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Chapter 9

Respiratory viral infections

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Introduction

Respiratory viruses are a major cause of morbidity and mortality throughout the world and affect persons of all ages [1–4]. In addition to >100 million office visits for upper respiratory infections each winter, hospitals fill to capacity with admissions due to community acquired pneumonia, acute exacerbations of chronic obstructive pulmonary disease, asthma and bronchitis and many of these illnesses are due to viral infection. “Pneumonia and Influenza” consistently ranks as the fourth most common discharge diagnosis, and each year, 270,000 to 540,000 hospitalizations and 7600 to 72,000 deaths in the United States are attributable to influenza [3, 5–8]. Due to their epidemic nature influenza and RSV are widely recognized as pathogens in adults and children, respectively. However, the true burden of disease and the contributions of other viruses such parainfluenza viruses (PIV), human metapneumoviruses (hMPV), coronaviruses (CoV), and rhinoviruses (HRV) now being more fully recognized using modern molecular detection methods [9–13]. In addition to sensitive and rapid diagnostic testing, new molecular techniques allow an understanding of viral evolution, mechanisms and predictors of severe disease, interrogation of vaccine responses, improved bacterial and viral diagnostics and associations of viral infections with non-respiratory medical events. In this chapter the many ways molecular and precision medicine have impacted the field of respiratory viral disease will be reviewed.

Molecular virology of respiratory viruses

Viral diagnosis

In the past defining the epidemiology and impact of viral respiratory pathogens was significantly hampered by slow and/or insensitive diagnostic techniques such as cell culture and antigen detection [13, 14]. Polymerase chain reaction (PCR) has revolutionized the study of respiratory viruses and provides
extremely sensitive, specific and rapid means for the detection of fastidious and non-cultivatable respiratory viruses [13]. PCR based epidemiologic studies now provide a more complete understanding of the clinical spectrum and age ranges of populations affected [15–20]. In one study, conventional methods yielded a viral diagnosis in 14% of pneumonia cases, while use of PCR increased the yield to 56% [21]. Technology has rapidly evolved from single-plex PCR and gel electrophoresis to multiplex real time assays where products are detected by luminescent signals proportional to the target amplified [14]. There are currently a variety of commercially available assays that detect from 2 to 20 viral respiratory pathogens and maintain excellent sensitivity [14]. Many clinical microbiology laboratories are now moving to primarily molecular methods for viral detection and PCR formats are becoming increasingly simple so that nucleic acid extraction and PCR is fully automated with little operator input. Molecular point of care assays will soon be feasible [22].

Viral discovery

In addition to providing more sensitive means of detecting known viruses, molecular methods are extremely useful for viral discovery [23–25]. Over the past several decades a number of new respiratory viruses or variants have been identified including hMPV, novel strains of coronaviruses (HKU1, NL63, SARS-CoV, MERS-CoV), rhinovirus C, Human Boca virus, parechoviruses and new strains of avian influenza viruses. Molecular methods have been critical for the rapid identification of new viruses associated with dramatic lethal outbreaks but also for pathogen discovery for routine respiratory illnesses. Despite intensive investigation, in 12–62% of lower respiratory illnesses no pathogen can be identified suggesting additional agents may yet be discovered [26–28]. Several different genomic approaches for pathogen discovery have been used successfully and include random primer amplification, pan-viral DNA microarray and next generation sequencing (Fig. 1) [24].

