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Systems biology and systems genetics — novel innovative approaches to study host–pathogen interactions during influenza infection☆

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Influenza represents a serious threat to public health with thousands of deaths each year. A deeper understanding of the host–pathogen interactions is urgently needed to evaluate individual and population risks for severe influenza disease and to identify new therapeutic targets. Here, we review recent progress in large scale omics technologies, systems genetics as well as new mathematical and computational developments that are now in place to apply a systems biology approach for a comprehensive description of the multidimensional host response to influenza infection. In addition, we describe how results from experimental animal models can be translated to humans, and we discuss some of the future challenges ahead.

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Introduction

Every year, about 500 million people worldwide are infected with the influenza A virus (IAV), and about 0.5 million die from the infection. The most severe pandemic in 1918 resulted in about 30–50 million deaths worldwide. Newly emerging H5N1 and H7N9 avian IAV cause lethal infections in humans but have not yet acquired the potential to spread from human to human. Our options for treatment of IAV infections are very limited since only two drugs for therapeutic interventions are available, and resistance to both has been observed. Furthermore, no reliable biomarkers exist for early prediction of severe progression of disease, co-infection or host risk factors. Classical virology has mostly investigated molecular mechanisms at the cellular level, such as adhesion, entry, replication and assembly of viruses. However, not only viral but also many host factors, like the quality and quantity of immune cell responses, bacterial co-infections, genetic predisposition and other health risks are crucial determinants for the course of an infection and potential lethal outcomes.

Thus, it is necessary to identify the host factors that are required to successfully fight an infection or that cause adverse responses. For this, a highly integrated research strategy is needed to understand all aspects of the complex interplay between the host and invading pathogen. This systematic approach has to go way beyond *in vitro* cell culture systems and needs to address all aspects of host–virus interactions at the molecular, cellular, organ, and organism level.

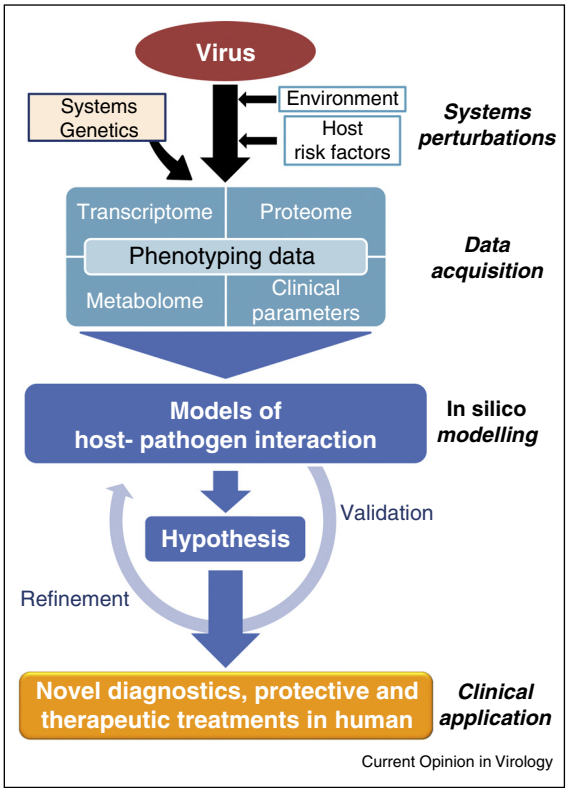
In this review, we summarize recent advances in omics data collection and systems genetics that were used to reveal crucial molecular interaction networks. In addition, we describe how systems biology in experimental models should be combined with analyses in patients to create predictive *in silico* models for humans, and we address some of the challenges that still need to be solved.

Omics data as the basis for systems biology

The first necessary step for a systems biology approach (Figure 1) is the gathering of large amounts of data that should be as comprehensive as possible. Analysis of the transcriptome is presently one of the few omics technologies that can be easily performed, and that records all changes for all annotated, transcribed regions.

Global gene expression changes using microarrays have been investigated in lungs of mice, ferrets or macaques after infection with different IAV subtypes and variants [1–4,5•,6–12]. Also, the expression of miRNAs has been studied [13–15]. Thus far, only few studies have used high-throughput RNA sequencing (RNAseq) for analyses of the host transcriptome after IAV infection [9,11,12] but more may be expected in the near future, because this new technology has several advantages, for example the analysis of virus gene expression in parallel with host genes. The overall conclusions from these transcriptome analyses were that the induced host

Figure 1



Systems analysis approach to study host–pathogen interactions during influenza infection. Infection by influenza A virus causes massive perturbation of the biological system (host). Here, infection dose and virulence of the virus are the most important variables. In addition, other influences, such as environment (diet, exposure) and host risk factors (age, sex, life style, co-morbidities) also influence the systems response. These variables and influences can be standardized and modified in a controlled fashion in animal models, whereas in humans they represent unknown confounding factors. Host genetic variations represent another host factor modifying the system response. It can be exploited in a systems biology approach in animal models as an additional controlled variable to systematically perturb the system. All perturbations result in systems responses which can be recorded at the level of transcriptome, proteome, metabolome and clinical parameters (body weight loss, survival, viral load, or immune response). Based on these large data sets, mathematical and computational methods are used to develop *in silico* models of host–pathogen interactions. The *in silico* models then allow for development of hypotheses about crucial networks, hubs, bottlenecks which are validated in animal models and humans, and then refined. The ultimate outcome of a systems biology approach is the identification of new targets and strategies for prevention, diagnosis, risk assessment and treatment of severe influenza disease in humans.

inflammatory response correlates with the severity of disease and depends on viral subtype and strain, viral dose and host genetic background. Severe and mild infections do not differ much in the type of activated genes, but rather in the magnitude of up-regulation of host response genes. Infections with highly pathogenic viruses resulted in an earlier and more sustained up-regulation of inflammatory genes (reviewed in [16,17]). Thus, in infections with a severe outcome, both virus spread and a strong host immune response result in the destruction of lung cells and eventually the failure of the respiratory system [18].

