



REVIEW

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What can we learn about influenza infection and vaccination from transcriptomics?

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ABSTRACT

Transcriptomics studies the set of RNA transcripts produced by the genome using high-throughput sequencing and bioinformatics. This growing field has revolutionized our understanding of host-pathogen interactions, revealing new insights into the host response to influenza infection and vaccination. Studies using transcriptomics have identified a unique immunosignature for influenza discernable from other bacterial and viral pathogens, key transcriptional factors that discriminate early from late, mild versus severe, and symptomatic versus asymptomatic infection. Recent studies evaluating the host response to influenza vaccines have revealed key differences in live versus inactivated influenza vaccines, identified early transcriptional signatures that predict hemagglutinin antibody production following vaccination, increased our understanding of how adjuvants enhance the immune response to influenza vaccine antigens, and demonstrate biologic variability in the response to vaccination due to host factors. These studies demonstrate the potential for influenza transcriptomics to be applied to clinical care, understanding the mechanisms of infection, and informing vaccine development.

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Transcriptomics is the study of the complete set of RNA transcripts that are produced by the genome (under specific circumstances or in a specific cell or group of cells) using high-throughput sequencing. Comparing transcriptomes within groups enables the identification of genes that are differentially expressed in specific cell populations or in response to different treatments. Technologies used to conduct transcriptomics include microarrays and RNA sequencing. Microarrays are a fixed-probe technology, while RNA sequencing is more dynamic, and measures both known as well as new transcripts in a given sample. Microarrays measure the relative amount of mRNA activity of target genes with existing sequences. RNA sequencing uses high-throughput sequencing to record all transcripts and provides information regarding the gene sequence in addition to the expression level. Such tools generate a transcriptional profile (gene expression signature), representing a snapshot of genes expressed at a specific point in time. The steps involved in transcriptomics utilizing RNA sequencing are outlined in [Figure 1](#). Briefly, after sample collection, cells of interest are isolated, RNA is extracted, and converted to a library consisting of cDNA fragments. Each molecule is then sequenced with or without amplification. The resulting reads are then aligned to a reference genome or transcripts (or assembled de novo) to produce a genome-scale transcription map that consists of the level of expression for each gene. RNA sequencing data can produce over 10^9 short DNA sequences, which must then be analyzed using bioinformatics.

Transcriptomics has revolutionized our understanding of how genes are expressed, by providing a comprehensive,

unbiased and integrated analysis of the complexities of cellular activity. However, transcriptomic analyses require significant computation and proper experimental design to produce meaningful data, and this technology assumes that mRNA transcription is a proxy for protein products of a cell, which is not always the case. Not only is RNA unstable, but post-transcriptional modifications further modulate protein synthesis, such that mRNA and protein abundance do not always correlate. Further, gene expression is highly tissue-specific, and caution is needed in interpretation of gene expression patterns from a mix of cell populations.

Transcriptomics is emerging as an important tool in immunological and infectious diseases research. The transcriptomic study of peripheral blood mononuclear cells – including B cells, T cells, monocytes, dendritic cells, and natural killer cells – can provide a comprehensive summary of the immune response to infection. More recently, newer platforms enable whole blood analysis and single cell sorting to allow for a more comprehensive analysis, and immune cells found in respiratory secretions are being used to study the local host response to influenza infection.

Immunopathology of influenza infection

Complex co-ordinated immune responses are triggered in the host following an acute influenza infection, involving both innate and adaptive immunologic processes.

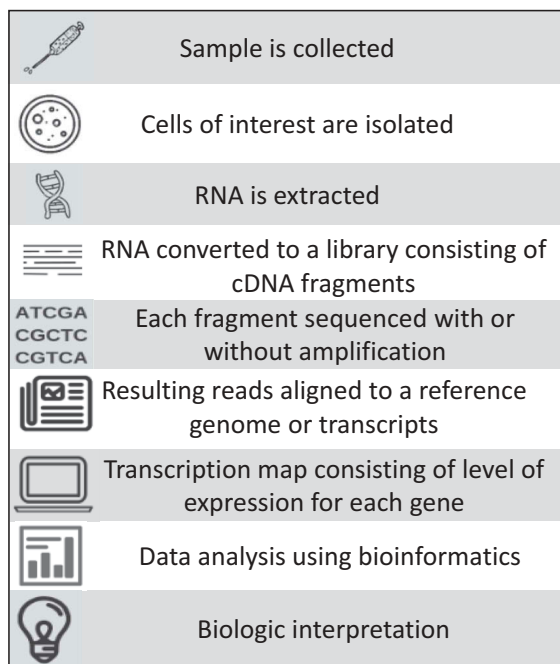


Figure 1. Steps involved in transcriptomic analyses using RNA sequencing.