If a viral class of an unknown pathogen or variant virus is suspected, consensus PCR using degenerate primers to detect sequences broadly conserved between members of a group can be used as was done to identify two new coronaviruses, HKU1 and MERS-CoV [29, 30]. Another technique for viral discovery is random primer amplification with conventional shotgun sequencing of PCR products [31–33]. Such was the case when Van den Hoogen discovered a new respiratory virus in 2001, in young children with bronchiolitis who tested negative for RSV [31]. After detecting paramyxovirus like particles in cell culture, RNA was subjected to random primer PCR and viral sequences were compared to all known pathogens. The new virus most closely aligned to avian pneumovirus but was determined to be a unique human pathogen and named human metapneumovirus (HMPV). Similarly, in 2003 Peiris identified a novel coronavirus as the cause of severe acute respiratory syndrome (SARS-CoV) using degenerate/random primers PCR
amplification [32]. Using pan viral micro array investigators at the Center for Disease Control and Prevention independently identified the same SARS-CoV [34]. In this technique, after random primer amplification, PCR products are hybridized to microarrays consisting of 70mer oligonucleotides derived from every fully sequenced viral genome. Hybridized sequences are scraped from the microspot, amplified, cloned and finally sequenced [25]. Identification of completely novel infectious agents requires unbiased and sequence independent methods for universal amplification [23, 24, 35]. Conventional Sanger sequencing may have poor sensitivity for genomes at low quantity. Next Generation Sequencing (NGS) involves the analysis of millions of sequences and can detect small amounts of novel nucleic acid sequences in clinical samples. Continuous sequences are assembled, host sequences are subtracted and the residual sequences are analyzed for similarity to known microbial sequences. NGS has led to the discovery a numerous novel human and animal pathogens [24]. A recent study of nasopharyngeal aspirates of Thai children with respiratory illness using NGS identified a number of mammalian viral sequences belonging to newly described families of viruses such Anelloviridae as well as novel strains of HRV, enteroviruses
and HBoV [35]. A critical step in viral discovery is the availability of bioinformatic tools to efficiently identify unique viral sequences in complex mixtures of host, bacterial and fungal sequences. New computational tools for analysis of the virome such as “VirusSeeker” are being developed [36]. Of note, detection does equate with causation and after discovery further studies are necessary to infer more than association.

**Viral evolution**

The genetic and antigenic evolution of error prone RNA respiratory viruses, particularly influenza, has been of interest for several decades [37, 38]. Understanding the selective pressure exerted by pre-existing immunity on viral evolution may help design more effective influenza vaccines and surveillance of animal populations can be critical for early identification of emerging influenza viruses [39]. Advances in deep sequencing make it possible to measure low frequency within host viral diversity and factors such as antigenic diversity, antiviral resistance, and tissue specificity can now be studied to understand the complexities of viral evolution [40]. Influenza evolution at a population level has been studied years, yet, new antigenic variants are initially generated and selected at the level of the individual infected host. Within a host, influenza viruses exist as a “swarm” of genetically distinct viruses [41]. Sanger sequencing defines consensus sequences and cannot resolve minority variants below 20% of the viral population. Deep sequencing has been used in natural infection and human challenge studies to characterize between and within host genetic diversity [41, 42]. The identification of low frequency mutations in the hemagglutinin (HA) antigenic sites or near the receptor-binding domain in vaccinated and unvaccinated influenza infected persons highlight viral evolution within a host due to selective immune pressure [41]. Similarly, NGS can reveal the rapid evolution of drug resistant variants during therapy [43]. Using samples collected over time, the mutational spectrum of H3N2 influenza A virus in an immunocompromised child was delineated [44]. Individual resistance mutations appeared weeks before they became dominant, evolved independently on cocirculating lineages. The within host evolution of antiviral resistance reflected a combination of frequent mutation, natural selection, and a complex pattern of segment linkage and reassortment. Within host sequencing diversity has also been examined in an infant with severe combined immune deficiency with persistent RSV infection [45]. NGS was performed on 26 samples obtained before and after bone marrow transplantation. The viral population appeared to diversify after engraftment with most variation occurring in the attachment protein (G). In addition, minority viral populations with palivizumab resistance mutations emerged after its administration. Deep sequencing of HRV during human challenge studies has shown that HRV generates new variants rapidly during the course of infection with accumulation of changes in “hot spots” in the capsid, 2C, and 3C genes [46].
Host genetic variation and genomic response to respiratory viral infection

Genome-wide association studies (GWAS)