The first studies in experimental models that use more advanced mathematical and computational methods for the analysis of host–pathogen interactions have just been published. Integration of phenotypic and transcriptomic data from H1N1-infected mice identified sets of transcript modules that correlate with body weight, clinical score, viral load, histopathology, and weight loss at day 4 post infection [5•]. Another study used expression data from pre-CC lines with extreme phenotypes, expression quantitative trait loci (eQTL) mapping and structural equation modeling. The authors identified three genes that were highly connected with putative causal correlations to many other genes in the lungs of H1N1 infected mice [6]: *Ifi27l2a* related to downstream genes involved in signal transduction and transportation, *Sh3gl3* to cellular growth and development genes, and *Kcmf1* to genes involved in metabolic processes. However, the role and contribution of other loci is less clear and interpretation is often limited because of poor annotations of the respective genes. Thus, a broad analysis of gene functions, for example large scale phenotyping in mouse knock-out mutants, is urgently needed to close this knowledge gap [19,20]. Other studies related transcriptome analysis with clinical, histopathological and viral parameters in macaques [21,22]. Clinical signs were consistent with those observed in humans and transcriptional changes revealed activation of the interferon pathways and innate immune responses as well as mediators for cell migration that related to the activation of inflammatory cells, histopathological signs of inflammation and tissue damage [22]. First transcriptional changes were observed after 6 h, inflammatory, antiviral and apoptotic genes were up-regulated after 12 h, and a shift to acquired immunity was observed after day 6 post infection [22].

Almost all studies thus far have concentrated on the innate phase of the host response. Only one publication presented a long-term time series transcriptome analysis for up to 60 days post infection in lungs after a non-lethal H1N1 infection in mice [23•]. In this publication, the different phases of the host response to an influenza infection were described as temporal changes in gene expression patterns. Further analysis of the time-series data using dynamic and time-varying gene regulatory network methodologies revealed the role of cell cycle genes both in innate and adaptive immunity. The pathogen–sensory pathways (e.g. RIG-I, NOD-like) showed a long-lasting association with other innate immune responses (e.g. NK cell cytotoxicity, cytokine/chemokine signaling [24]).

Global gene expression profiles were also used to determine sets of signature genes that are indicative of an IAV

infection. One report has collected results from various experimental studies in mice infected with either highly pathogenic H5N1, reconstructed 1918 influenza A virus or SARS, and has defined a set of influenza-specific signature genes by meta-analysis [25]. Another study described a set of 10 host signature genes that identified the infecting IAV strain in ferrets [10]. Moreover, the analysis of blood cell transcriptomes in experimentally infected volunteers revealed a set of influenza-specific signature genes in humans [26•,27,28]. Furthermore, a clinical investigation in patients with severe pneumonia has identified blood transcriptome signatures that distinguish IAV from other respiratory infections [29,30].

In addition, global gene expression profiles were used to follow immune cell infiltration in an infected lung by using cell-type specific gene expression profiles [23•,31]. These studies revealed that the infiltration of lymphoid cells can be followed by signature genes whereas it is more difficult to obtain profiles for myeloid cells. Recent attempts to create a comprehensive set of specific signature genes for immune cell populations should facilitate this approach in the future [32].

Proteomics or combined proteome and transcriptome analyses in experimental IAV infections of macaques demonstrated that in most cases changes at the transcriptional and protein levels were highly correlated. Also, some differences were found emphasizing the need for complementary proteome analyses [33,34]. Studies in H5N1-infected mice revealed that the host response, after infection with viruses of different virulence, mainly differed in the magnitude and velocity of the host response kinetics, rather than specific sets of regulated host genes [35].

Furthermore, bioactive lipid mediators have been analyzed by liquid chromatography/mass spectrometry and the results were integrated with gene expression. Data generated from animal studies as well as human patients, showed an increase in 5-lipoxygenase metabolites that correlated with the pathogenic phase, whereas 12/15-lipoxygenase metabolites were increased during the resolution phase [36].

The most advanced systems biology approach in which a full circle of omics studies, modeling, prediction of interactions, formulation of a hypothesis and finally experimental testing of the hypothesis has been performed was reported recently [37••]. The authors combined transcriptome with imaging and flow cytometry studies in mice and identified a chemokine-driven feed-forward circuit that triggered strong neutrophil responses in lethally infected mice. They then validated their findings by showing that experimental attenuation of neutrophil responses resulted in reduction of tissue damage and increased survival.