Innate immune response

The first mechanisms of defense against influenza infection come into play at the portal of entry in the respiratory tract. The virus must cross the mucous layer that covers the respiratory epithelium in order to attach to the cell membrane and invade the cell. Infected epithelial cells, tissue macrophages and plasmacytoid dendritic cells (pDC) identify the viral RNA as a foreign element through pattern recognition receptors (PRRs), which activate an innate immune cascade resulting in the downstream secretion of type I interferons, other inflammatory cytokines, and chemokines.¹⁻³ The type I interferons stimulate hundreds of genes collectively known as interferon-stimulated genes (ISGs) in the surrounding cells, establishing potent innate local antiviral activity.⁴ The inflammatory mediators released at this stage of infection may result in systemic symptoms including fever and malaise. They also instruct the adaptive immune response. The chemokines released by epithelial cells and local immune cells attract natural killer cells (NKs), monocytes and neutrophils at the site of infection. These recognize the virally infected cells and eliminate them through NK-mediated cytotoxicity followed by monocyte and neutrophil phagocytosis of the dead cells.⁵ This process is generally sufficient to eliminate the virus in most immune-competent individuals.^{6,7}

Adaptive immunity – T cell response

If the influenza virus overcomes the innate protective immune responses and establishes a successful infection in the respiratory tract, the ultimate clearance of the virus requires the participation of the adaptive immune system. The adaptive immune response is generated in the lymphoid tissue. Typically, conventional dendritic cells (cDC) carry antigen to the draining lymph node where both T cells and B cells become primed. Primed CD4+ and CD8+ T cells differentiate

into T helper 1 (Th1), T follicular helper (Tfh) and cytotoxic T cells (CTL), all of which contribute to the clearance of influenza infection.⁸ Regulatory T cells (Treg) and other types of T helper cells may also be generated, but they contribute to the overall outcome of the viral clearance process mostly through their interactions with the influenza-specific Th1, CTL, Tfh and B cells.^{9,10} T cells migrate to the site of infection where they continue to proliferate and differentiate, activate themselves and local NKs and phagocytes until the virus is eliminated. The process of viral clearance is accompanied by tissue destruction and systemic inflammation. Once the foreign antigenic stimulus disappears, T helper cell proliferation ceases, effector T cells die or differentiate into memory cells and the tissue repair process predominates. Influenza-specific tissue-resident memory T cells (Trm) have been recently recognized.¹¹ While these cells lack the receptors necessary to leave the lung, they have a relatively long life and high replicative capacity like memory T cells, but also have the ability to accelerate cytokine production after encountering cognate antigens, similar to effector T cells. These T cells will quickly generate an adaptive immune response upon re-exposure to influenza viruses. It is important to note that T cells recognize small epitopes that are shared not only by homotypic, but also by heterosubtypic influenza viruses, thus enabling cross-protection against different influenza subtypes. Treg are another important component of the antiviral process. They are essential in quenching the effector immune response and redirecting the Th1 cell differentiation from effector to memory. They also play an important role in tissue repair that has only recently been recognized.¹² The T cell response must strike a fine balance between tissue destruction and viral elimination.

Adaptive immunity – antibody response

Tfh and Th1 cells cross into the germinal center of the lymph node where they provide support for the influenza-specific plasmablasts to differentiate into memory B cells and long-lived plasma cells, which are the main producers of high-affinity antibodies against influenza. Antibodies against the major glycoproteins of the viral envelope, including the hemagglutinin (HA), neuraminidase (NA) and matrix protein 2 (M2), mediate several adaptive immune mechanisms. Antibodies against the HA block the influenza virus attachment to the target cell thus neutralizing the virus. Most antibodies generated against the HA recognize the variable portion of the HA. These antibodies confer protection against influenza A and B homotypic viruses but very limited protection against heterosubtypic viruses. There are conserved areas in the HA of influenza A viruses that have been extensively studied because they generate broadly neutralizing antibodies.^{13,14} However, broadly neutralizing antibodies are rarely synthesized by the host during infection. The NA has a critical role in the release of newly formed viruses from the infected cells that controls the spread of the infection. The NA contains a higher proportion of conserved amino acid sequences compared with the HA and; therefore, anti-NA antibodies can neutralize a larger spectrum of viruses.^{15,16} Anti-M2 antibodies do not induce neutralizing antibodies,

