Differential evolution of peripheral cytokine levels in symptomatic and asymptomatic responses to experimental influenza virus challenge

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SUMMARY

Exposure to influenza virus triggers a complex cascade of events in the human host. In order to better understand the evolution of this intricate response over time, human volunteers were inoculated with influenza A/Wisconsin/67/2005 (H3N2), and then had serial peripheral blood samples drawn and tested for the presence of 25 major human cytokines. Nine out of 17 (53%) inoculated subjects developed symptomatic influenza infection. Individuals who will go on to become symptomatic demonstrate increased circulating levels of IL-6, IL-8, IL-15, MCP-1, and IP-10 as early as 12-29 hours post-inoculation (during the pre-symptomatic phase), whereas challenged patients who remain asymptomatic do not. Overall the immunologic pathways of leukocyte recruitment, TLR-signaling, innate antiviral immunity and fever production are all overrepresented in symptomatic individuals very early in disease, but are also dynamic and continuously evolve over time. Comparison with simultaneous peripheral blood genomics demonstrates that some inflammatory mediators (MCP-1, IP-10, IL-15) are being actively expressed in circulating cells while others (IL-6, IL-8, IFN-α and IFN-γ) are likely effectors produced locally at the site of infection. Interestingly, asymptomatic exposed subjects are not quiescent either immunologically or genomically but instead exhibit early and persistent downregulation of important inflammatory mediators in the periphery. The host inflammatory response to influenza infection is variable but robust and evolves over time. These results offer critical insight into pathways driving influenza-related symptomatology and offer the potential to contribute to early detection and differentiation of infected hosts.

INTRODUCTION

Influenza infection is one of the leading causes of acute respiratory illness worldwide and causes substantial morbidity and mortality(1). The ongoing global impact of clinical influenza infections, coupled with the continued evolution of the virus resulting in periodic pandemics, highlights the need for better understanding of the nature of the host response to this ubiquitous and ever-changing pathogen. Analysis of how humans respond to influenza infection is a key to understanding virus-mediated immunopathology and resultant clinical disease.(2) Respiratory viruses such as Influenza are some of the most common causes of airway inflammation and acute lung injury but mechanisms underlying this injury have not been fully elucidated. Influenza infection initiates in the host a cascade of increased biosynthesis of proinflammatory mediators (cytokines and chemokines) by airway inflammatory and epithelial cells.(3) These chemotactic, pro and anti-inflammatory cytokines have pleiotropic effects that in a concentration-dependent manner mediate proliferation, differentiation, receptor and leukocyte recruitment, can act as secondary messengers, hormones, ligands and function in positive and negative feedback. Studies involving influenza H1N1 pdm09 found correlations between disease severity and circulating levels of IL-6, IL-10, IP-10, and MCP-1(4). Another recent study of individuals with influenza H7N9 infection revealed elevated levels of IP-10, IL-2, IL-6, IL-17(5, 6). Fatal outcomes following human infection with avian influenza A virus (H5N1) are associated with high levels of inflammatory cytokines in the peripheral blood including IP-10, MCP-1 (CCL2), MIG (CXCL9), and IL-8 (7, 8) while other recent data demonstrate that agents which modulate some of these key host inflammatory pathways show promise as adjunctive

therapies(9). Thus, understanding the mechanisms of chemokine and cytokine responses to influenza infection is of high priority, as excessive cytokine production seems to directly contribute to clinical pathogenesis.

Unfortunately, the bulk of available data regarding cytokine expression in influenza-infected humans are from single-timepoint clinical studies, although there are some limited temporal human data focusing on a small number of specific targets.(10-12) Such studies, while powerful, fail to shed light on very early (pre-symptomatic) timepoints in disease, or on the development and progression of host responses over time. In order to more accurately and completely characterize the temporal dynamics of the host response to acute influenza infection, we have utilized our own human influenza challenge cohorts with a defined inoculation event and typical seasonal influenza virus strain coupled with frequent serial sampling in order to explore the ability of modern immunologic techniques to accurately identify and classify individuals with both symptomatic and asymptomatic responses to influenza infection as early as possible following viral exposure, as well as to explore the potential mechanisms and pathogenic impact of these responses through simultaneous monitoring of gene expression in PBMCs(13).

MATERIALS AND METHODS

Viral Challenge

For the human viral challenge study, in collaboration with Retroscreen Virology, Ltd (London, UK), we intranasally inoculated 17 healthy volunteers aged 22-41 (avg. age 27) with influenza A/Wisconsin/67/2005 (H3N2) as previously described in detail(13). All volunteers provided informed consent and underwent extensive pre-enrollment health screening, and inclusion required negative baseline hemagglutination inhibition titers to the specific strain of influenza utilized in the study. After 24 hrs in quarantine, we instilled 10^6 $TCID_{50}$ influenza A into bilateral nares of subjects using standard methods(14, 15). At pre-determined intervals (q8h for the first 5d following inoculation), we collected blood, serum, and plasma. We obtained nasal lavage samples from each subject daily for qualitative viral culture and and/or quantitative influenza RT-PCR to assess viral shedding. Blood and nasal lavage collection continued throughout the duration of the quarantine. Symptom scores were tabulated twice daily throughout the study(13, 16), where subjects ranked upper respiratory and systemic symptoms (runny nose, sinus stuffiness, sneezing, sore throat, earache, cough, shortness of breath, malaise, myalgias, fever) on a scale of 0-3 of “no symptoms”, “just noticeable”, “bothersome but can still do activities” and “bothersome and cannot do daily activities”. All subjects were negative by rapid antigen detection (BinaxNow Rapid Influenza Antigen; Inverness Medical Innovations, Inc) at time of discharge, and no additional post-challenge adverse events were reported.