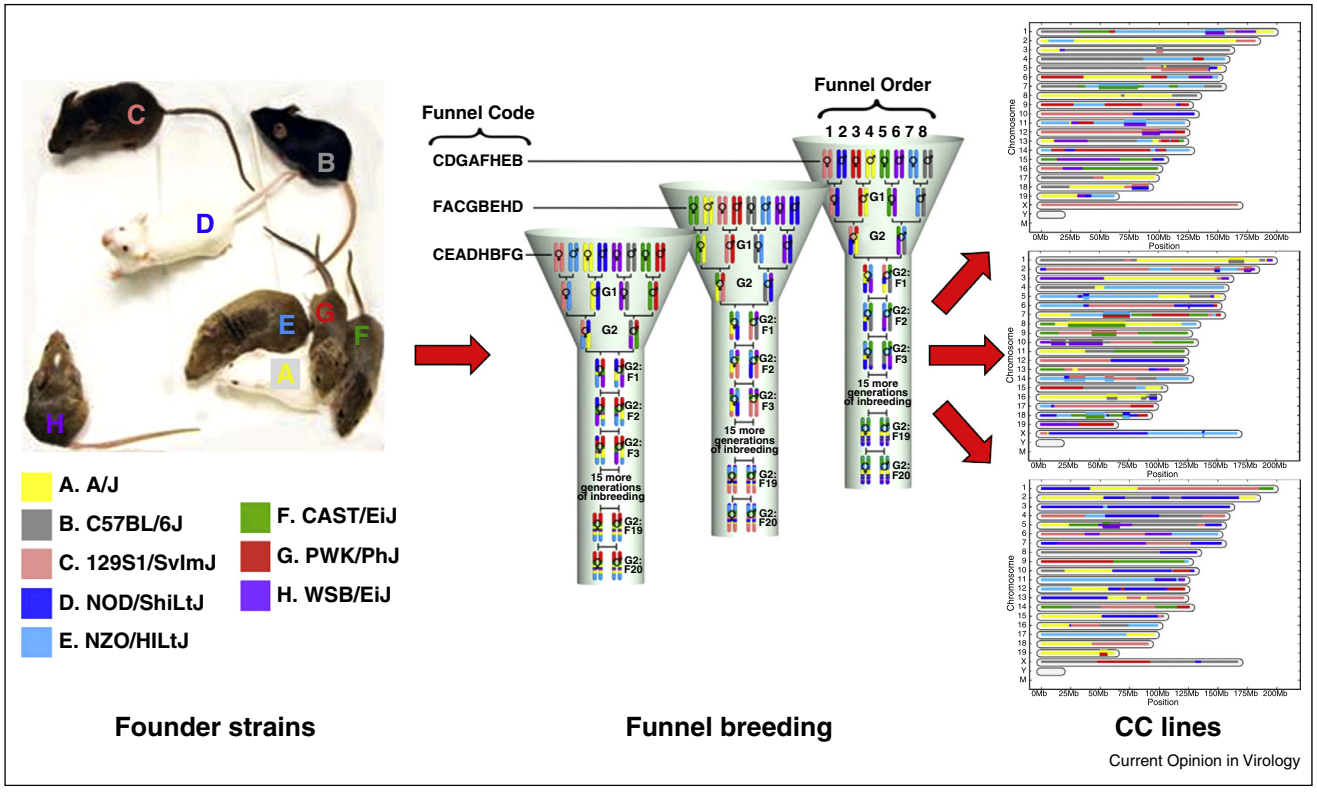
In conclusion, systems biology for influenza host–pathogen interactions in experimental animal models has just begun. The first integrated studies and network analyses have clearly demonstrated the advantage of a systems biology approach to better understand the complexity and multitude of host–pathogen interactions and to predict disease outcome. Although major challenges lie ahead, systems biology offers strong potential for new discoveries.

Systems genetics — an important new avenue

Systems genetics represents a new approach to evaluate the contribution of host genetics to the outcome of infectious diseases. Multiple phenotypes are collected in a genetic reference population (GRP) and subsequently associated with genetic variations. Using this approach, genomic regions (quantitative trait loci, QTLs) that regulate a given phenotypic trait can be identified. Many traits, including molecular omics data such as transcriptomes and proteomes can be collected on the same GRP. In this way, systems genetics connects traits with genes and gene networks, and will therefore become an important element in systems biology. Furthermore, systems genetics represents a hypothesis-free experimental approach in which completely new and previously unknown associations can be discovered.

The BXD and the AXB/BXA mouse strain collections are two GRPs that were created from two parental mouse strains. BXD are a set of recombinant inbred strains from C57BL/6J and DBA/2J as parents [38]. AXB/BXA represents a collection of recombinant congenic strains derived from A/J and C57BL/6J [39]. Analyses of these GRPs identified several QTLs after infection with H1N1, H3N2 or H5N1 [40–43]. Five QTLs on chromosomes 2, 7, 11, 15 and 17 associated with body weight loss and survival after infection of BXD strains with H5N1 virus [41], and the hemolytic complement gene (*Hc*) located in the chromosome 2 QTL was subsequently shown to influence viral titer at day 7 post infection. Infection of 53 BXD strains with H1N1 identified two significant QTLs on chromosomes 5 and 19 that regulated body weight loss, survival and mean time to death in a time-dependent manner [42]. Infection of 29 AcB/BcA recombinant congenic strains with mouse-adapted H3N2 virus revealed sex-specific clinical QTLs for survival on chromosomes 2 and 17 [40]. Using cis-eQTLs as a means to search for the most likely quantitative trait genes, the authors identified also *Hc* as a candidate on chromosome 2, and *Tnfrsf21* and *Pla2g7* in the chromosome 17 QTL intervals. Further studies with more mice and congenic lines will be required in the future to identify additional quantitative trait genes and to confirm their causal role. In this context, it is important to note that the results from these GRPs have been deposited in a publically available database, GeneNetwork [44], that greatly facilitates the correlation of influenza host responses to thousands of other phenotypes and transcriptomes.

Figure 2



Generation of the Collaborative Cross resource. The Collaborative Cross (CC) [45,67] is the result of a ten-year collaborative effort by the mouse genetics community to create a large, genetically highly diverse population (CC lines) for phenotyping and mapping studies. The population has been generated from eight founder strains, A/J, C57BL/6J, 129S1/SvImJ, NOD/ShiLtJ, NZO/HlLtJ, WSB/EiJ, PWK/PhJ, and CAST/EiJ (Photo: Brynn H. Voy, University of Tennessee). These strains represent the three major *Mus musculus* subspecies: *M. m. domesticus*, *M. m. musculus*, and *M. m. castaneus*. Five of the founder strains are common laboratory strains, and three are wild-derived inbred strains. After inbreeding of the founder strains for two generations, and subsequent inbreeding for more than 20 generations (breeding scheme: [45] with permission of the Genetics Society of America) has resulted in the generation of CC lines (http://csbio.unc.edu/CCstatus/). The genetic diversity of the CC lines is similar to that of the human population and thus represents an unprecedented and unique resource for genetic mapping and correlation studies [45,68].