but are expressed in abundance on the surface of the infected cells and may be an important target for viral clearance through phagocytosis or antibody-mediated cellular cytotoxicity through the recruitment of NK cells, monocytes, macrophages, and phagocytic DC.¹⁷⁻¹⁹

What have we learned about influenza pathogenesis through transcriptomics?

Each stage of the immune response against influenza infection may be characterized through its gene expression, cytokine release, and cell activation signatures or patterns, which can be studied using transcriptomics.²⁰ Transcriptomics has helped elucidate immune pathways specific to influenza infection, and has delineated immune profiles that differentiate asymptomatic from symptomatic infection, mild from severe disease, and early versus late infection. Further, transcriptional profiling enables the detection of infection prior to the onset of peak respiratory symptoms and has identified strain-specific differences in the host response to infection. A summary of the most recent findings from studies using transcriptomics to study influenza infection and vaccination and their potential clinical and research applications are provided in Table 1. A summary of study methodology and main findings from studies evaluating the host response to influenza infection are provided in Table 2.

Immune profiling of host PBMCs can differentiate bacterial versus viral infections in febrile adults with 89% sensitivity and 94% specificity.²¹ Moreover, transcriptional profiling can also identify signatures unique to influenza infection,²²⁻²⁴ which can be distinguished from uninfected individuals with 94% accuracy. Compared with other viral pathogens, infection with influenza was associated with a higher magnitude and longer duration of the illness biosignature, which reflected upregulation of interferon pathway and innate immunity genes. Gene expression patterns 21 days post-infection were identical to baseline gene expression.²³ A unique molecular signature consisting of eight gene clusters was shown to correlate with symptomatic disease. This included genes coding for innate viral RNA sensors (TLR7, RNA helicases, and interferon induced with helicase C domain 1), which were transcribed 36 h before the peak symptoms. The expression of suppressor of cytokine signaling genes (SOC) 1 and 3 (which are important inhibitory modulators in limiting the inflammatory effect of interferon signaling during viral infection) declines early among asymptomatic individuals but strongly increases among symptomatic individuals. Further, ribosomal protein gene transcription was upregulated in asymptomatic compared with symptomatic individuals.²⁵

Several key immune pathways have been found to discriminate early from late phases of infection. For example, a large increase in components of the type-1 interferon antiviral response and innate immunity were upregulated, whereas the expression of genes involved in translational elongation and protein synthesis were decreased during acute infection in one study.²³ Four days later, there was a characteristic recovery phase, with the upregulation of genes involving antigen binding and antibody secretion, and genes regulating cell morphogenesis.

Several transcriptional patterns associated with the outcome of infection have also been described. Signatures characterized by decreased type I interferon and ubiquitination gene transcription were associated with a more severe outcome of influenza A infection.²⁶ In contrast, transcription of interferon-induced transmembrane protein genes were associated with less severe disease.

Transcriptomics studies have the additional advantage of identifying infection based on immune signatures prior to the onset of symptoms. In a study of adults experimentally infected with influenza A H1N1 or H3N2, there was a specific genomic signature for infection that was present as early as 53-h post viral exposure, over 24 h before the onset of symptoms. Predictive genes included interferon response elements, the myxovirus-resistance gene MX1, and cytokine response pathways.²⁷ When applied to a population visiting the Emergency Department, this signature differentiated H1N1 pdm-infected from other patients with 92% accuracy.