Cytokine Quantification:

Serum chemokine and cytokine levels were evaluated using the Invitrogen Human Cytokine 25-plex assay (Carlsbad, CA, USA) per manufacturer's instructions. Briefly, beads are conjugated to cytokine-specific capture antibodies and added along with the sample of interest into the wells of a filter-bottom microplate. After washing the beads, biotinylated detector antibodies are added followed by addition of Streptavidin-RPE. The Streptavidin-RPE binds to the biotinylated detector antibodies associated with the immune complexes on the beads, forming a four-member solid phase sandwich. After washing to remove unbound Streptavidin-RPE, the beads are analyzed on a Luminex detection system.

RNA purification and microarray analysis

For each challenge, we collected peripheral blood at 24 hours prior to inoculation with virus (baseline), immediately prior to inoculation (pre-challenge) and at set intervals following challenge as described(13). RNA was extracted at Expression Analysis (Durham, NC) from whole blood using the PAXgene™ 96 Blood RNA Kit (PreAnalytiX, Valencia, CA) employing the manufacturer's recommended protocol. Hybridization and microarray data collection was performed at Expression Analysis (Durham, NC) using the GeneChip® Human Genome U133A 2.0 Array (Affymetrix, Santa Clara, CA).

Viral titration

Confluent monolayers of MDCK cells were inoculated with replicate (n = 4) serial 10-fold dilutions of virus stocks or clinical samples (nasopharyngeal washes) in 96-well

microtiter format. After 90 min at 37°C in a humidified 5% CO2 incubator, the inocula were removed and the cells were washed with IM and cultured for 6 days in a humidified incubator at 37°C and 5% CO2, followed by enumeration and calculation of $TCID_{50}$ by standard practice(17).

Statistical Analysis

Preprocessing. Prior to data log-transformation, cytokine values above (0.03% of measured samples) or below (0.3% of samples) measurement range were imputed to either twice or half of the maximum or minimum value of any given cytokine, respectively.

Cytokine profile model. Our model for cytokine time series assumes that a subset of subjects sharing a phenotype also share a cytokine time profile hidden within measurements. More specifically, we assume that due to subject-to-subject variability any observed time series is a time shifted, amplitude shifted and scaled version of a phenotype-specific cytokine profile, that is in addition naturally subject to measurement noise. The Bayesian model, fully described in the Supplementary Material estimates subject-specific shifts and scale parameters, and a phenotype-specific subject-shared cytokine profile function. For each cytokine two models are considered: symptomatic and asymptomatic profile models.

Symptom score correlation model. We can assess the level of collinearity between

symptom scores and individual cytokines by simply computing Spearman correlation coefficients between them. When symptom scores are a monotone function of some cytokine we obtain a correlation coefficient of either 1 or -1. Alternatively, we can try to predict symptom scores based on all cytokines using an ordinal regression model such as Bayesian rank-likelihood regression(18). In order to avoid overfitting,the predictions of the regression model were obtained within the context of a leave-one-out (LOO) cross-validation scheme. If the LOO-based predictions correlate with symptom scores then there exists a non-trivial linear combination of cytokines that also correlates with symptom scores. Model development and analyses were primarily performed using MATLAB (The Mathworks, Inc). The Student's *t*-test, Mann-Whitney U, and χ^2 tests were used for univariate comparisons where appropriate. Correlations between immunological parameters (cytokine levels), symptoms, and viral load were calculated using Spearman's rank correlation test. In all analyses a p-value of <0.05 was used to indicate statistical significance where appropriate.

RESULTS

Temporal development of the host response to viral challenge

In our previously completed H3N2 (A/Wisconsin/67/2005) challenge trial, we inoculated 17 volunteers (mean age 27 years; range 22-41 years), of whom 9 (53%) developed symptomatic influenza infection(13). For the 9 symptomatic-infected subjects, symptom onset began at an average of 45-49 hours after inoculation, and they experienced maximal symptoms on average 85-93 hours after inoculation followed by a slow steady

improvement. Viral shedding increased rapidly with an early peak at 48 hours post-inoculation followed by a comparatively more rapid decline compared to symptom resolution (Fig. 1). For the current study, we assayed 25 cytokines in peripheral blood at each of 17 different time points ranging from pre-inoculation through the entire course of clinical disease and eventual symptom resolution. Levels of the various cytokines in peripheral blood exhibited significant variability amongst individuals and across time. This variability in the cytokine responses amongst individuals manifests quantitatively and, to a lesser degree, temporally. However, the character, direction, relative magnitude, and shape of the temporal responses seen for each cytokine are remarkably conserved amongst symptomatic individuals. This can be visualized by examining a composite curve for all individuals which illustrates this conserved motif for each significantly altered cytokine (Supp. Fig. 1). The majority of this variation is quantitative in nature and can be dealt with by calculating fold change compared to baseline for each individual, cytokine, and timepoint studied (Fig. 2), which reveals the overall conserved character of the host response.

Pre-symptomatic times (0-45 hours post-inoculation)

There were no significant differences in baseline, pre-inoculation cytokines between those who would go on to become symptomatic or asymptomatic. In symptomatic individuals, however, some cytokines commonly increased well before the average time of symptom onset (45 hrs, Fig. 2). The primary cytokines which experienced the earliest spikes in symptomatic individuals are IL-6, which shows increased circulating levels of

as much as 70% by as early as 5 hours post-inoculation, IL-12, (21hrs), MCP-1 and Eotaxin (29 hrs), IP-10 (36 hrs), and IL-15 (45hrs post-inoculation, Fig. 2).