The Collaborative Cross (CC [45]) is a novel mouse GRP with unprecedented genotypic and phenotypic variation generated from eight founder strains, including three wild-derived strains (Figure 2). The CC population is genetically as diverse as the human population. This resource has just become available, and we can expect the first results to appear within the next years. In the meantime, several research groups have started to phenotype mice from emerging CC lines (pre-CC lines) that were not yet fully inbred. Analysis of the host responses at day 4 post infection with IAV , such as inflammation, viral replication, body weight loss and survival studies identified several QTLs regulating body weight loss, viral titer, pulmonary edema, neutrophil recruitment and expression levels of host genes [5•]. A highly significant QTL on chromosome 16 that explained most of the phenotypic variance for body weight loss contained the well-known *Mx1* resistance gene representing the most likely candidate. A surprising finding was that the wild-derived CAST/EiJ founder strain carrying a presumably functional *Mx1* allele showed reduced ability to inhibit viral replication at day 4 post infection [5•]. Additional significant QTLs were identified on chromosome 7 after controlling for the *Mx1* QTL and on chromosome 1 by performing a mapping analysis only in the subpopulation that carried a non-functional *Mx1* allele. Furthermore, immunophenotyping of 66 pre-CC lines and the CC founder strains revealed highly significant QTLs controlling B/T cell ratio, CD8 T-cell numbers, and expression of CD11c and CD23. The CD23 regulating QTL represented a cis-QTL containing the CD23 encoding gene *Fcer2a* [46]. Thus, it should soon be possible to correlate the genetically controlled variations in immune cells and other phenotypic traits in CC mice with influenza-associated host responses.

In conclusion, systems genetics has collected first sets of omics data and correlated them with clinical phenotypes. In the future, we can expect that many more phenotypic traits will be accumulated from the CC population and

thereby contribute significantly to a systems analysis of host–pathogen interactions.

From animal models to human patients

The next step will be to translate the knowledge from systems biology into systems medicine to predict disease outcome in humans. First omics results from the blood of humans have been obtained from experimentally infected volunteers [26•,27,28] and from infected patients [29,30,47]. These studies revealed a pronounced activation of granulocytes and increase in chemokines and cytokines. However, studies in human patients are compromised because they have an uncertain history and are often confounded by many unknown intrinsic and extrinsic factors. Furthermore, controlled experimental infection in human volunteers can only be performed to a very limited extend for obvious ethical reasons. Therefore, experimental animal models must lead the way. Initial *in silico* models will have to be generated from extensive datasets obtained in animal models because they allow collection of large datasets from infected organs and body fluids simultaneously and at a very high spatial and temporal resolution. Crucial pathways and players can then be identified, tested experimentally and finally integrated with data obtained from human patients.

It should be noted that the value of the mouse as model system for studying inflammatory responses in humans has recently been debated [48]. However, in the case of influenza, the responses in mice are very similar to those in human and non-human primates, results are very reproducible, and the kinetics of pathological symptoms are identical. Environmental factors like nutrition, age, sex, among others and even genetics can be easily controlled in murine models. A comparative analysis of the transcriptional regulatory networks in human cell culture and experimental mouse and macaque model systems after H5N1 infections observed conserved host responses [49•]. Similar clinical signs were observed in mouse, macaque and swine after pH1N1 infection, whereas differences were detected for the kinetics of expression of inflammatory genes. In addition genes associated with the retinoid X receptor signaling pathway were found to be differentially regulated between species [50].

There is an urgent clinical need for biomarkers that allow predicting the severe course of disease in humans. A study in patients with severe and mild infections found elevated levels of MCP-3 and IFNα2 in nasal lavage, and increased IL-10 levels in plasma, that were correlated with progression to severe disease [51]. Plasma appeared to provide a poor reflection of the immune profile in the lung [51]. Until now, only few omics studies in experimental animal models have been performed from peripheral blood [11], and only a limited number of chemokines and cytokines have been measured in broncho-alveolar lavages (BAL). It will now be important to relate and complement these findings by including a systems approach in animal models where proteome, transcriptome and metabolome markers in the blood, broncho-alveolar lavages or nasal washes are measured. These changes have to be correlated with clinical parameters as well as with pathology and viral load in the infected lung. In the future it will be necessary to address clinical needs more precisely by designing even more advanced experimental models that offer better transferability to humans.

The development of more efficient vaccines, especially for the elderly or very young, is another important goal in preventing influenza disease in humans. Several studies systematically profiled host responses to vaccination in humans [52•,53,54]. One report identified early molecular signatures in the transcriptomes of peripheral blood monocytes that correlated with induction of hemagglutinin-neutralizing antibody responses [52•]. Their theoretical model predicted CAMK4 as a negative regulator, and its function could subsequently be confirmed in *Camk4* knock-out mice. Future systems biology studies in animal models may contribute substantially to the development of more efficient vaccines by studying the host response at the interface of innate and adaptive immunity in more detail.

In conclusion, the mouse and other experimental animal models represent excellent systems to use systems biology for a comprehensive description of host–pathogen interactions during IAV infections, leading the way to systems medicine in humans.

Challenges ahead

Clearly, systems biology has entered the field of influenza virology but there are still major challenges ahead.

Proteomics is as important as transcriptomics for systems analysis because many signaling pathways are activated by posttranslational modifications or by protein degradation [55]. However, proteomics (especially at the organ and tissue level) still has major limitations to overcome, such as detection of low-abundant species, incomplete proteome coverage, and narrow dynamic range.

