tract infections and hospitalizations [7–9]. Most studies have revealed that earlier treatment results in improved efficacy, with the greatest benefit in outpatients being seen when given within 48 hours after onset of symptoms; however, little is known about how such treatment effects molecular markers of host recovery [9, 10]. We have previously used human influenza challenge cohorts with a defined inoculation event coupled with dense serial sampling to explore the ability of modern genomic and statistical techniques to accurately classify individuals with influenza infection as early as possible after viral exposure [11–13]. Through this method, we have demonstrated the potential for a robust host gene response signature in identifying presymptomatic human infection [13]. In the current work, we undertook to use this experimental human challenge model to further explore the utility of genomic signatures for monitoring and directing therapeutic interventions in cases of acute influenza virus infection.

METHODS

Human Viral Challenges

In collaboration with hVivo (London, United Kingdom), we intranasally inoculated 21 healthy volunteers with influenza A H3N2 (A/Wisconsin/67/2005). All volunteers provided informed consent and underwent extensive pre-enrollment health screening, and they were excluded for positive baseline antibody titers to the strain of influenza used. After 24 hours in quarantine, we instilled 10^6 of median tissue culture infectious dose ($TCID_{50}$) influenza A into bilateral nares of subjects using standard methods [12]. At predetermined intervals (q8h for the first 5 days after inoculation), we collected blood into RNA PAXGene collection tubes (PreAnalytix, Franklin Lakes, NJ) as well as standard plasma or serum tubes, according to manufacturers' specifications. We obtained nasal lavage samples from each subject daily for qualitative viral culture and and/or quantitative influenza reverse transcription-polymerase chain reaction (RT-PCR) to assess the success and timing of infection [14]. Blood and nasal lavage collection continued throughout the duration of the quarantine. All subjects received oral oseltamivir (Roche Pharmaceuticals) 75 mg by mouth twice daily as

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treatment either at 36 hours postinoculation (Early Treatment arm) or at day 5 after inoculation (Standard Treatment arm). Gene expression was assessed in peripheral blood samples utilizing GeneChip Human Genome U133A 2.0 Arrays (Affymetrix, Santa Clara, CA) as previously described [11, 13] (see Supplementary Methods for full experimental details).

RESULTS

Clinical Response to Viral Challenge

Before inoculation, 21 subjects were randomized to receive either Early Treatment (oral oseltamivir given 36 hours postinoculation) or Standard Treatment (oral oseltamivir given 120 hours postinoculation) in a 2:1 ratio (see Supplementary Methods). The Early timepoint was selected based upon prior challenge trials that demonstrated that with influenza infection, a diagnostic gene signature begins to significantly diverge from baseline in symptomatic individuals between 29 and 38 hours postinoculation [13]. After nasal inoculation with influenza virus (A/Wisconsin/67/2005), subjects were serially monitored and sampled for 7 days (see Supplementary Methods for details) and 11 of 21 subjects (52%) developed symptomatic influenza infection. On average, they developed symptoms 44 hours after inoculation (range, 24–96 hours) and experienced maximal symptoms 84 hours postinoculation (range, 48–120 hours). Symptomatic infection developed in 6 of 14 (43%) individuals in the Early Treatment arm and 5 of 7 (71%) individuals in the Standard Treatment arm.

Early Treatment With Oseltamivir at the Time of Genomic Signature Development Attenuates Clinical Symptoms and Reduces Viral Shedding

Symptomatic subjects receiving Standard oseltamivir demonstrated symptom onset approximately 46 hours postinoculation, and they experienced maximal symptoms at approximately 112 hours (Figure 1). Symptomatic subjects receiving Early oseltamivir developed initial symptoms at a similar time after inoculation, on average 43 hours postinoculation. However, these patients experienced peak symptoms earlier than the Standard Treatment group (73 hours vs 94 for Standard Treatment, $P = .07$), returned to baseline more quickly (124 hours postinoculation vs 144 hours for Standard Treatment, $P = .1$), and experienced fewer total symptoms over the course of the study (aggregate symptom scores of 23.5 for the Early Treatment group, and 46.3 for the Standard Treatment group, $P = .18$; Figure 1). The Early Treatment group also experienced more rapid