Symptomatic times (45-91 hours post-inoculation)

Between the time of symptom onset and symptom max, spikes in the circulating levels (minimum 50% increase compared to baseline) of IL-4, and IL-7 (small, 60 hrs), IL-10 (69hrs), IFN-a (69 hrs), TNF-a (60 hrs) were seen. A slightly larger increase is seen in IL-1RA at 53 hrs, 3-fold increase. Additionally, further evolution is seen in of the elevation of the most active cytokines as they reach their peak values - IP-10 (peak 53-69 hrs, almost a 10-fold increase), and IL-6 (peak 60 hrs, 6-fold increase), MCP-1 (peak 53 hrs at 4.3-fold), and IL-15 (peak 60 hrs at 4.3 fold increase).

Interestingly, starting at around the time of maximal symptoms and cytokine expression (84-96 hours) and extending even later (132-168 hrs), symptomatic subjects exhibit not only a gradual reduction in the levels of previously elevated cytokines, but also show specific downregulation (relative to baseline) of a number of important mediators including IL-1b, IL-13, IL-17, GM-CSF, IL-2, IL-7, and IL-4.

Cytokine changes relative to symptoms

Individual cytokine expression levels did not directly correlate with any individual symptom, nor were there any specific cytokines that distinguished ‘upper respiratory’ (runny nose, sinus stuffiness, sneezing, sore throat, earache, cough, shortness of breath) , or ‘systemic’ (malaise, myalgias, fever) symptom subgroups. Even the best-performing individual cytokine (IP-10) showed only a moderate correlation with overall

symptom score (Correlation coefficient 0.64). However, peripheral cytokine levels tended to broadly increase prior to the time of symptom onset and peaked just prior to the time of maximal symptoms, and it is possible to derive a model utilizing the combined score of differentially weighted individual cytokine levels which achieves much closer correlation with overall symptom scores (Correlation coefficient 0.84, Fig. 3). There were no significant correlations between cytokine levels achieved and d28 Influenza HAI titers (data not shown).

Cytokine changes relative to viral load.

Viral loads on average peaked around 48 hours post-inoculation, around the mean time of symptom onset, but interestingly showed no significant correlation at the individual level with the degree of clinical disease (as determined by symptom scoring). However, when divided into the individuals with the highest level of viral shedding and those with the least (top/bottom thirds, as determined by quantitative culture) there were several key differences noted in peripheral cytokine levels (Fig. 4). Those individuals with the highest levels of viral shedding exhibited higher circulating levels of many of the cytokines tested. The largest increases were seen with IL-6 (3-fold higher in high viral shedders), IL-1RA (3-fold higher), IL-10 (2-fold higher), and IP-10 (3-fold). The relatively higher levels of peripheral cytokine expression seen in those with the highest viral loads is most prominent around the time of peak viremia (36-53 hours, Fig. 1 and 4), further supporting a direct relationship between these variables.

Comparison to peripheral gene expression

We have previously published on the temporal development of gene expression signatures in circulating white blood cells which are capable of diagnosing acute respiratory viral infection both at the time of clinical presentation (13, 14), as well as much earlier in the presymptomatic state(13). Specifically, in this cohort of H3N2 challenge patients, a gene expression signature capable of accurately detecting infected subjects was detectable in symptomatic subjects as early as 29 hours after inoculation, but absent from those would remain asymptomatic (13). This gene signature included interferon stimulated pathways such as those including RSAD2, IRF7, MX1, OAS3, MDA-5, RIG-I and others which are thought to drive both innate and, to a lesser degree, adaptive immune responses to viral infection. In order to examine the hypothesis that increases in some circulating peripheral serum cytokines are driven by production in circulating immune cells (while others are not), we examined differential gene expression levels for those peripheral cytokines which were most significantly elevated in infected subjects. In a subset of individuals (5 Asymptomatic and 7 Symptomatic) both cytokine and transcriptomic data were available from the same blood draws, and these were utilized for the analysis depicted in Figure 5. At the time of symptom onset (45-49 hours post-inoculation) there are already marked increases in the levels of IP-10, MCP-1, IL-15 and IL-6 (Fig. 2). However, interestingly, of these cytokines only IP-10, MCP-1 (4-5-fold) and to a lesser degree IL-15 (1.9-fold) have undergone similar increases in gene expression in PBMCs themselves to that point (Fig. 5). Genes driving production of IL-6 are not upregulated in the peripheral cells themselves. Also, while there are modest changes in the concentration of circulating

IFN-α and IFN-γ (on the order of 50-60% increases in each), their gene expression remains completely unchanged in the PBMCs (Fig. 5). However, interferon-inducible genes (such as IFIT1, IFIT3, IFI44L and others) are among the most strongly upregulated genes in these peripheral cells(13). Later, at the time of maximal symptoms, the levels of some of these peripheral cytokines have decreased substantially (MCP-1, IL-6, IL-8), while the level of gene expression of these cytokines in peripheral cells is only mildly lower. However, at these late times the level of expression of interferon-inducible genes is reaching its highest level and these IFI genes dominate the Influenza-specific host gene signature at those times. Together, these suggest that while some of the circulating cytokines seen likely originate in circulating peripheral blood leukocytes, others seem more likely to be produced at a distal site (such as locally in the upper respiratory tract).