Thus far, we record omics changes at the level of the whole lung, but we need to understand in which cell types these changes occur. If we want to understand the cross-talk between gene signaling pathways and identify crucial points of connectivity in the network, it will be essential to know the expression profiles of individual cells and the quantitative changes of immune cell populations over time in the infected organ. For this, future developments in single cell transcriptome, metabolome and proteomics analysis (reviewed by [56]) (e.g. cells isolated by laser capture from tissue sections or cell sorting) should provide a higher resolution that is required for improved modeling.

Various computational approaches have been used to identify relevant host–pathogen-interaction networks in cell cultures [4,57–63]. These studies represent a highly valuable knowledge base that will have to be integrated into *in silico* models from whole organisms. Thus, a future challenge will be to develop the appropriate computational models that enable this integration into a single model.

Systems genetics builds on the integration of large data sets which should be easily accessible. The value of meta-analyses of different studies has recently been demonstrated by comparing transcriptome results from mice infected with four different respiratory viruses [25]. There is an urgent need to establish publically available comprehensive databases. First initiatives for transcriptome data have started [64], and the NIH-funded 'Systems Biology Program for Infectious Disease Research' consortium represents an essential step in this direction [65]. However, existing resources need to be expanded in the future to also include results from phenotypic studies from experimental model systems and humans.

Furthermore, it will be necessary to have a knowledge base that describes all known interactions at the molecular and cellular level, and that is constantly updated and validated by the scientific community. A first influenza map has been created for host–pathogen interactions at the level of an infected cell [66••]. In the future, it will be essential to expand these interaction maps to also include host responses at the level of the whole organism, such as cross talk between infected epithelial cells and immune cells, immune cell activation, tissue destruction and tissue remodeling.

Conclusion

Systems biology of influenza host–pathogen interactions has just begun. Large data sets have been collected, and more sophisticated mathematical and computational approaches are being used to provide a holistic view of the many molecular and cellular interactions involved, and to correlate them with clinical outcomes. The ultimate goal is to generate *in silico* models where crucial pathways, hubs and bottlenecks can be identified, leading to new targets and strategies for prevention, diagnosis, risk assessment and treatment of severe influenza disease in human. Although there is still a long way to go, exciting times in this emerging field of systems virology are lying ahead.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Cilloniz C, Shinya K, Peng X, Korth MJ, Proll SC, Aicher LD, Carter VS, Chang JH, Kobasa D, Feldmann F *et al.*: **Lethal influenza virus infection in macaques is associated with early dysregulation of inflammatory related genes**. *PLoS Pathog* 2009, **5**:e1000604.

2. Alberts R, Srivastava B, Wu H, Viegas N, Geffers R, Klawonn F, Novoselova N, do Valle TZ, Panthier JJ, Schughart K: **Gene expression changes in the host response between resistant and susceptible inbred mouse strains after influenza A infection**. *Microbes Infect* 2010, **12**:309-318.

3. Ding M, Lu L, Toth LA: **Gene expression in lung and basal forebrain during influenza infection in mice**. *Genes Brain Behav* 2008, **7**:173-183.

4. Li C, Bankhead A 3rd, Eisfeld AJ, Hatta Y, Jeng S, Chang JH, Aicher LD, Proll S, Ellis AL, Law GL *et al.*: **Host regulatory network response to infection with highly pathogenic H5N1 avian influenza virus**. *J Virol* 2011, **85**:10955-10967.

5. • Ferris MT, Aylor DL, Bottomly D, Whitmore AC, Aicher LD, Bell TA, Bradel-Tretheway B, Bryan JT, Buus RJ, Gralinski LE *et al.*: **Modeling host genetic regulation of influenza pathogenesis in the collaborative cross**. *PLoS Pathog* 2013, **9**:e1003196.
The first extensive investigation of influenza phenotypes in the emerging Collaborative Cross (pre-CC lines) in which many phenotypic responses were correlated with transcriptome changes. The study reveals expected but also unexpected trait correlations.

6. Bottomly D, Ferris MT, Aicher LD, Rosenzweig E, Whitmore A, Aylor DL, Haagmans BL, Gralinski LE, Bradel-Tretheway BG, Bryan JT *et al.*: **Expression quantitative trait Loci for extreme host response to influenza a in pre-collaborative cross mice**. *G3 (Bethesda)* 2012, **2**:213-221.

7. Cilloniz C, Pantin-Jackwood MJ, Ni C, Carter VS, Korth MJ, Swayne DE, Tumpey TM, Katze MG: **Molecular signatures associated with Mx1-mediated resistance to highly pathogenic influenza virus infection: mechanisms of survival**. *J Virol* 2012, **86**:2437-2446.

8. Bruder CE, Yao S, Larson F, Camp JV, Tapp R, McBrayer A, Powers N, Granda WV, Jonsson CB: **Transcriptome sequencing and development of an expression microarray platform for the domestic ferret**. *BMC Genomics* 2010, **11**:251.

9. Leon AJ, Banner D, Xu L, Ran L, Peng Z, Yi K, Chen C, Xu F, Huang J, Zhao Z *et al.*: **Sequencing, annotation, and characterization of the influenza ferret infectome**. *J Virol* 2013, **87**:1957-1966.

10. Ljungberg K, McBrayer A, Camp JV, Chu YK, Tapp R, Noah DL, Grimes S, Proctor ML, Liljestrom P, Jonsson CB *et al.*: **Host gene expression signatures discriminate between ferrets infected with genetically similar H1N1 strains**. *PLoS ONE* 2012, **7**:e40743.

11. Camp JV, Svensson TL, McBrayer A, Jonsson CB, Liljestrom P, Bruder CE: **De-novo transcriptome sequencing of a normalized cDNA pool from influenza infected ferrets**. *PLoS ONE* 2012, **7**:e37104.