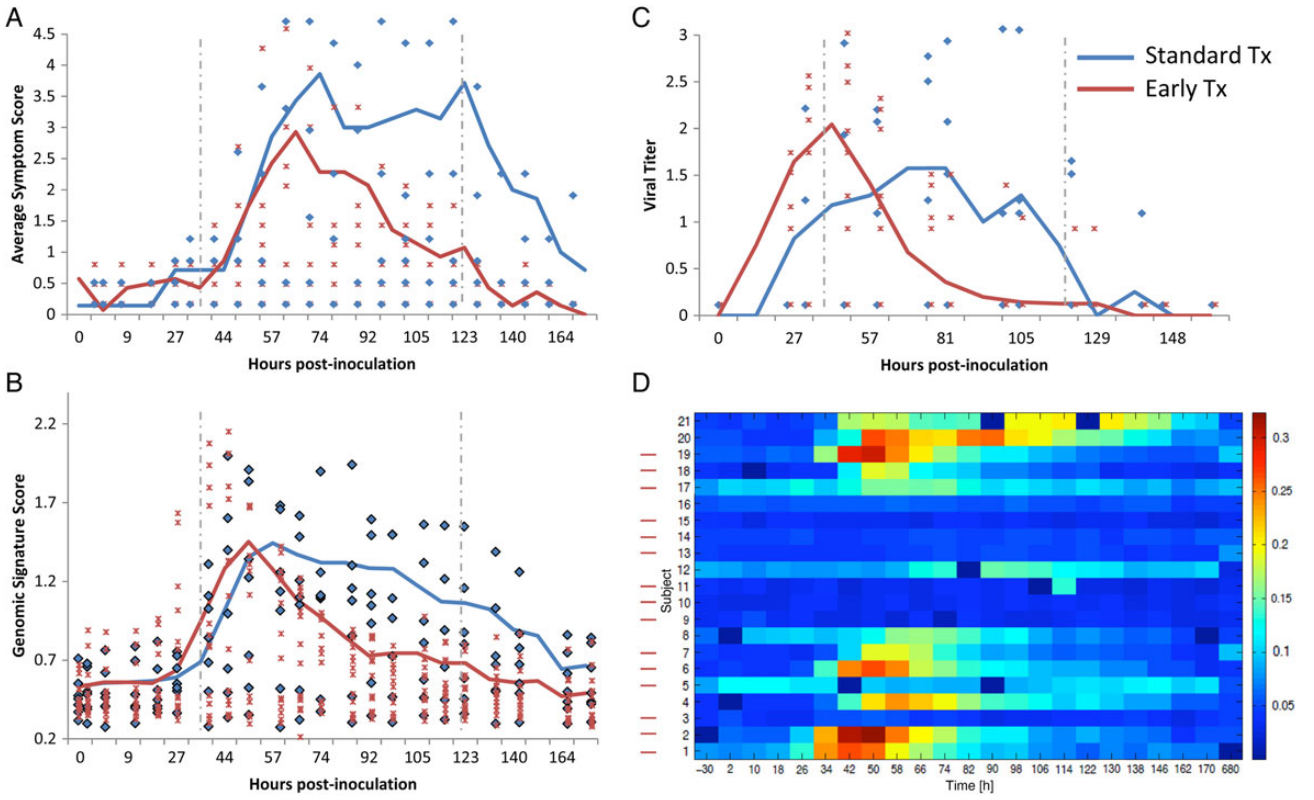

Figure 1. Response to influenza challenge followed by Early (red) and Standard (blue) Treatment (TX) with oseltamivir as measured by clinical symptoms (A) and viral shedding (B). Individual subject results (*, ◇) and mean values (lines) are shown. Time of administration of oseltamivir in the 2 groups is marked by vertical dotted gray lines. Early abrogation of the influenza genomic signature over time in subjects after Early treatment (red) and Standard treatment (blue) is similarly shown in panel (C). A heatmap demonstrates genomic signature score over time after inoculation in all subjects involved in the study (D). Subjects who received Early Treatment are marked with an (–).

reduction in levels of viral shedding as determined by quantitative culture and shed less virus overall (aggregate $TCID_{50}$ per subject 7.4 for Early Treatment vs 9.7 with Standard Treatment, $P = .12$; Figure 1).

Early Treatment Reduces Markers of the Host Inflammatory Response

Individuals who received Early Treatment with oseltamivir also exhibited abrogated expression of a number of key inflammatory cytokines over the course of the study. Over time, individuals randomized to Early oseltamivir exhibited significantly lower aggregate levels of a number of cytokines including interleukin (IL)-4 (average 21.1 vs 26.4 pg/mL for Early Treatment vs Standard Treatment, $P = .04$), IL-5 (16.7 vs 22.0 pg/mL, $P = .01$), IL-6 (17.2 vs 24.1 pg/mL, $P = .01$), interferon-γ (19.3 vs 25.5 pg/mL, $P = .01$), and tumor necrosis factor-α (15.7 vs 21.1 pg/mL $P = .02$; Supplementary Table 1). It is interesting to note that a few cytokines such as the classically anti-inflammatory IL-10 were increased in the Early Treatment group (46.7 vs 35.3 pg/mL, $P = .001$). The cytokine levels between the 2 groups parallel each other at very early times postinoculation, whereas the observed relative changes in cytokine levels occurs primarily after the administration of the Early oseltamivir at 36 hours.

