Supplemental Methods

Institutional Review Board Approvals

The Influenza challenge protocols were approved by the East London and City Research Ethics Committee 1 (London, UK), an independent institutional review board (WIRB: Western Institutional Review Board; Olympia, WA), the IRB of Duke University Medical Center (Durham, NC), and the SSC-SD IRB (US Department of Defense; Washington, DC) and were conducted in accordance with the Declaration of Helsinki. All subjects enrolled in viral challenge studies provided written informed consent per standard IRB protocol. Funding for this study was provided by the US Defense Advanced Research Projects Agency (DARPA) through contract N66001-07-C-2024 (P.I., Ginsburg).

Human viral challenges

In collaboration with Retroscreen Virology, Ltd (London, UK), 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 were excluded for positive baseline antibody titers to the strain of influenza utilized. After 24 hours in quarantine, we instilled of 10^6 $TCID_{50}$ influenza A into bilateral nares of subjects using standard methods.[1] The virus was manufactured and processed under current good manufacturing practices (cGMP) by Baxter BioScience, (Vienna, Austria). At pre-determined intervals (q8h for the first 5d following 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 RT-PCR to assess the success and timing of infection[2]. 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 treatment either at 36 hours post-inoculation (Early Treatment arm) or at day 5 following inoculation (Standard Treatment arm). All subjects were negative by rapid antigen detection (BinaxNow Rapid Influenza Antigen; Inverness Medical Innovations, Inc) at time of discharge.

Clinical Case Definitions

Symptoms were recorded twice daily using a modified standardized symptom score [3]. The modified Jackson Score requires subjects to rank symptoms of upper respiratory infection (stuffy nose, scratchy throat, headache, cough, etc) on a scale of 0-3 of “no symptoms”, “just noticeable”, “bothersome but can still do activities” and “bothersome and cannot do daily activities”. For all cohorts, modified Jackson scores were tabulated to determine if subjects became symptomatic from the respiratory viral challenge. Symptom onset was defined as the first of 2 contiguous days with score of 2 or more. A modified Jackson score of ≥ 6 over a consecutive five day period was the primary indicator of symptomatic viral infection[4] and subjects with this score and a positive qualitative viral culture or quantitative RT-PCR for at least 2 consecutive days (beginning 24 hours after inoculation) were denoted as ”symptomatic infection” and included in the signature performance analyses.[3-5]. Subjects were classified as “asymptomatic, not infected” if the symptom score was less than 6 over the five days of observation and viral shedding was not documented after the first 24 hours subsequent to inoculation as above. Standardized symptom scores were tabulated at the end of each study to determine attack rate and time of maximal symptoms. Some subjects in each study demonstrated an overall picture that fell in between these two categories. These individuals were either ‘asymptomatic viral shedders’ or ‘symptomatic non-viral shedders’. Given the heterogeneity of their overall ‘infected’ status these individuals were not included in performance analyses.

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. 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 also performed at Expression Analysis (Durham, NC) using the GeneChip® Human Genome U133A 2.0 Array (Affymetrix, Santa Clara, CA) as previously described[1]. Microarray data used for this study will be deposited in GEO prior to publication.

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.

Statistical analyses

Comparison of distributions of continuous variables (aggregate symptoms, total viral shedding, time to onset and resolution of symptoms) by treatment group were evaluated with means and ranges and the statistical significance of differences by treatment group were evaluated

with the Wilcoxon rank-sum test.

Factor score derivation

The mRNA abundance levels were extracted from 482 gene chips and abundance levels were normalized utilizing robust multi-array (RMA)[6] with the custom definition file Hs133Av2_Hs_ENTREZG.cdf (Brainarray version 10), as in our previous studies. Combat software, which implements the parametric and nonparametric empirical Bayes frameworks, was then used to compensate for batch effects[7]. Following RMA normalization and batch correction of the raw probe data, similarly to previous studies [Huang PLoS Genetics], we applied a positivity and sum-to-one constrained factor analysis method[8] to perform dimensionality reduction, decomposing the data into different components or pathways. Factor analysis factors the data matrix **Y** of mRNA abundances into a matrix product **MA** where each column of **M** is a factor loading and each column of **A** is a set of chip-specific factor scores corresponding to relative contributions of the individual factors (columns of **M).** Factor analysis can be interpreted as the following factor regression model

$$\mathbf{Y} = \mathbf{MA} + \mathbf{N}$$

where the columns of **M** plays the role of regressor variables and the columns of **A** play the role of regression coefficients. Positivity and sum-to-one constrained factor analysis constrains both **M** and **A** to be non-negative and the columns of **A** to sum to one. Thus each column of **M** represents a set of factor weights whose magnitudes specify the relative contribution of each gene present in that factor, and each column of **A** specifies the proportions of these factors that are present in each chip. Such positivity constraints are natural in gene microarray analysis as the expression intensity measurements of genes are always non-negative.