There is evidence of strain-specific variability in gene signatures involved in the host response to influenza infections. Seasonal influenza A H1N1 and H3N2 infections result in gene expression profiles that share 44 out of the top 50 expressed genes but significantly differ from the transcriptional profile of the avian H5N1. Infection with H7N9 induces a gene transcription profile that was more similar to seasonal than avian influenza infection indicating better adaptation of H7N9 to human hosts compared with other avian viruses.²⁸

Immunity conferred by influenza vaccines

Vaccination is the primary strategy for protection against influenza infections. Although there are multiple influenza vaccine preparations, they can be largely grouped into live attenuated (LAIV) and inactivated vaccines (IIV). In addition, selective IIV are adjuvanted but currently are not licensed for use in children. LAIV and IIV differ in their immune mechanisms and correlates of protection. Influenza vaccines are licensed based on the hemagglutination inhibition antibody (HAI) titers that they generate. This is based on an older study in adults that showed that HAI titers $\geq 1:40$ after vaccination were associated with 50% decrease in the incidence of symptomatic influenza infections.²⁹ Recent studies have challenged this dogma, but this continues to be a licensure criterion of influenza vaccines and is also considered the main mechanism of protection of IIV. IIV also generate T cell responses and IgA antibodies that are not measured by the HAI assay but may contribute to protection against influenza infection. Inactivated vaccines generate demonstrable, but limited heterosubtypic protection against influenza A and B viruses. The most obvious reasons for the decreased cross-protection potential of IIV are the low T cell responses and the emphasis on the antibodies against the highly variable HA at the expense of the more conserved NA or M2.³⁰ The addition of adjuvants increases the antibody production and/or decreases the amount of antigen that is needed to generate HAI titers $\geq 1:40$ in response to vaccination. Less is known about CMI responses to adjuvanted IIV but they arguably confer higher cross protection than non-adjuvanted IIV, suggesting that they may also generate higher CMI responses. It is important to understand that HAI antibodies are not necessary nor sufficient for protection against influenza infection.³¹ This

Table 1. Transcription profiles of host responses to influenza infection and vaccination with potential clinical and research applications.

Outcome measure	Genes/immune pathways involved	Potential clinical/research application	References
Differentiate influenza from other respiratory viral infections	Type I interferon (IFI44, IFI44L, OAS2, IRF7) and type II interferon (IFI35, IFITM1–3) were overexpressed in children with influenza compared to children with RSV or HRV LRTI	Diagnosis of influenza infection; potential for elucidating the main pathogen in viral co-infections Target antiviral therapy	Mejias et al. ²¹ Parnell et al. ²² Zhai et al. ²³ Andres-Terre et al. ²⁴ Parnell et al. ²²
Differentiate influenza infection from bacterial pneumonia and systemic inflammatory response syndrome	Influenza gene-expression profile -upregulation of genes from cell-cycle regulation, apoptosis, and DNA-damage-response pathways, e.g. Cell cycle mitosis, Cell cycle core, Proteolysis Ubiquitin-proteasomal proteolysis, Cell cycle S phase, Cell cycle G2M	Identification of presence or absence of secondary bacterial infections Target therapy	Parnell et al. ²²
Differentiate symptomatic from asymptomatic infection	Asymptomatic infection characterized by transcriptional regulation of the inflammasome genes; transcriptional activation of suppressor of cytokine signaling (SOCS) family genes and downregulation of JAK-STAT signaling; transcriptional activation of virus interacting proteins, antioxidant and cell-mediated innate immune responses.	Disease surveillance Host transmission studies Identify desired host immune responses for new vaccine development	Huang et al. ²⁵
Identify transcription profiles associated with more severe clinical outcomes	Decreased type I interferon and ubiquitination gene transcription associated with severe outcome of influenza A infection Transcription of interferon-induced transmembrane protein genes associated with mild disease	Identify patients at high risk of developing severe disease and design early interventions to ameliorate the outcome	Hoang et al. ²⁶
Identify infection prior to the onset of symptoms	Transcription of genes coding for innate viral RNA sensors, interferon response elements and cytokine response pathways 24–36 hours before symptoms	Studying outbreaks, illness clusters, epidemiologic surveillance, transmission studies	Hoang et al. ²⁶
Distinctive biosignatures that represent acute and recovery phase of infection	Acute phase- activation of gene transcription on the interferon pathway and innate immunity; decreased translational elongation and protein biosynthesis gene transcription Recovery phase – activation of genes involved in antigen binding, antibody secretion and cell morphogenesis	Devise and monitor response to novel therapeutic interventions	Zhai et al. ²³ Huang et al. ²⁵
Human cell responses to avian influenza viruses	H7N9 infection of human epithelial cells generates a transcriptional profile intermediate between H3N2 and H5N1	Identify the potential of avian influenza viruses to spread in humans	Josset et al. ²⁷
Genome-based identification of influenza-specific immune modifiers	Kinase inhibitor SB-203580, genistein, troglitazone, minocycline, and LY-294002- capable of reverting gene transcription induced by avian influenza viruses	Identifying novel therapeutic interventions	Josset et al. ²⁷
Differences in IIV- and LAIV-induced responses	LAIV activates gene transcription of the interferon pathway TIV generates plasmablast activation and antibody response gene transcription	Novel vaccine design based on attributes of existing vaccines	Nakaya et al. ²⁸ Cao et al. ²⁹ Cole et al. ³⁰ Nakaya et al. ³¹ Olafsdottir et al. ³²
Adjuvant-specific signatures	Adjuvanted vaccine induced higher magnitude of gene expression compared with non-adjuvanted vaccine, including antiviral interferon, dendritic cell signature, Toll-like receptor (TLR) and inflammatory signaling pathways	Identify adjuvants that elicit most protective immune response	Nakaya et al. ³¹ Olafsdottir et al. ³²
Early Transcriptional signature that correlates with HAI responses to vaccines	Early upregulation of Interferon response and antigen presentation pathways is associated with a higher antibody response	Development of new vaccines and adjuvants	Tan et al. ³³ Bucasas et al. ³⁴