Key cytokine changes in the Asymptomatic state

Despite extensive pre-screening and identical exposures, eight of the 17 individuals in this study (47%) exhibited no symptoms or viral shedding following inoculation, which is similar to the rate reported in previous challenge studies(12, 19, 20). However, we have previously demonstrated that the peripheral gene expression profile of these individuals indicatesthat there is still a significant host response in these asymptomatic individuals(21). The asymptomatic but exposed state is not passive but involves a significant genomic response in peripheral white blood cells – different from that seen in symptomatic subjects - yet these individuals do not exhibit clinical signs of influenza infection. Concordantly, in the current work we also see temporal changes in their

peripheral cytokine expression patterns which are different from those seen in symptomatic subjects. There is some minor and variable upregulation of some cytokines starting immediately post-inoculation and this is seen most prominently in the same cytokines which dominate the symptomatic response, although at significantly lower levels (Fig. 2). However, the asymptomatic cytokine response is dominated by a significant early downregulation of many of the cytokines tested. There was a generalized downregulation of IL-7, IL-5, IFN-y (all essentially immediately following inoculation), and to a lesser degree IL-17, IL-15, IL-4, and MIP-1a. While there is also downregulation of most of these cytokines in symptomatic subjects, it does not occur until late (84-96 hours) in the process when symptoms are high and viral load is decreasing. In symptomatic subjects this time corresponds to a natural correction of the immune response towards regulation and a return to homeostatic levels – however, in asymptomatic subjects this downregulation occurs almost immediately and persists throughout the study period. Interestingly, despite a lack of detectable viral shedding 3 asymptomatic individuals seroconverted to challenge virus by day 28. No significant differences were noted in the cytokine profiles of these 3 relative to the other asymptomatic subjects.

DISCUSSION

We have utilized a human viral challenge study with influenza A (H3N2) to define the host-based peripheral blood cytokine expression patterns characteristic of the temporal response to influenza infection. The results provide clear evidence that unique, biologically relevant peripheral blood cytokine expression patterns are characteristic of

symptomatic influenza in humans. For the first time we have further undertaken to explore the development and evolution of such a diverse cytokine panel over time throughout the course of clinical disease. The results highlight the profound immune activation which occurs in this disease state, and provide key insights into the mechanisms that likely drive the symptomatic response in human hosts.

In symptomatic individuals, the major cytokine which undergoes the earliest increase is IL-6, which shows increased levels as early as 5-12 hours post-inoculation. The early increase in IL-6 is consistent with its role as an acute phase reactant and known mediator of the febrile response in a number of disease states, including severe influenza infection (1, 5, 7), although the increased levels here precede symptom onset by as much as 2 days. Around 29-36 hours we begin to see increased circulating levels of chemoattractants IP-10 and MCP-1, the timing of which also suggests a possible role for these cytokines in driving the initiation of some early pathology. At slightly later times (45-60 hrs), corresponding to the time of average symptom onset but still well before symptom max, a number of other cytokines begin to be significantly expressed in symptomatic-infected individuals. These include T_H1 cytokines IFN-y, IL-15, and the chemoattractant IL-8 as increases in circulating levels of these early mediators crescendo and become quantitatively more robust. Overall the immunological pathways of leukocyte recruitment, TLR-signaling, innate antiviral immunity and fever production are all overrepresented in symptomatic individuals very early in disease, but are also dynamic and continuously evolve over time. We see elevations in symptomatic subjects of many of the same cytokines which have been reported to closely correlated with severe disease in hospitalized pH1N1, H5N1, and H7N9 infections(4, 5, 22, 23).

Influenza infection induces activation of chemoattractants IP-10, MCP-1, MIP-1β, MIG and IL-8 which are driven by adaptive and innate responses. Unchecked, the induction of certain pro-inflammatory cytokines like TNF-α and chemoattractants IP-10, MIP-1β, MCP-1 and MIG is known to contribute to the pathogenesis of severe H5N1 and H3N2 influenza infection.(7, 8, 24) However, the individuals in our challenge model exhibit fairly mild-to-moderate symptoms overall, suggesting that determination of severe symptomatology is due to more than simple activation of these common inflammatory response pathways.

Interestingly, despite the fairly rapid and profound upregulation of interferon-response genes in the circulating cells, there are only minor increases (40-50%) in circulating levels of measured interferons, almost none of which appear to be expressed by peripheral cells, and these do not occur for several days following inoculation (Figs. 3, 5). This suggests that there is relatively greater physiologic downstream effect of quantitatively smaller increases in some cytokines. Also, the high circulating levels of some cytokines such as IL-6 in the absence of significant upregulation of the genes driving IL-6 cytokine production in circulating PBMCs (Fig 5) suggests that while peripheral cells may be contributing to the levels of some cytokines others are likely being produced locally at the site of inoculation and then released into the periphery(19).