Once the factor analysis was completed, we explored the biological relevance of any particular factor by examining the genes that are "in" that factor -- the genes that show significantly non-zero factor loadings. Notably, the initial models built to determine factors that distinguish symptomatic infected individuals from asymptomatic individuals were derived using an unsupervised process (i.e., the model classified subjects based on gene expression pattern alone, without a priori knowledge of infection status).

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2. Gharabaghi F, Tellier R, Cheung R, et al. Comparison of a commercial qualitative real-time RT-PCR kit with direct immunofluorescence assay (DFA) and cell culture for detection of influenza A and B in children. J Clin Virol **2008**; 42:190-3.
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4. Turner RB. Ineffectiveness of intranasal zinc gluconate for prevention of experimental rhinovirus colds. Clin Infect Dis **2001**; 33:1865-70.
5. Barrett B, Brown R, Voland R, Maberry R, Turner R. Relations among questionnaire and laboratory measures of rhinovirus infection. Eur Respir J **2006**; 28:358-63.
6. Dai M, Wang P, Boyd AD, et al. Evolving gene/transcript definitions significantly alter the interpretation of GeneChip data. Nucleic Acids Res **2005**; 33:e175.
7. Johnson WE, Li C, Rabinovic A. Adjusting batch effects in microarray expression data using empirical Bayes methods. Biostatistics **2007**; 8:118-27.
8. Bazot C, Dobigeon N, Tourneret JY, Zaas AK, Ginsburg GS, Hero AO, 3rd. Unsupervised Bayesian linear unmixing of gene expression microarrays. BMC Bioinformatics **2013**; 14:99.

Supplemental Table 1: Cytokine levels in peripheral blood

Cytokine	Std (pg/mL)	Early (pg/mL)
IL-1b	8.0	5.0*
IL-1RA	616.6	548.1*
IL-2	9.0	8.6
IL-2R	338.2	320.6
IL-4	26.4	21.1*
IL-5	22.0	16.7*
IL-6	24.1	17.2*
IL-7	77.2	53.9*
IL-8	17.8	15.0
IL-10	35.3*	46.7
IL-12	173.6*	195.4
IL-13	93.5	74.3*
IL-15	157.2	137.7
IL-17	56.3	48.5
IFN-α	102.9	94.6
IFN-γ	25.5	19.3*
TNF-α	21.1	15.7*
IP-10	1970.7	1794.7
MCP-1	186.5*	222.3
MIG	128.5	107.3
MIP-1α	70.4	61.4*
MIP-1β	161.6	133.4*
RANTES	1433.0	1672.9
GM-CSF	89.1	82.8
Eotaxin	26.8*	33.3

Average total levels of cytokine expression across the study for individuals who received either Standard Oseltamivir (120 hours post-inoculation, 'Std') or Early Oseltamivir (36 hours post-inoculation,' Early').
* denotes statistically lower levels compared to alternate treatment regimen (p-value <0.05).

Supplemental Table 2: Top 50 genes in the influenza gene signature

Woods et al*	Current Signature		Woods et al*
APOL6	**AIM2**	**LY6E**	LOC26010
ATF3	**ATF3**	**MS4A4A**	LY6E
CXCL10	**CXCL10**	**MT2A**	MX1
DDX58	**DDX58**	**MX1**	MX2
DDX60	**DDX60**	**OAS1**	OAS1
EIF2AK2	**EIF2AK2**	**OAS2**	OAS2
GBP1	**GBP1**	**OAS3**	OAS3
HERC5	**GCH1**	**OASL**	OASL
HERC6	**HERC5**	**PARP12**	PARP12
IFI16	**HERC6**	**PLSCR1**	PLSCR1
IFI27	**IFI27**	**RSAD2**	RSAD2
IFI35	**IFI35**	**RTP4**	RTP4
IFI44	**IFI44**	**SAMD4A**	SCO2
IFI44L	**IFI44L**	**SCO2**	SERPING1
IFI6	**IFI6**	**SERPING1**	SIGLEC1
IFIH1	**IFIH1**	**SIGLEC1**	STAT1
IFIT1	**IFIT1**	**STAT1**	TNFAIP6
IFIT2	**IFIT2**	**TNFAIP6**	TNFSF10
IFIT3	**IFIT3**	**TNFSF10**	TOR1B
IFIT5	**IFIT5**	**TOR1B**	TRIM22
IFITM3	**IRF7**	**TRIM22**	TRIM5
IRF7	**ISG15**	**UBE2L6**	UBE2L6
ISG15	**LAMP3**	**XAF1**	XAF1
LAMP3	**LAP3**	**ZBP1**	ZBP1
LAP3	**LOC26010**	**ZCCHC2**	ZCCHC2

*Top 50 genes from an Influenza gene signature derived from a prior challenge trial with the same strain of influenza virus - Woods et al, PLoS One 2013;8(1):e52198