IIV, inactivated influenza vaccine; TIV, trivalent influenza vaccine; LAIV, live attenuated influenza vaccine; HAI, hemagglutinin antibody inhibition; RSV, respiratory syncytial virus; HRV, human rhinovirus; LRTI, lower respiratory tract infection.

was well demonstrated by studies in children and older individuals in whom CMI or nasal IgA correlated with protection against influenza and HAI titers did not.^{32–35} Conversely, hematopoietic stem cell transplant recipients have regular infusions of HAI-containing IVIG for the first 6 months after transplantation but continue to be at high risk of severe influenza infection. LAIV exemplifies best this paradigm. HAI titers generated by LAIV are low and do not correlate with protection against influenza infection. Conversely, LAIV generates stronger CMI responses and broader cross-protection compared with IIV. LAIV is more efficacious in children than in adults.

Insights into the immunobiology of influenza vaccines using transcriptomics

Recent studies have described gene expression signatures associated with influenza vaccination. Advanced bioinformatics analytical tools allowed classifying the gene expression patterns

in modules characteristic for activation and proliferation of different immune cells, such as B cells, T cells, NK cells, and dendritic cells.³⁶ Differences have been observed in the immune profiles of IIV compared with LAIV recipients.^{37–39} IIV has generally been associated with increased transcription of B cells, plasmablasts, plasma cells, and conventional DC modules.^{40,41} Furthermore, the early transcriptional signatures (involved in interferon signaling, antigen processing, and presentation and IL-6 regulation) of IIV recipients predicted HAI production after vaccination.⁴⁰ The molecular signatures associated with antibody responses to IIV have been reproduced in different populations and seasons, utilizing different techniques, underscoring the solidity of the data.^{37,42}

The addition of adjuvants to IIV may create signature patterns specific for each adjuvant in addition to the antigen. This is suggested by initial studies of MF59- and ASO3-adjuvanted vaccines.^{43–45} ASO3 induces NK cell division activity and interferon signaling and antigen processing and

Table 2. Summary of findings from studies evaluating the host response to influenza infection.