12. Zou W, Chen D, Xiong M, Zhu J, Lin X, Wang L, Zhang J, Chen L, Zhang H, Chen H *et al.*: **Insights into the increasing virulence of the swine-origin pandemic H1N1/2009 influenza virus**. *Sci Rep* 2013, **3**:1601.

13. Li Y, Chan EY, Li J, Ni C, Peng X, Rosenzweig E, Tumpey TM, Katze MG: **MicroRNA expression and virulence in pandemic influenza virus-infected mice**. *J Virol* 2010, **84**:3023-3032.

14. Li Y, Li J, Belisle S, Baskin CR, Tumpey TM, Katze MG: **Differential microRNA expression and virulence of avian reassortant, and reconstructed 1918 influenza A viruses**. *Virology* 2011, **421**:105-113.

15. Peng X, Gralinski L, Ferris MT, Frieman MB, Thomas MJ, Proll S, Korth MJ, Tisoncik JR, Heise M, Luo S *et al.*: **Integrative deep**

sequencing of the mouse lung transcriptome reveals differential expression of diverse classes of small RNAs in response to respiratory virus infection. *MBio* 2011, **2**.

16. Tisoncik JR, Korth MJ, Simmons CP, Farrar J, Martin TR, Katze MG: **Into the eye of the cytokine storm**. *Microbiol Mol Biol Rev* 2012, **76**:16-32.

17. Korth MJ, Tchitchek N, Benecke AG, Katze MG: **Systems approaches to influenza–virus host interactions and the pathogenesis of highly virulent and pandemic viruses**. *Semin Immunol* 2013, **25**:228-239.

18. Short KR, Kroeze EJ, Fouchier RA, Kuiken T: **Pathogenesis of influenza-induced acute respiratory distress syndrome**. *Lancet Infect Dis* 2014, **14**:57-69.

19. Brown SD, Moore MW: **Towards an encyclopaedia of mammalian gene function: the International Mouse Phenotyping Consortium**. *Dis Model Mech* 2012, **5**:289-292.

20. Brown SD, Wurst W, Kuhn R, Hancock JM: **The functional annotation of mammalian genomes: the challenge of phenotyping**. *Annu Rev Genet* 2009, **43**:305-333.

21. Baskin CR, Garcia-Sastre A, Tumpey TM, Bielefeldt-Ohmann H, Carter VS, Nistal-Villan E, Katze MG: **Integration of clinical data, pathology, and cDNA microarrays in influenza virus-infected pigtailed macaques (*Macaca nemestrina*)**. *J Virol* 2004, **78**:10420-10432.

22. Shinya K, Gao Y, Cilloniz C, Suzuki Y, Fujie M, Deng G, Zhu Q, Fan S, Makino A, Muramoto Y *et al.*: **Integrated clinical, pathologic, virologic, and transcriptomic analysis of H5N1 influenza virus-induced viral pneumonia in the rhesus macaque**. *J Virol* 2012, **86**:6055-6066.

23. • Pommerenke C, Wilk E, Srivastava B, Schulze A, Novoselova N, Geffers R, Schughart K: **Global transcriptome analysis in influenza-infected mouse lungs reveals the kinetics of innate and adaptive host immune responses**. *PLoS ONE* 2012, **7**:e41169.

A comprehensive analysis of changes at the transcriptome level in a non-lethal infection in mice until day 60 post infection providing a description of the different phases of the host response such as innate, adaptive immunity and recovery stages.

24. Dimitrakopoulou K, Dimitrakopoulos GN, Wilk E, Tsimpouris C, Sgarbas KN, Schughart K, Bezerianos A: **Influenza A immunomics and public health omics: the dynamic pathway interplay in host response to H1N1 infection**. *OMICS* 2014, **18**:167-183.

25. Chang ST, Tchitchek N, Ghosh D, Benecke A, Katze MG: **A chemokine gene expression signature derived from meta-analysis predicts the pathogenicity of viral respiratory infections**. *BMC Syst Biol* 2011, **5**:202.

26. • Woods CW, McClain TA, Chen M, Zaas KA, Nicholson PB, Varkey J, Veldman T, Kingsmore FS, Huang Y, Lambkin-Williams R *et al.*: **A host transcriptional signature for presymptomatic detection of infection in humans exposed to influenza H1N1 or H3N2**. *PLoS ONE* 2013, **8**:e 52198.

This study analyzed blood transcriptome from experimentally H1N1 and H3N2 infected human volunteers.

27. Chen M, Zaas A, Woods C, Ginsburg GS, Lucas J, Dunson D, Carin L: **Predicting viral infection from high-dimensional biomarker trajectories**. *J Am Stat Assoc* 2011, **106**:1259-1279.

28. Zaas AK, Burke T, Chen M, McClain M, Nicholson B, Veldman T, Tsalik EL, Fowler V, Rivers EP, Otero R *et al.*: **A host-based rt-PCR gene expression signature to identify acute respiratory viral infection**. *Sci Transl Med* 2013, **5**:203ra126.

29. Parnell GP, McLean AS, Booth DR, Armstrong NJ, Nalos M, Huang SJ, Manak J, Tang W, Tam OY, Chan S *et al.*: **A distinct influenza infection signature in the blood transcriptome of patients with severe community-acquired pneumonia**. *Crit Care* 2012, **16**:R157.

30. Herberg JA, Kaforou M, Gormley S, Sumner ER, Patel S, Jones KD, Paulus S, Fink C, Martinon-Torres F, Montana G *et al.*: **Transcriptomic profiling in childhood H1N1/09 influenza reveals reduced expression of protein synthesis genes**. *J Infect Dis* 2013, **208**:1664-1668.