A genome-wide association study (GWAS) involves rapidly scanning sets of DNA, or genomes, of many people to find genetic variations associated with a particular disease. Typically, the genomes of cases are compared to non-affected controls and search for single nucleotide polymorphisms (SNPS) or polygenic changes that are associated with risk or protection from susceptibility or severity of the condition. GWAS have been useful to find genetic variations and risk for asthma, cancer, diabetes, heart disease and autoimmune illnesses with relatively limited studies relating to infectious diseases [47, 48]. Recent studies examining host genetic factors conferring susceptibility to respiratory viruses such as pandemic H1N1 2009 influenza A, SARS-CoV and RSV now provide some insight into host genetic factors for respiratory viral infections [49–51]. Previously most influenza research focused on viral genetics of novel viruses, yet experience with H1N1 2009 and H5N1 clearly indicate host factors also influence disease severity [49, 50]. A number of candidate genes influencing respiratory virus susceptibility have been identified in animal and human studies and involve host virus interactions, innate immune signaling, interferon related pathways and cytokine responses (Table 1) [49–51, 69–75]. Over 20 studies have evaluated genetic polymorphisms associated with severe RSV disease and none demonstrates dramatic results [51]. Most focused on one or a few candidate genes resulting in only modestly increased odds ratios of severe illness. A relatively large study of almost 500 hospitalized children that examined 384 SNPS in 220 candidate genes demonstrated that susceptibility to RSV is complex with a several associations to a few innate immunity genes. These included a Vitamin D receptor gene associated with down regulating interleukin 12 (IL-12), gamma interferon (IFN-γ), nitrous oxide synthase (NOS2A), the JUN oncogene, an important transcriptional regulator for innate immune pathways, and IFN-α (IFNA5) an antiviral cytokine [68].

Host response to investigate mechanisms of disease

The host transcriptional response can be analyzed to investigate disease pathogenesis using a variety of methods including in-vitro studies of bronchial epithelial cells (BEC), animal models and infection both natural and experimental challenge [76–79]. In addition, two compartments, the respiratory epithelium and blood can be sampled in human studies and interrogated using different viruses or viral strains to develop gene signatures for prognosis, as indicators of severity and to identify potential therapeutic targets.

Mechanisms of disease

Most respiratory viral mechanistic studies have been performed using influenza viruses, RSV, HRV and coronaviruses [80–83]. Using BEC, the common and
| Gene  | Polymorphism                        | Significance                                                                 | Virus       | References |
|-------|------------------------------------|------------------------------------------------------------------------------|-------------|------------|
| CCR5  | CCR5Δ32                            | Increased allele frequency in Canadian ICU cases                             | H1N1, H5N1  | [52, 53]   |
| KIR   | 2DL2/L3                            | Increased allele frequency in Canadian ICU cases                             | H1N1        | [54]       |
| IFTIM3| rs12252 altered splice receptor    | Increased in hospitalized English and Scottish cases                         | H1N1        | [55]       |
| FcyRlla| IGHG2 *n/*−n                       | IgG2 subclass deficiency                                                     | H1N1        | [56, 57]   |
| NLRP3 | rs4612666(intron 7)                | Dysregulation of inflammasome                                                | H1N1        | [58, 59]   |
|       | rs10754558 (3′UTR)                 | Alteration of NLRP3 mRNA stability and enhancer activity                     |             |            |
| HLA   | Various alleles                    | Influenza specific CTL                                                       | H1N1        | [60]       |
| MBL2  | 230G/A                             | Mannose-binding lectin                                                       | SARS        | [61, 62]   |
| MxA   | -88G/T(rs2071430)                  | Encode IFN induced antiviral proteins                                       | H5N1, SARS  | [63, 64]   |
|       | -123C/A (rs17000900)               |                                                                              |             |            |
| OAS1  | rs2660(3′UTR A/G)                  | IFN induced antiviral proteins                                               | H5N1, SARS, West Nile | [64–66]   |
|       | rs3741981 (exon 3A/G)              |                                                                              |             |            |
|       | rs1077467                          |                                                                              |             |            |
| Gene   | SNP    | Function                                                      | Virus | Reference  |
|--------|--------|---------------------------------------------------------------|-------|------------|
| TLR3   | 908T/C | Missense mutation in patient with encephalopathy             | H5N1  | [67]       |
| VDR    | Thr1met| Vitamin D receptor, downregulate IL-12 and IFNγ                | RSV   | [68]       |
| JUN    | G750A  | Transcriptional regulator for innate immune pathways          | RSV   | [68]       |
| IFNA5  | C435T  | Antiviral cytokines                                           | RSV   | [68]       |
| NOS2A  | G275A  | Antimicrobial and anti-inflammatory                            | RSV   | [68]       |
| FCER1A | T-66C  | Innate immunity                                               | RSV   | [68]       |