Contrary to individuals who will go on to become symptomatic, asymptomatic individuals exhibit immediate and persistent downregulation of many circulating cytokines in the immediate early phase following infection, while simultaneously allowing a much more muted increase in the cytokines which drive the symptomatic

response. In effect, our results demonstrate that the asymptomatic (but still virally inoculated) state is not passive, and suggest that perhaps the immune response in those individuals represents a focused, limited, but still effective early response, whereas the response in symptomatic individuals is much more robust but may also therefore incidentally lead to the development of clinical disease. Unfortunately, study of the peripheral cytokine response alone does not identify the driving force behind this muted (but still successful), quantitatively appropriate response in asymptomatic individuals. Individuals were pre-screened for vaccine history as well as anti-HA and neutralizing antibody titers to the challenge virus as a condition for inclusion, but there are data suggesting non-humoral pre-existing immunity (primarily T-cell mediated and directed towards conserved, immunodominant epitopes)also plays a role in this observed divergence(15, 25). It seems likely that complex interactions at the site of inoculation, under the control of pre-existing, targeted immune cell types such as these, aid in determining which pathway exposed individuals will follow. Also, our data suggest that the determination of which inflammatory pathway an individual will follow is likely determined at very early, even presymptomatic times, highlighting the need for early diagnosis and the potential for early host-targeted intervention(26-28).

The differences between symptomatic and asymptomatic cytokine responses are profound and a model that involves weighting of individual cytokines (Fig. 3) permits distinction between symptomatic and asymptomatic individuals not only at the time of maximal symptoms (where patients might present to a clinician) but at much earlier times when the symptomatic cytokine response is already profound but clinical disease is only just beginning. Whether such a lag time might be beneficial for predicting future

development of severe disease is unclear, for while many of same cytokines known to be associated with severe clinical disease are found in our model(4, 5, 8), our patients never progress beyond moderate symptoms (Fig 1)(13).

Despite the benefits of using a human challenge model, including controlled variables and availability of dense temporal sampling, such models do convey certain limitations. The mechanism of disease initiation through direct nasal inoculation with high levels of virus is contrived rather than natural, and it is unknown how this may affect host responses. Also, the degree of clinical disease seen is often not severe and tends to be less than those symptoms which typically lead patients to seek clinical care or hospitalization, which can limit broad applicability of the findings. The variability of absolute cytokine levels between individuals coupled with the relatively small number of subjects in the study limit the statistical significance of some findings, which could be alleviated by future studies of larger cohorts. Although the unique types of changes demonstrated herein have not been seen with previous 'sham' viral challenges in either our hands or others'(29) without a 'sham'-inoculated control the downregulation of cytokines in asymptomatic subjects cannot be definitively shown to represent a muted host antiviral responses rather than the general effects of inoculation and study procedures. Furthermore, this work also focuses on those conclusions which can be made from analysis of peripherally circulating cytokines during the presymptomatic and clinical phases of illness, which represent only a subset of the many-faceted totality of the human immune response.

In conclusion, the host cytokine response to experimental influenza challenge seems to follow one of two divergent paths. In individuals who will go on to become

symptomatic the peripheral cytokine response is robust, detectable well prior to the onset of symptoms, and manifested through pathways typically seen in severe clinical disease. In those who will remain asymptomatic, however, the host response is characterized by immediate control with rapid and focused downregulation of inflammatory pathways. These differences highlight the complexity of the host response to influenza challenge and suggest that directing the body's early immune response towards appropriate control of the relevant inflammatory pathways may be central to improving clinical outcomes.

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Author Contributions:

MTM, ELT, GSG, and CWW wrote the manuscript

RLW, AG, GSG, MTM, and CWW designed and ran the viral challenge

RH and MTM performed data analysis

JW, BN, TL, and LH designed the study and performed the experiments

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Figure Legends:

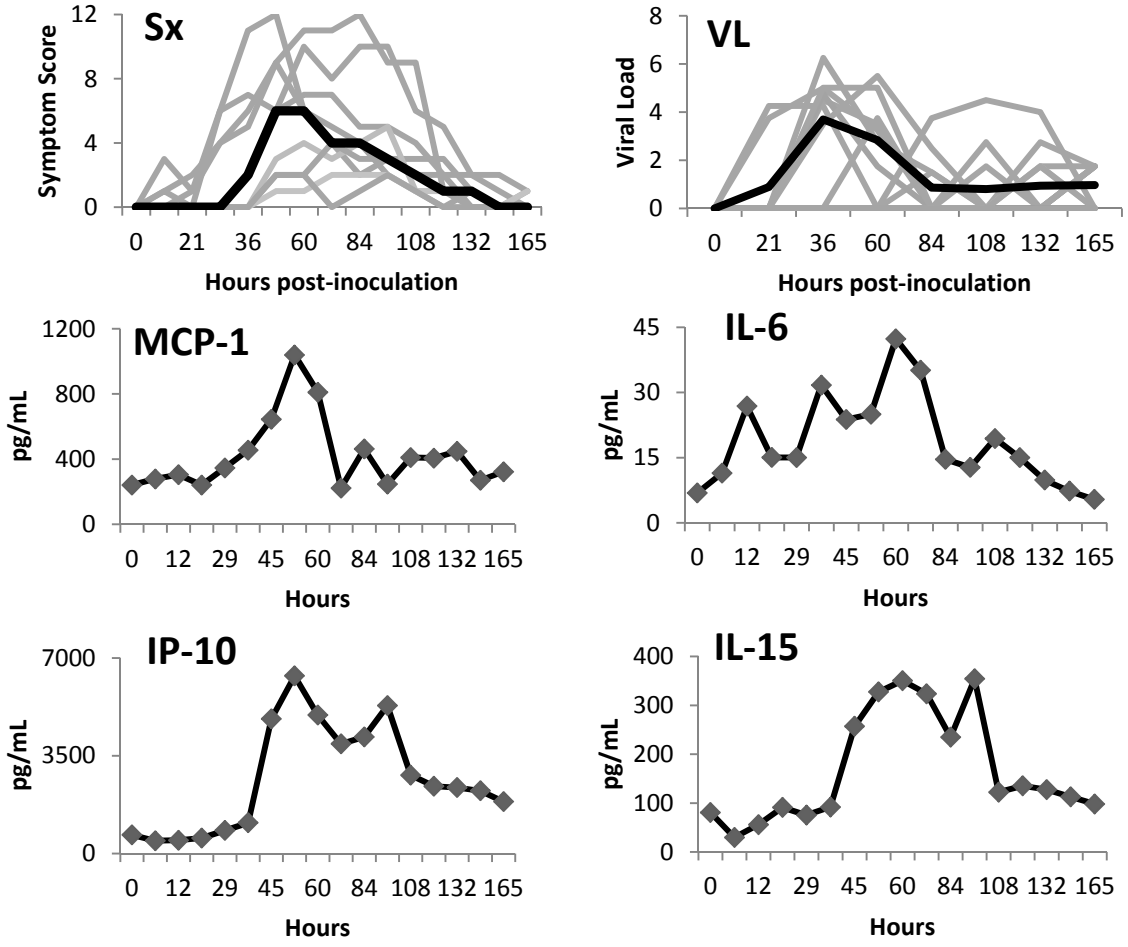
Figure 1: Temporal development of symptoms, viral shedding, and absolute levels of circulating cytokines in symptomatic subjects experimentally infected with Influenza A virus. Time courses of individual subjects (light gray) and median values (black) are represented.