31. Shoemaker JE, Lopes TJ, Ghosh S, Matsuoka Y, Kawaoka Y, Kitano H: **CTen: a web-based platform for identifying enriched cell types from heterogeneous microarray data**. *BMC Genomics* 2012, **13**:460.

32. Kim CC, Lanier LL: **Beyond the transcriptome: completion of act one of the Immunological Genome Project**. *Curr Opin Immunol* 2013, **25**:593-597.

33. Baas T, Baskin CR, Diamond DL, Garcia-Sastre A, Bielefeldt-Ohmann H, Tumpey TM, Thomas MJ, Carter VS, Teal TH, Van Hoeven N *et al.*: **Integrated molecular signature of disease: analysis of influenza virus-infected macaques through functional genomics and proteomics**. *J Virol* 2006, **80**:10813-10828.

34. Brown JN, Palermo RE, Baskin CR, Gritsenko M, Sabourin PJ, Long JP, Sabourin CL, Bielefeldt-Ohmann H, Garcia-Sastre A, Albrecht R *et al.*: **Macaque proteome response to highly pathogenic avian influenza and 1918 reassortant influenza virus infections**. *J Virol* 2010, **84**:12058-12068.

35. Tchitchek N, Eisfeld AJ, Tisoncik-Go J, Josset L, Gralinski LE, Becavin C, Tilton SC, Webb-Robertson BJ, Ferris MT, Totura AL *et al.*: **Specific mutations in H5N1 mainly impact the magnitude and velocity of the host response in mice**. *BMC Syst Biol* 2013, **7**:69.

36. Tam VC, Quehenberger O, Oshansky CM, Suen R, Armando AM, Treuting PM, Thomas PG, Dennis EA, Aderem A: **Lipidomic profiling of influenza infection identifies mediators that induce and resolve inflammation**. *Cell* 2013, **154**:213-227.

37. •• Brandes M, Klauschen F, Kuchen S, Germain RN: **A systems analysis identifies a feedforward inflammatory circuit leading to lethal influenza infection**. *Cell* 2013, **154**:197-212.

This study describes the full circle of experimental studies, integration of results into a mathematical frame work, identification of crucial components and their subsequent experimental validation in animal models.

38. Peirce JL, Lu L, Gu J, Silver LM, Williams RW: **A new set of BXD recombinant inbred lines from advanced intercross populations in mice**. *BMC Genet* 2004, **5**:7.

39. Marshall JD, Mu JL, Cheah YC, Nesbitt MN, Frankel WN, Paigen B: **The AXB and BXA set of recombinant inbred mouse strains**. *Mamm Genome* 1992, **3**:669-680.

40. Boivin GA, Pothlichet J, Skamene E, Brown EG, Loredo-Osti JC, Sladek R, Vidal SM: **Mapping of clinical and expression quantitative trait loci in a sex-dependent effect of host susceptibility to mouse-adapted influenza H3N2/HK/1/68**. *J Immunol* 2012, **188**:3949-3960.

41. Boon AC, deBeauchamp J, Hollmann A, Luke J, Kotb M, Rowe S, Finkelstein D, Neale G, Lu L, Williams RW *et al.*: **Host genetic variation affects resistance to infection with a highly pathogenic H5N1 influenza A virus in mice**. *J Virol* 2009, **83**:10417-10426.

42. Nedelko T, Kollmus H, Klawonn F, Spijker S, Lu L, Hessmann M, Alberts R, Williams RW, Schughart K: **Distinct gene loci control the host response to influenza H1N1 virus infection in a time-dependent manner**. *BMC Genomics* 2012, **13**:411.

43. Trammell RA, Liberati TA, Toth LA: **Host genetic background and the innate inflammatory response of lung to influenza virus**. *Microbes Infect* 2012, **14**:50-58.

44. Chesler EJ, Lu L, Wang J, Williams RW, Manly KF: **WebQTL: rapid exploratory analysis of gene expression and genetic networks for brain and behavior**. *Nat Neurosci* 2004, **7**:485-486.

45. Collaborative Cross Consortium: **The genome architecture of the collaborative cross mouse genetic reference population**. *Genetics* 2012, **190**:389-401.

46. Phillippi J, Xie Y, Miller DR, Bell TA, Zhang Z, Lenarcic AB, Aylor DL, Krovi SH, Threadgill DW, de Villena FP *et al.*: **Using the emerging Collaborative Cross to probe the immune system**. *Genes Immun* 2014, **15**:38-46.

47. Berdal JE, Mollnes TE, Waehre T, Olstad OK, Halvorsen B, Ueland T, Laake JH, Furuseth MT, Maagaard A, Kjekshus H *et al.*: **Excessive innate immune response and mutant D222G/N in severe A (H1N1) pandemic influenza**. *J Infect* 2011, **63**:308-316.

48. Seok J, Warren HS, Cuenca AG, Mindrinos MN, Baker HV, Xu W, Richards DR, McDonald-Smith GP, Gao H, Hennessy L *et al.*: **Genomic responses in mouse models poorly mimic human inflammatory diseases**. *Proc Natl Acad Sci U S A* 2013, **110**:3507-3512.

49. • McDermott JE, Shankaran H, Eisfeld AJ, Belisle SE, Neuman G, Li C, McWeeney S, Sabourin C, Kawaoka Y, Katze MG *et al.*: **Conserved host response to highly pathogenic avian influenza virus infection in human cell culture, mouse and macaque model systems**. *BMC Syst Biol* 2011, **5**:190.

A comparative study of transcriptional networks in human cell culture, mouse and macaque after H5N1 infection demonstrating conserved responses between *in vivo* and *in vitro* studies and also between animal models and human cell lines.