Study methodology	Findings	Summary of Main findings	References
Prospective observational study of infants with RSV (n = 135), HRV (n = 30), influenza (n = 16) and healthy age matched controls, using microarray analysis	Children with influenza displayed a stronger activation of genes related to interferon, inflammation, monocytes, and innate immune response compared with children with RSV or HRV. Several type I interferon (IFIH1, IFIT1–5, STAT2, MX1) and type II interferon (IFI16, CXCL10, CCL8, GBP1–5, STAT1, SOCS1) genes were only expressed in influenza and RSV infection. The magnitude of the type I interferon (IFI44, IFI44L, OAS2, IRF7) and type II interferon (IFB5, IFITM1–3) response present was 2- to 22-fold higher in children with influenza compared with children with RSV or HRV. Genes related to inflammation, monocytes, and innate immune response were overexpressed in children with influenza compared to those with RSV or HRV LRTI.	Type I interferon and type II interferon were overexpressed in children with influenza compared to children with RSV or HRV LRTI	Mejias et al. ²¹
39 critically ill patients with severe community-acquired pneumonia, 12 patients with non-infective systemic inflammatory response syndrome and 18 healthy volunteers evaluated using microarray analysis	Biological pathway analysis of the 1,416 genes uniquely upregulated in H1N1 influenza A infection showed that the influenza gene-expression profile is characterized by upregulation of genes from cell-cycle regulation, apoptosis, and DNA-damage-response pathways. In contrast, a specific gene-expression signature was not found in bacterial pneumonia. Activation and signaling pathways of interleukins (IL-8, IL-2, IL-15, IL-6, IL-10, IL-7, IL-3, IL-13, IL-17, and IL-23) were downregulated in influenza H1N1 cohort. Influenza gene-expression profile showed upregulation of genes from cell-cycle regulation, apoptosis, and DNA-damage-response pathways, e.g. Cell cycle mitosis, Cell cycle core, Proteolysis Ubiquitin-proteasomal proteolysis, Cell cycle S phase and Cell cycle G2M	Gene-expression profile of H1N1 influenza A pneumonia was distinctly different from those of bacterial pneumonia and systemic inflammatory response syndrome. More interleukin suppression is present in severe H1N1 influenza infection	Parnell et al. ²²
Prospective observational study of 1610 healthy adults; of these 133 developed influenza-like illness, and 73 had PCR-confirmed influenza and were followed for 3 weeks; changes in peripheral blood gene expression evaluated using microarray analysis	In the acute phase, there was a large increase in components of the interferon pathway and innate immunity (e.g. IFI44L, IFIT1, MX1, IFITM3, OAS2, IFI27 and IFIT3), as well as decreased expression of genes involved in translational elongation and protein biosynthesis (e.g. RPS4X, RPS18, RPS6, RPS8 and RPL5). This pattern was most intense on the first day of illness and continued for 2–4 days. This phase was followed by a characteristic recovery phase in which there was a transition to genes involved in antigen binding and antibody secretion (IGJ, LOC652694, IGLL1 and MZB1) and genes regulating cell morphogenesis (STRADB, DPYSL5, EPB42, LST1 and MAP1S).	There is upregulation of interferon pathway and innate immunity genes in the early phase of infection lasting 2–4 days, followed by cell proliferation and repair gene overexpression at days 4–6. Day 21 gene expression patterns were indistinguishable from baseline patterns. Influenza virus infection induced a higher magnitude and longer duration of the expression signature of illness compared to other viral infections. There is an intense activation of NK cells during the acute phase of infection.	Zhai et al. ²³
Analyses of 18 publicly available transcriptional datasets of host whole blood, PBMCs or epithelial cells of patients with viral infections, bacterial infections, SIRS, vaccinated patients	An 11-gene 'influenza meta-signature' was identified, that could discriminate symptomatic from asymptomatic subjects, influenza infection from other respiratory viral infections, and patients with mixed influenza and/or bacterial pneumonia from those with bacterial pneumonia alone. (CD38, HERC5, HERC6, IFI6, IFIH1, LGALS3BP, LY6E, MX1, PARP12, RTP4, and ZBP1)	Gene signature that can differentiate influenza infections from other viral infections. The host signature was predictive of response to influenza vaccine	Andres-Terre et al. ²⁴

(Continued)

Table 2. (Continued).