Figure 2: Heatmap showing changes in circulating cytokine levels over time in symptomatic (A) and asymptomatic (B) subjects challenged with Influenza A virus. Numbers depicted represent fold change relative to baseline expression levels.

Figure 3: Weighted cytokine model of symptomatic responses. Presented are the relative weight assigned to each cytokine in the model (A), and Spearman correlations between weighted cytokine levels (y-axis) and symptom scores of each timepoint in the study (x-axis) for both symptomatic (black) and asymptomatic (red) subjects (B). Panel C presents a heatmap demonstrating the similarity between actual symptom scores over time for symptomatic subjects (C, top) and predicted symptom scores from the weighted model for the same subjects and timepoints (C, bottom).

Figure 4: Differential development of circulating cytokine levels over time in the top and bottom 1/3rd of symptomatic subjects as determined by total viral shedding. Colors depicted represent fold change relative to baseline expression levels.

Figure 5: Simultaneous gene expression in circulating PBMCs and serum levels of some cytokines (IP-10 and MCP-1) suggest production of these analytes is driven by peripheral cells, while dysregulation of expression of others (IL-6 and IFN-α) suggests production at an alternate site. Temporal changes in levels of circulating cytokines (red) or levels of expression of the gene(s) for that cytokine (blue) in circulating PBMCs at the same timepoint. Changes in levels over time (as $\log_2$ fold change relative to baseline) are shown for each symptomatic (right of dotted line) and asymptomatic subject (left of dotted line). Only the subset of individuals (5 Asx, 7 Sx) for whom both cytokine and transcriptomic data from the same blood draws are pictured.



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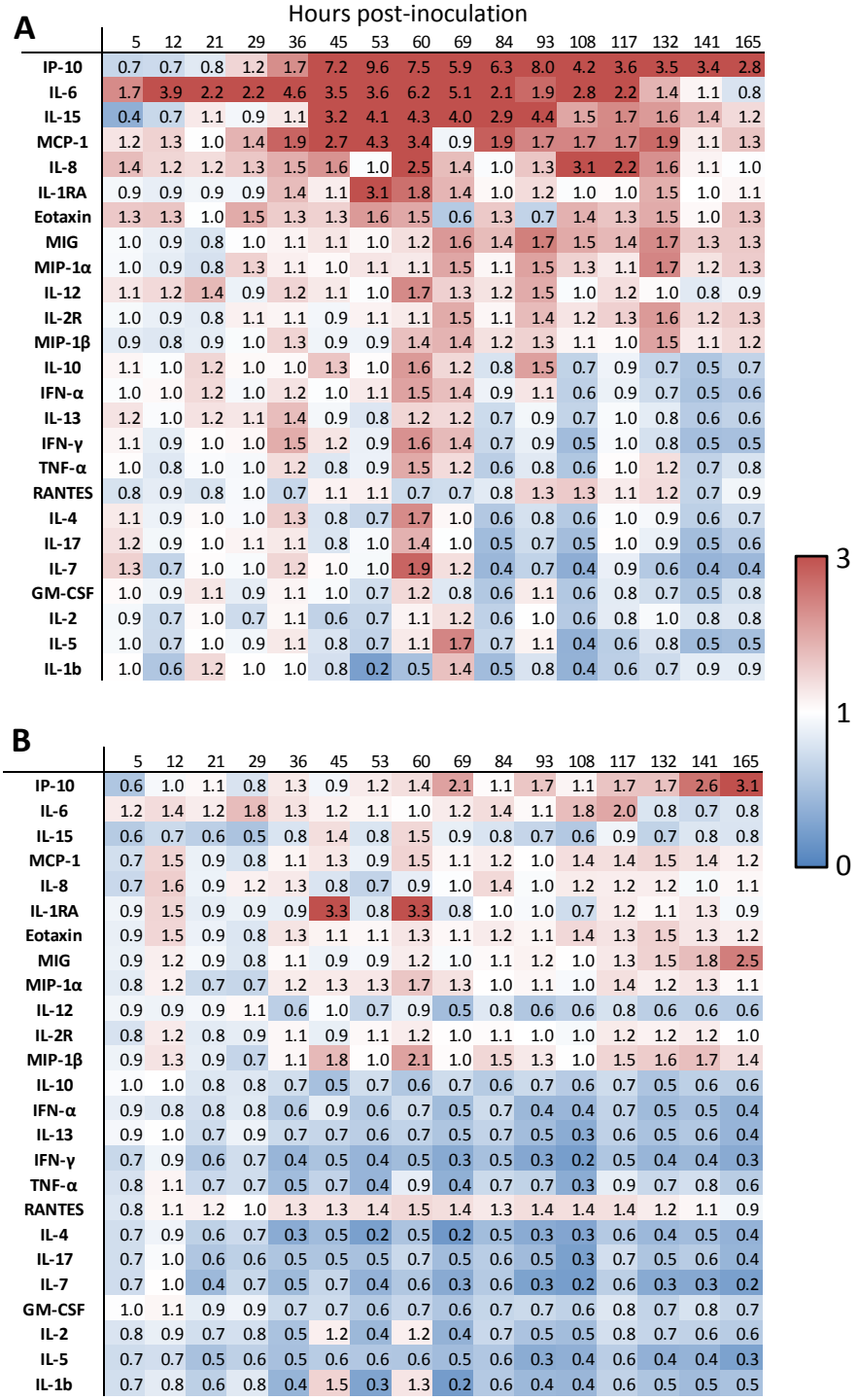