50. Go JT, Belisle SE, Tchitchek N, Tumpey TM, Ma W, Richt JA, Safronetz D, Feldmann H, Katze MG: **2009 pandemic H1N1 influenza virus elicits similar clinical course but differential host transcriptional response in mouse, macaque, and swine infection models**. *BMC Genomics* 2012, **13**:627.

51. Oshansky CM, Gartland AJ, Wong SS, Jeevan T, Wang D, Roddam PL, Caniza MA, Hertz T, Devincenzo JP, Webby RJ *et al.*: **Mucosal immune responses predict clinical outcomes during influenza infection independently of age and viral load**. *Am J Respir Crit Care Med* 2014, **189**:449-462.

52. • Nakaya HI, Wrammert J, Lee EK, Racioppi L, Marie-Kunze S, Haining WN, Means AR, Kasturi SP, Khan N, Li GM *et al.*: **Systems biology of vaccination for seasonal influenza in humans**. *Nat Immunol* 2011, **12**:786-795.

Early molecular signatures in the transcriptome of peripheral blood monocytes were identified and correlated with induction of hemagglutinin-neutralizing antibody responses. Their theoretical model predicted CAMK4 as a negative regulator, and its function could subsequently be confirmed in *Camk4* knock-out mice.

53. Tan Y, Tamayo P, Nakaya H, Pulendran B, Mesirov JP, Haining WN: **Gene signatures related to B-cell proliferation predict influenza vaccine-induced antibody response**. *Eur J Immunol* 2014, **44**:285-295.

54. Henn AD, Wu S, Qiu X, Ruda M, Stover M, Yang H, Liu Z, Welle SL, Holden-Wiltse J, Wu H *et al.*: **High-resolution temporal response patterns to influenza vaccine reveal a distinct human plasma cell gene signature**. *Sci Rep* 2013, **3**:2327.

55. Cox J, Mann M: **Quantitative, high-resolution proteomics for data-driven systems biology**. *Annu Rev Biochem* 2011, **80**:273-299.

56. Wang D, Bodovitz S: **Single cell analysis: the new frontier in 'omics'**. *Trends Biotechnol* 2010, **28**:281-290.

57. Zaslavsky E, Nudelman G, Marquez S, Hershberg U, Hartmann BM, Thakar J, Sealfon SC, Kleinstein SH: **Reconstruction of regulatory networks through temporal enrichment profiling and its application to H1N1 influenza viral infection**. *BMC Bioinformatics* 2013, **14Suppl6**:S1.

58. Mitchell HD, Eisfeld AJ, Sims AC, McDermott JE, Matzke MM, Webb-Robertson BJ, Tilton SC, Tchitchek N, Josset L, Li C *et al.*: **A network integration approach to predict conserved regulators related to pathogenicity of influenza and SARS-CoV respiratory viruses**. *PLoS ONE* 2013, **8**:e69374.

59. Madrahimov A, Helikar T, Kowal B, Lu G, Rogers J: **Dynamics of influenza virus and human host interactions during infection and replication cycle**. *Bull Math Biol* 2013, **75**:988-1011.

60. Heldt FS, Frensing T, Pflugmacher A, Gropler R, Peschel B, Reichl U: **Multiscale modeling of influenza a virus infection supports the development of direct-acting antivirals**. *PLoS Comput Biol* 2013, **9**:e1003372.

61. Viemann D, Schmolke M, Lueken A, Boergeling Y, Friesenhagen J, Wittkowski H, Ludwig S, Roth J: **H5N1 virus activates signaling pathways in human endothelial cells resulting in a specific imbalanced inflammatory response**. *J Immunol* 2011, **186**:164-173.

62. Chakrabarti AK, Vipat VC, Mukherjee S, Singh R, Pawar SD, Mishra AC: **Host gene expression profiling in influenza A virus-infected lung epithelial (A549) cells: a comparative analysis between highly pathogenic and modified H5N1 viruses**. *Virol J* 2010, **7**:219.

63. Lee SM, Gardy JL, Cheung CY, Cheung TK, Hui KP, Ip NY, Guan Y, Hancock RE, Peiris JS: **Systems-level comparison of host-responses elicited by avian H5N1 and seasonal H1N1 influenza viruses in primary human macrophages**. *PLoS ONE* 2009, **4**:e8072.

64. Squires RB, Noronha J, Hunt V, Garcia-Sastre A, Macken C, Baumgarth N, Suarez D, Pickett BE, Zhang Y, Larsen CN *et al.*: **Influenza research database: an integrated bioinformatics resource for influenza research and surveillance**. *Influenza Other Respir Viruses* 2012, **6**:404-416.

65. Aderem A, Adkins JN, Ansong C, Galagan J, Kaiser S, Korth MJ, Law GL, McDermott JG, Proll SC, Rosenberger C *et al.*: **A systems biology approach to infectious disease research: innovating the pathogen–host research paradigm**. *MBio* 2011, **2**:e00310-e00325.

66. •• Matsuoka Y, Matsumae H, Katoh M, Eisfeld AJ, Neumann G, Hase T, Ghosh S, Shoemaker JE, Lopes TJ, Watanabe T *et al.*: **A comprehensive map of the influenza A virus replication cycle**. *BMC Syst Biol* 2013, **7**:97.

A comprehensive graphical map of the influenza A virus replication cycle presenting the knowledge of host–pathogen interactions at the cellular level. The corresponding website allows continuous updating and provides interactive online access.

67. Threadgill DW, Churchill GA: **Ten years of the collaborative cross**. *G3 (Bethesda)* 2012, **2**:153-156.

68. Aylor DL, Valdar W, Foulds-Mathes W, Buus RJ, Verdugo RA, Baric RS, Ferris MT, Frelinger JA, Heise M, Frieman MB *et al.*: **Genetic analysis of complex traits in the emerging Collaborative Cross**. *Genome Res* 2011, **21**:1213-1222.