(Adapted from references Juno J, Fowke KR, Keynan Y. Immunogenetic factors associated with severe respiratory illness caused by zoonotic H1N1 and H5N1 influenza viruses. Clin Dev Immunol 2012;2012:797180; Keynan Y, Malik S, Fowke KR. The role of polymorphisms in host immune genes in determining the severity of respiratory illness caused by pandemic H1N1 influenza. Public Health Genomics 2013;16(1–2):9–16; Miyairi I, DeVincenzo JP. Human genetic factors and respiratory syncytial virus disease severity. Clin Microbiol Rev 2008;21(4):686–703.)
divergent pathways used by four virulent viruses (H1N1 2009, H5N1, SARS Co-V and MERS Co-V) to antagonize interferon stimulated genes (ISG) responses was demonstrated (Fig. 2) [84]. H5N1 exhibited early strong up and down regulation of ISG subsets, whereas, less virulent H1N1 did not. SARS Co-V and MERS Co-V also demonstrated delayed ISG allowing early viral replication. In a similar experiment Josset et al. infected BEC with different influenza viruses (H5N1, H7N7, H3N2 and H7N9) and analyzed cellular responses using microarray [83]. Common proinflammatory cytokines and antigen presentation were identified although each viral response was unique and notably, H7N9 responses were most similar to H3N2. The response of different clinical isolates of RSV in A549 cells, and monocyte derived human macrophages
demonstrated that the pattern of innate immune activation was both host cell and viral strain specific [85]. Using RNA seq, differences in IL-6 and CCL5 were noted among the responses to different clinical isolates suggesting different RSV strains may vary in inherent virulence. Human studies have shown significant differences in the blood transcriptional profiles which change over time and differ depending on the infecting respiratory virus. Mejias and colleagues were able to differentiate RSV, HRV and influenza in young children based on the blood gene profile (Fig. 3). HRV infection exhibited the mildest innate and adaptive responses compared to RSV and influenza and neutrophil gene expression was greatest in RSV infection with marked suppression of B and T cell and lymphoid responses [79]. Notably, gene expression changes persisted up to 1 month after infection. Similarly, studies of H7N9 infected patients showed transcriptional profile changes persisting up to 1 month with a transition from innate to adaptive immunity [86].

**Rhinovirus (HRV) association with asthma**

Because of the association of HRV and exacerbations of asthma, the host response to HRV has been of particular interest [87–90]. Studies using BECs from asthmatic and healthy donors demonstrate different transcriptional profiles when infected with HRV [87]. HRV, similar to other picornaviruses induces gene expression down regulation by the 2A and 2C proteins. In both asthmatic and healthy control derived cells the majority of genes were down regulated after exposure to HRV. However, some significant expression differences in inflammatory, tumor suppressor, airway remodeling and metalloproteinase pathways have been noted in asthmatic derived cells. Asymptomatic HRV infection is quite common and its role in asthma pathogenesis has been questioned. Interestingly, Heinonen et al. did not find a difference in the blood transcriptome of asymptomatic HRV infected children compared to non-infected controls [91]. Whereas, Wesolowska-Anderson and colleagues demonstrated over 100 differentially expressed genes in the nasal epithelium of asymptomatic infected HRV patients [90]. Thus, the blood transcriptome may not be as informative as the nasal epithelial transcriptional response for asymptomatic HRV infection. Given the significant host response to asymptomatic infection, HRV may play a role in asthma exacerbations in the absence of clinically evident disease. Lastly, it may be possible to identify patients with asthma who are prone to frequent HRV related exacerbations by examining the gene expression response of their PBMCs stimulated with HRV [89].