An Influenza Gene Signature Defines the Infected State and Tracks the Rapid Resolution Seen When Early Treatment Is Given

Whole blood RNA was isolated from each individual every 8 hours from inoculation through day 7 and assayed by Affymetrix U133a 2.0 human microarrays. Coexpressed gene transcript factors (or signatures) were generated through sparse latent factor regression analysis to provide an unbiased (unlabeled) examination of gene expression (see Supplementary Methods). Similar to our previous work, gene expression analysis allowed for development of a single genomic signature (or factor) as best able to discriminate symptomatic subjects from asymptomatic subjects. The signature developed for this study closely mirrors the gene composition detected in our prior work (sharing 44 of the top 50 genes with the previously reported signature; Supplementary Table 2) [12]. This gene signature begins to diverge from baseline during the presymptomatic phase between 24 and 28 hours postinoculation (Figure 1), which is 18–24 hours before the onset of symptoms. The gene signature then reaches its quantitative peak at similar times in the Standard and Early Treatment groups (54 and 50 hours, respectively). However, the gene signature demonstrates a more rapid decline in the Early Treatment group, reaching baseline levels by 130 hours after inoculation, whereas in the Standard Treatment group the signature had not yet returned to baseline by the last timepoint of the study (168 hours). This rise and decline in the quantitative strength of the gene signature closely mirrors the worsening and subsequent resolution of symptoms over the same time period. Overall, individuals receiving early treatment demonstrated lower aggregate signature factor scores over the course of their illness (aggregate mean factor score 0.97 vs 1.36, $P = .09$), again similar to the differences seen in symptom scores between the 2 treatment groups.

DISCUSSION

As expected from previous work in both experimental and natural infection [7–10], early treatment with oseltamivir triggered a reduction in the duration and overall severity of clinical illness as well as a reduction in the amount of viral shedding that occurred. However, for the first time, we have been able to define the effect of early treatment on temporal dynamics of the host peripheral blood genomic responses that underlie this process.

Genomic analyses of experimental infection with this strain of influenza (both herein and in our prior work [13]) have demonstrated the potential to identify viral infection either before symptoms emerge or simultaneous to onset of what otherwise are mild, common, nonspecific upper respiratory symptoms. It was previously hypothesized that intervention with antiviral medications at these early times (flagged by signature positivity) could have a profound impact on both individual symptoms and disease transmission, and the current study now provides further evidence that such genomics-led predictive early treatment may be possible and furthermore may offer improved outcomes compared with standard postsymptomatic treatment timing. Administration of antiviral medications at the time of divergence of the influenza genomic signature from baseline resulted in marked reduction in key clinical endpoints and biomarkers of inflammation compared with the Standard Treatment group. The change in clinical response is similar to the result of a prior interventional trial in which subjects were given oseltamivir at a defined timepoint 28 hours after inoculation [7] and confirms that earlier intervention shows promise for improved clinical outcomes in influenza infection. Although there were a smaller proportion of symptomatic infections in the Early Treatment group (6 of 14 or 43% compared with 5 of 7 or 71% of the Standard group, $P = .21$), the number of patients in the trial is too small to definitively state whether Early intervention prevented any symptomatic cases from developing. It is clear that the perfect test of genomic signature-directed therapy would be to design a trial wherein treatment decisions were made with signature data in hand rather than through randomization, but the current nature of the technology (arrays with complex statistical analysis) makes this problematic until a more rapid and accessible platform (such as RT-PCR [11]) can be developed.

These data further suggest that gene signatures of acute infectious processes can provide a useful correlate of disease activity. The overall trajectory of the influenza gene signature tracks closely with symptom scores over time, both in the Standard Treatment and Early Treatment groups. Symptom scoring, although elucidating a clinically relevant variable, is by its nature extremely subjective, and clinical trials could benefit from more

quantitative measures of the host response to illness or therapeutic intervention. Based upon these observations, genomic signatures may well provide just such a means.

It is clear that care must be taken when analyzing and applying host genomic data from such studies. Experimental challenge trials do not perfectly mimic natural human exposure or disease, and they are performed in a homogenous population. Hosts in these studies are young, healthy individuals, which, along with the overall mild-to-moderate symptoms seen in the trial, may limit the broad applicability of such findings, although this is somewhat mitigated by the strong discriminative performance of the gene signature despite significant clinical symptom variability in infected subjects. Although the trends in the data suggest a promising role for genomic studies in differentiating these states, the small numbers of infected subjects (6 symptomatic subjects in the Early Treatment arm, and only 5 in the Standard arm) coupled with high levels of intersubject variability limit the strength of some of these conclusions. In addition, the microarray analysis used herein is too time-intensive to become a practical clinical platform, and further work with other modalities such as RT-PCR of an important subset of genes would be required to create a clinically useful test [11].

CONCLUSIONS

Despite these limitations, we have for the first time defined the temporal dynamics of a genomic signature driving the host response to early treatment of influenza infection in humans. This work demonstrates that analyses of the temporal development of gene expression signatures shows promise for creating diagnostics for early detection, which may drive therapeutic decisions as well as provide insight into the biology of the host response to the onset, progression, and eventual resolution of influenza infection.

Supplementary Data

Supplementary material is available online at Open Forum Infectious Diseases online (http://OpenForumInfectiousDiseases.oxfordjournals.org/).

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Potential conflicts of interest. M. T. M., E. T., G. S. G., A. K. Z., and C. W. W. have patents pending on host-based diagnostics for pathogen identification. G. S. G., E. T., and C. W. W. receive funding from Novartis to develop novel biomarkers for infectious disease. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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