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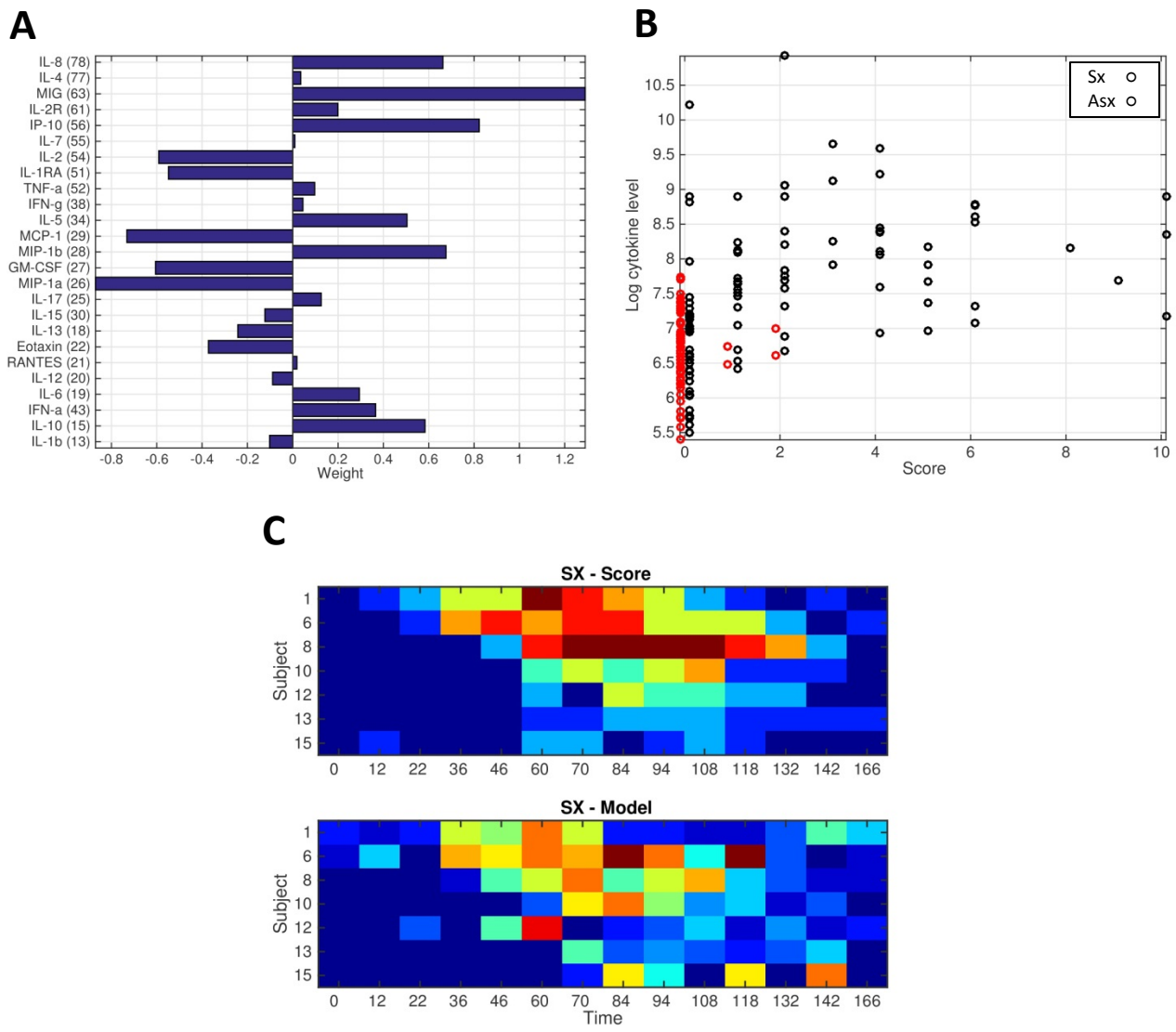