Study methodology	Findings	Summary of Main findings	References
Experimental challenge of 17 healthy adults with H3N2 and evaluation of peripheral blood gene expression at 16 timepoints over 132 hours.	Asymptomatic infection characterized by transcriptional regulation of the inflammasome genes; transcriptional activation of suppressor of cytokine signaling (SOCS) family genes and downregulation of JAK-STAT signaling; transcriptional activation of virus interacting proteins, anti-oxidant and cell-mediated innate immune responses. Early expression patterns of suppressor of cytokine signaling family genes and related JAK-STAT signaling in asymptomatic hosts with no sign of excessive inflammation Transcription of genes coding for innate viral RNA sensors, interferon response elements and cytokine response pathways 24–36 hours before symptoms	Transcriptome can differentiate symptomatic from asymptomatic infection. The host antiviral response is activated 36 hours before peak symptom time.	Huang et al. ²⁵
Evaluation of transcriptomic profiles of children and adults with influenza infection, classified as mild (N = 83) moderate (N = 40) and severe (N = 11), using microarray analysis; compared with febrile patients with unknown etiology	Genes in IL-1 signaling, IL-22 signaling, Production of Nitric Oxide and Reactive Oxygen Species in Macrophages and p38 MAPK signaling were only up-regulated in moderate and severe patients Transcription of interferon-induced transmembrane protein genes was associated with mild disease	Transcription profiles involving type I interferon and ubiquitination gene transcription were attenuated in patients with severe disease	Hoang et al. ²⁶
Experimental challenge of 24 healthy volunteers with influenza A H1N1 or H3N2, whole blood RNA isolated from blood samples collected every 8 hours and analyzed using microarrays	Gene signatures derived independently for the two different strains of influenza are highly similar, sharing 44 out of the top 50 genes, and characterize host response to viral infection. These genes include RSAD2, the OAS family, multiple interferon response elements, the myxovirus-resistance gene MX1, cytokine response pathways	Signature differentiates symptomatic influenza A H1N1 or H3N2 infection from asymptomatic individuals	Woods et al. ³⁵
Human bronchoepithelial cells were inoculated with influenza H3N2, H7N9 or H5N1, then extracted RNA was analyzed using microarrays	H7N9 infection of human epithelial cells generates a transcriptional profile intermediate between H3N2 and H5N1. Kinase inhibitor SB-203580, genistein, troglitazone, minocycline, and LY-294002 are capable of reverting gene transcription induced by avian influenza viruses	Higher potential of H7N9 avian influenza viruses to spread in humans Identifying several novel therapeutic interventions	Josset et al. ²⁷

RSV, respiratory syncytial virus; HRV, human rhinovirus; DNA, deoxyribonucleic acid; RNA, ribonucleic acid; NK, natural killer; PCR, polymerase chain reaction.

presentation several days after vaccination.⁴⁵ However, the relationship between the adjuvant signature and protection conferred by the vaccines still needs to be elucidated.

The transcriptional signature of LAIV was characterized by increased activity of plasmacytoid DC, T cell and NK cell modules. Interferon-signaling pathways were induced 7 days after vaccination.³⁸ Transcriptional signatures in LAIV recipients differed from those of IIV recipients and were not associated with the magnitude of the HAI responses to the vaccine.^{38,39} The LAIV signature revealed five upregulated genes that represented an interferon-stimulated gene response. It is interesting to note that the transcriptional signature of LAIV more closely resembled that of yellow fever vaccine, which is also a live attenuated virus vaccine, than the IIV signature. The transcriptional signature of yellow fever, however, is predictive of the antibody response to this vaccine which also correlates with protection against yellow fever viral infection.

Finally, transcriptional signatures following influenza vaccination differ among children, young adults and older adults,^{43,46} racial groups⁴⁷ and between men and women.^{48,49} Further research is required to understand key differences in the immune response among our more vulnerable populations.

Conclusion

Transcriptomics is a rapidly evolving discipline that provides an unbiased, accurate and sensitive method to study host-pathogen interactions. This approach requires significant computation, and thoughtful experimental design and data interpretation are required to ensure that meaningful conclusions can be reached. The studies presented in this review demonstrate the potential for transcriptomics to provide valuable applications for research and clinical use. This technology has provided novel insights into the host response to influenza natural infection and vaccination, which can help guide the development and selection of future influenza vaccines and therapeutics. Further, combining pathogen detection with the host immune response can improve interpretation of pathogens identified in a biological sample and enhance the classification of disease states. Currently, transcriptomics techniques are labor intensive and require high-level bioinformatics support, limiting their clinical application. However, further advancements in this technology may allow for faster acquisition of transcriptomic profiles in the clinical setting, in order to enhance diagnostics, monitor response to therapy, and identify markers of severity.

Disclosure of potential conflicts of interest

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