**Disease severity**

*Respiratory syncytial virus*

Gene expression studies focusing on illness severity may enhance our understanding of disease pathogenesis, can identify potential therapies to modulate
FIG. 3 Transcription profiles from blood samples of children with influenza, RSV and HRV Lower Respiratory Tract Infection (LRTI). 70 top ranked genes best discriminated influenza, RSV and HRV. Mean modular transcriptional fingerprint for influenza (n=16 and 10 matched controls), RSV (n=44 and 14 matched controls), and HRV LRTI (n=30 and 14 matched controls). The outer dark circles highlight the disease group (influenza, RSV, or HRV) with greater (red) or lower (blue) modular activation. Children with HRV infection demonstrated a milder activation of the innate and adaptive immune responses, compared with children with influenza or RSV infection. Children with influenza displayed a stronger activation of genes related to interferon (M1.2, M3.4, M5.12), inflammation (M4.6, M5.1, M6.13), monocytes (M4.14), and innate immune response (M3.2, M4.2, M4.13) compared with children with RSV or HRV. Several type I interferon and type II interferon genes were expressed only in influenza and RSV infection. In addition, the magnitude of the type I interferon and type II interferon response present was 2- to 22-fold higher in children with influenza compared with children with RSV or HRV. Similarly, genes related to inflammation, monocytes, and innate immune response were greatly overexpressed in children with influenza compared to children with RSV or HRV LRTI. Neutrophil-related genes (M5.15) were significantly overexpressed in RSV infection, followed by HRV infection and, at a lower level, influenza infection. The suppression of genes related to B cells (M4.10), T cells (M4.1, M4.15), lymphoid lineage (M6.19), and antimicrobial response (M2.1) observed in RSV infection was significantly milder or not present in children with influenza or HRV LRTI. (Reproduced with permission from Mejias A, Dimo B, Suarez NM, Garcia C, Suarez-Arrabal MC, Jartti T, et al. Whole blood gene expression profiles to assess pathogenesis and disease severity in infants with respiratory syncytial virus infection. PLoS Med 2013;10(11):e1001549. https://doi.org/10.1371/journal.pmed.1001549.g005.)
harmful host responses and can be used to develop biomarkers for predicting life-threatening disease [79, 92–94]. A number of studies have been undertaken to understand the pathogenesis of severe RSV in young children and have identified a variety of gene expression patterns in blood including under expression of T cell cytotoxicity/NK cells and plasma cell genes, as well as upregulation of JAK/STAT, prolactin, IL-9 signaling, cell to cell signaling, and immune activation pathways [79, 92]. Using nasal epithelial gene expression analysis, van den Kieboom identified 5 differentially expressed genes in 30 children with mild, moderate and severe RSV infection [81]. Ubiquitin D, tetraspanin 8, mucin 13, β microseminoprotein, chemokine ligand 7 were upregulated and differentiated mild from severe illness. Lastly, nasal gene expression is complicated by interactions of the nasal microbiota and host cell gene responses [95]. In nasal samples from children with RSV infection, *H. influenzae* and *S. pneumoniae* dominated microbiota, Toll like receptors and neutrophil/macrophage signaling were over expressed and the presence of *H. influenzae* and *S. pneumoniae* along with age and sex were predictive of risk of hospitalization due to RSV.

**Influenza**

Transcriptional profiling related to severity has been analyzed in seasonal influenza as well as emerging avian pathogens with a recognition that disease is not only due to an infection with a novel virus in a non-immune host but may also be due to an exaggerated host immune response [78, 96]. In a study of primarily seasonal influenza (H1N1, H3N2), influenza infection was associated with a significantly stronger antiviral, cytokine, attenuation of T/NK cell response compared to patients with respiratory illnesses of unknown etiology regardless of severity [96]. Notably, IFN and ubiquitination was significantly down regulated in those with severe vs. mild to moderate disease. In a study of the lethality of 1918 H1N1 influenza and H5N1 Vietnam influenza virus in Macaques, upregulation of key components of the innate immune response and cell death pathways were noted were noted with 1918 H1N1 infection but were down regulated with H5N1 [78]. Early up regulation of the inflammasome likely resulted in some of the severe tissue damage noted with the 1918 H1N1 influenza infections.