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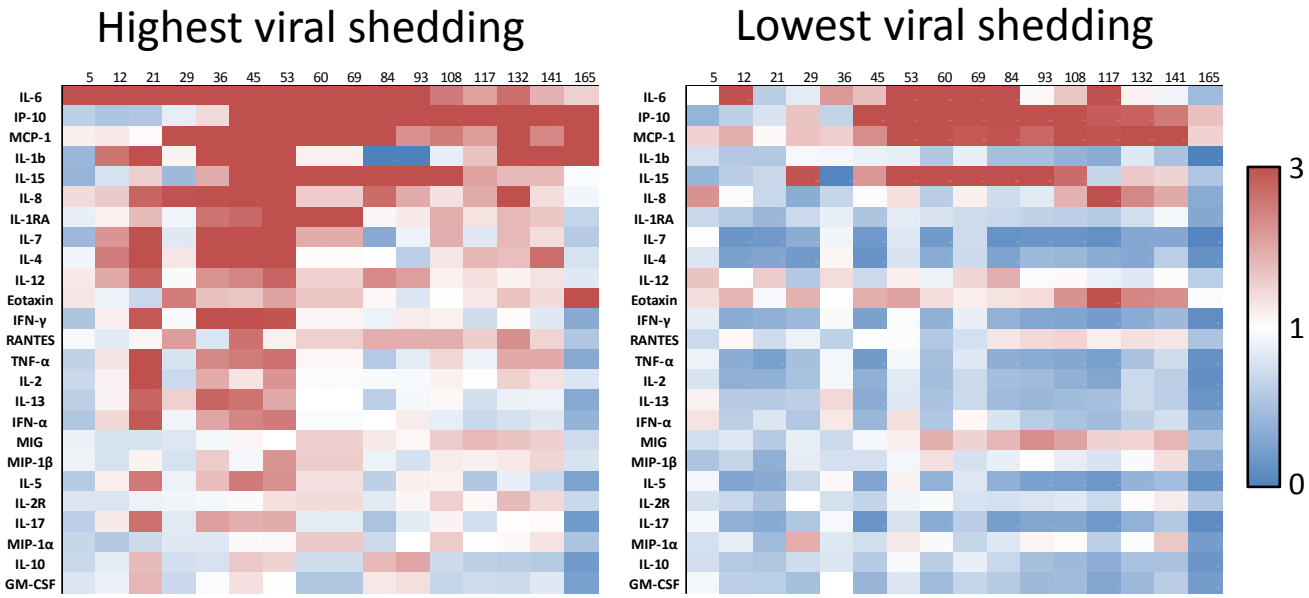

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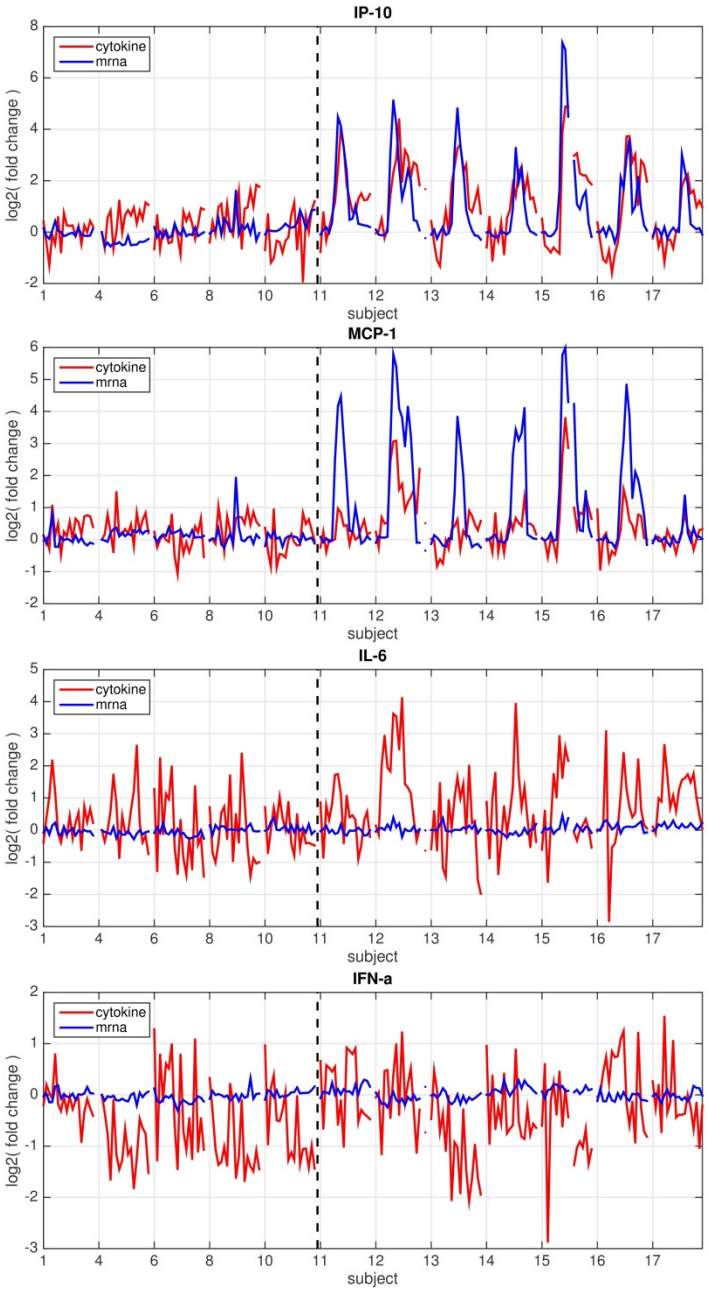

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