**Identification of potential therapeutic targets**

In vitro, animal and human challenge studies have been used to identify new strategies control or prevent symptomatic or severe infection [82, 97]. In HRV challenge studies, virperin expression correlated with rhinorrhea and chilliness. Knockdown of expression resulted in increased viral replication in BECs suggesting virperin has antiviral actions and might have potential therapeutic use. Influenza challenge studies clearly show a definable transcriptomic profile in the blood prior to the onset of symptoms offering the possibility of earlier and more effective oseltamivir treatment [77, 98].
Associations with respiratory viral infection with non-respiratory medical events

Lastly, host gene expression studies may allow investigation into links between respiratory viral infections and specific non-respiratory events. There is ample epidemiologic evidence that influenza epidemics are linked with increased rates of strokes and myocardial infarction (MI) [99, 100]. Increased rates of falls and functional decline in nursing homes have also been associated with increased influenza activity [101, 102]. However, direct links of events to viral infection are scarce in part due the event of interest may follow the infection by several weeks when the virus is no longer detectable by traditional testing. Several gene profiling studies have identified viral infection signatures that may persist up to 1-month post infection [79, 86]. Thus, it might be possible to study patients with falls or cardiac events for evidence of recent viral infection using a host response viral signature. In addition, evaluating the host response can provide information on mechanisms of disease. A viral gene signature was used to evaluate patients undergoing cardiac catheterization [103]. Notably, 25% vs. 12%, \( P = 0.04 \) of those with a viral gene signature present vs. those without viral signatures, suffered an MI. Furthermore, H1N1 infected patients showed an increased gene platelet expression signature providing insight into how infection may induce a prothrombotic state.

Host response for diagnosis

Diagnosis of viral infection based on host response

Given the availability of rapid and accurate Multiplex PCR for viral detection, host-based diagnostics might seem unnecessary. However, current PCR assays use conserved known viral sequences but can miss novel or significantly mutated viruses. This issue was seen in 2009 with pandemic H1N1 when influenza PCR assays had to be adapted to optimally detect the new influenza strain [104]. The emergence of novel respiratory viruses are a persistent threat and methods to detect a “viral signature” in the setting of clusters of severe pneumonia cases could be very useful. Zaas and colleagues developed an acute respiratory viral gene signature using microarray analysis of the blood from volunteers experimentally infected with influenza A, HRV or RSV [105]. The signature was subsequently 89% sensitive and 94% specific in classifying as viral 25 influenza and 3 HRV infected patients presenting to an emergency room. Additionally, a distinct blood transcriptome signature was noted in patients with severe H1N1 pneumonia [106]. Upregulated genes included those related to cell cycle, DNA damage, apoptosis, protein degradation, and T helper cells. Down regulated genes were primarily in immune response pathways suggesting immunosuppression as a mechanism of severe influenza pneumonia. Investigators developed a 29 gene classifier which predicted H1N1 influenza A regardless of concomitant bacterial infection and such a predicator could guide antiviral therapy in the face of negative pathogen detection methods.
Distinguishing viral and bacterial respiratory infections

In most cases of respiratory infection, the precise microbial etiology is unknown and antibiotics are frequently administered empirically [27, 107]. Although sensitive molecular diagnostics (PCR) now allow rapid diagnosis of a wide variety of respiratory viruses, their impact on patient management and antibiotic prescription has been modest primarily due to concern about bacterial co-infection [108–110]. Approximately 40% of adults hospitalized with a documented viral respiratory infection have evidence of concomitant bacterial infection and thus clinician concerns are reasonable [109]. Importantly, sensitive and specific diagnostic tests for bacterial lung infection are currently lacking [111, 112]. Although the site of infection is the respiratory tract, blood is a convenient sample comprised of components of the innate immune system (neutrophils, natural killer cells), as well as the adaptive immune system (B and T lymphocytes) [113]. Recent studies indicate that viral and bacterial infections trigger pathogen specific host transcriptional patterns in blood, yielding unique “bio-signatures” that may discriminate viral from bacterial causes of infection [114–117]. In the largest study to date, Tsalik et al. used gene expression in blood to discriminate bacterial from viral infection or non-infectious illness in 273 subjects with respiratory illness [118]. These investigators defined 130 predictor genes in a model with an accuracy of 87% to discriminate clinically adjudicated bacterial, viral, and non-infectious illness. Most studies to date have used micro array but recently RNAseq has been used to differentiate viral and bacterial respiratory illness and in one study 141 genes were noted to be differentially expressed [119]. Three pathways (lymphocyte, α-linoleic acid metabolism, IGF regulation pathways) which included 11 genes as predictors for bacterial infection from non-bacterial infection (naive AUC=0.94; nested CV-AUC=0.86). To date, a number of gene expression studies of adults and children have developed predictors with similar accuracy (AUC ranging from 78% to 94%), yet there has been little overlap in classifying genes identified [105, 106, 114–116, 118–122]. Diverse populations, types of infection, plus alternate analytic tools used, likely explain the different genes identified. More work needs to be done to refine predictive gene sets including patients with mixed viral-bacterial respiratory tract infection. Most studies to date have focused on blood; however, analysis of the nasal respiratory epithelium which is the site of infection might offer advantages. Although data are limited, several recent papers demonstrate that nasopharyngeal host response can also be used as a diagnostic for respiratory viruses [93, 123, 124].

Influenza vaccine response

Immune response to influenza vaccine is variable and influenced by a variety of factors including prior vaccinations and infections, age, the presence of underlying conditions and the type of vaccine administered. Yet, even among a relatively homogeneous cohort of young healthy adults, antibody responses to
vaccine can be variable [125]. Transcriptional profiling of whole blood provide insights into the mechanisms of variability, the effects of age, and vaccine types. The ability to predict vaccine response at baseline based on a transcriptomic signature would have significant clinical implications. To understand the biologic effects of live attenuated influenza vaccine (LAIV) compared to trivalent inactivated vaccine (TIV) blood transcription profiles from 85 young children were assessed by microarray at day 7 post vaccination [126]. Many more genes were differentially expressed in children receiving LAIV compared to TIV (245 vs. 49, respectively) and many modulated type 1 IFN. The efficacy of LAIV has been problematic in recent years and assessing stimulation of type 1 IFN genes could represent a potential biomarker for response to LAIV [126]. Bucasa and colleagues evaluated gene expression at multiple time points after vaccination of healthy young men with TIV [127]. They noted marked up regulation of gene expression of IFN signaling, IL-6 regulation, antigen processing and presentation genes within 24h of vaccination and were able to define a 494 gene expression signature that correlated with the magnitude of antibody response. In another study, a gene profile predictive of antibody response 28 days after influenza vaccination of young and older adults was developed [128]. Notably, the predictive genes were the same in young and old as well as a subgroup of subjects with diabetes suggesting similar pathways were involved despite differences in age and underlying medical conditions. Additionally, transcriptional profiling has been used to signatures in blood associated with B cell memory responses to vaccination. In a study of 150 older and middle aged adults vaccinated with TIV including an H1N12009 antigen, metabolic, cell migration/adhesion, MAP kinase and NF-κB cell genes correlated with peak memory B cell ELISPOTs [129]. Finally, in a study of over 500 subjects vaccinated over several seasons, a predictive signature of nine genes and three gene modules were significantly associated with the magnitude of the serum antibody response (Fig. 4) [130]. Interestingly and in contrast to a previous study, the signature was distinct to the younger cohort. For example, inflammatory genes were associated with better response in the young but a worse response in the elderly. In summary, gene expression studies could be used to evaluate new vaccines and develop predictors of vaccine response in different subgroups of patients based on age and disease state allowing for individualized vaccine regimens.

Conclusions

Molecular analysis of respiratory viruses and the host response to both infection and vaccination have transformed our understanding of these ubiquitous pathogens. The ability to accurately diagnosis viral infections has not only impacted patient care but also changed our perceptions of the burden of disease and populations effected. Transcriptional profiling of blood and nasal epithelium may provide therapeutic targets to prevent and ameliorate illness as well as offer predictors of severe disease.
FIG. 4  Gene expression signatures at baseline as a predictor of influenza response in young adults. (A) The geographic mean of predictive genes (GRB2, ACTB, MVP, DPP7, ARPC4, PLEKHB2, ARRB1) z-scored expression values (response scores) was calculated for low, moderate and high responders. (B) Receiver Operator Characteristic (ROC) curves for classifiers designed to separate high from low to moderate responders. (C) Temporal behavior of response score in the validation cohort for low, moderate and high responders. Each point = individual subject and each group at a time point is summarized by a boxplot with significant P values for high vs. low responders above the date. (Reproduced with permission from HIPC-CHI Signatures Project Team and HIPC-I Consortium. Multicohort analysis reveals baseline transcriptional predictors of influenza vaccination responses. Sci Immunol 2017;2(14).)

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