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Systems biology: a tool for charting the antiviral landscape

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Abstract

The host antiviral programs that are initiated following viral infection form a dynamic and complex web of responses that we have collectively termed as “the antiviral landscape”. Conventional approaches to studying antiviral responses have primarily used reductionist systems to assess the function of a single or a limited subset of molecules. Systems biology is a holistic approach that considers the entire system as a whole, rather than individual components or molecules. Systems biology based approaches facilitate an unbiased and comprehensive analysis of the antiviral landscape, while allowing for the discovery of emergent properties that are missed by conventional approaches. The antiviral landscape can be viewed as a hierarchy of complexity, beginning at the whole organism level and progressing downward to isolated tissues, populations of cells, and single cells. In this review, we will discuss how systems biology has been applied to better understand the antiviral landscape at each of these layers. At the organismal level, the Collaborative Cross is an invaluable genetic resource for assessing how genetic diversity influences the antiviral responses. Whole tissue and isolated bulk cell transcriptomics serves as a critical tool for the comprehensive analysis of antiviral responses at both the tissue and cellular levels of complexity. Finally, new techniques in single cell analysis are emerging tools that will revolutionize our understanding of how individual cells within a bulk infected cell population contribute to the overall antiviral landscape.

Keywords

Systems biology; virology; antiviral response; Collaborative Cross; transcriptomics; single cell analysis

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1. The antiviral landscape

Following pathogen recognition, a series of well-orchestrated and dynamic immune responses are triggered, resulting in the rapid generation of antiviral effectors and pathogen-specific responses. The central function of these responses is to restrict viral replication and spread to neighboring uninfected cells, ultimately promoting viral clearance. Conventional approaches for investigating antiviral responses have focused on defining the mechanism of action for either a single or closely related set of genes through experimental perturbation-based studies. However, these approaches often overlook the complexities and redundancies built into the antiviral host response, providing only a narrow viewpoint. Holistic approaches, such as systems biology, instead provide a comprehensive understanding of the host response during viral infection. One of the major features of this approach is that it considers the biological system as a whole, providing a powerful tool for the unbiased and thorough analysis of the antiviral response. A unique feature of systems level approaches is their ability to reveal emergent properties that are only evident when considering the system as a whole, rather than focusing on the individual components of a system. Through this type of approach, computational and network analyses are integrated into predictive models that can be experimentally tested and refined through an iterative process. We have referred to this latter step as “biological validation” and consider it to be an integral component of any systems biology based investigation (Suthar and Pulendran, 2014). The use of systems level approaches complements and guides conventional studies by revealing novel host molecules or pathways. The scope of systems biology based studies can be designed to span the organism, tissue, and cell levels, probing distinct but complementary compartments of the host response (Figure 1). We feel that it is beneficial to use the term “antiviral landscape” to represent the entire defense response process, from the onset of viral infection to clearance. In this review, we will highlight recent studies that have employed systems biology based approaches to unravel the host antiviral response, focusing on transcriptional profiling studies from whole tissues, heterogeneous cell populations, and single cells.

2. Organism level: genetic diversity impacts the antiviral landscape

Epidemiological and clinical studies have revealed that host genetics strongly influences immunity and disease severity in response to viral infection. Studies in humans infected with WNV have identified single nucleotide polymorphisms (SNPs) within CCR5R, MX1, IRF3, and OAS1 as strong risk factors for enhanced susceptibility and disease severity (Bigham et al., 2011; Glass et al., 2006). Similarly, genetic risk factors have been identified for hepatitis C virus (Venegas et al., 2012), Human immunodeficiency virus (Vannberg et al., 2011), Influenza virus (Juno et al., 2012), and other human viral pathogens. However, human genetic studies are often confounded by variable environmental factors, lack of genomic data, and difficulty in dissecting the mechanisms of a causal genetic variant on a complex trait. Inbred mouse strains have proven to be tractable models for studying viral pathogenesis. Indeed, the initial development and refinement of systems biology approaches focused on simple perturbations of classic inbred systems, such as the discovery of Serpine-1 and the larger urokinase pathway in driving SARS-coronavirus lung injury and pathogenesis (Gralinski et al., 2013). While inbred mouse strains are more tractable models for studying viral pathogenesis, they often overlook the complex genetic traits that influence
disease and symptomatic infection outcomes in humans. To overcome these challenges, the highly genetically diverse Collaborative Cross (CC) recombinant inbred (RI) mouse panel was generated to identify, characterize, and dissect the mechanisms of naturally occurring genetic variants (e.g. genes and gene networks) that influence diverse clinically relevant traits (Aylor et al., 2011; Campbell et al., 2012; Gelinas et al., 2011; Kelada et al., 2012; Kovacs et al., 2011; Mathes et al., 2011; Patel et al., 2013; Philip et al., 2011; Rogala et al., 2014; Sun et al., 2012; Svensson et al., 2012; Zombeck et al., 2011), including susceptibility to fungal (Durrant et al., 2011), bacterial (Shusterman et al., 2013) and viral infections (Bottomly et al., 2012; Ferris et al., 2013; Josset et al., 2014; Peng et al., 2010; Peng et al., 2011; Xiong et al., 2014). The CC is a multi-parental RI panel derived from eight inbred mouse strains (5 classic laboratory strains, and 3 wild-derived strains from the 3 major Mus musculus subspecies: musculus, domesticus and castaneus), and has >45 million naturally occurring polymorphisms (SNPs, small insertions/deletions) segregating uniformly across the genome, with minor allele frequencies of >12%, and averaging six distinct haplotypes per locus (Collaborative Cross, 2012; Keane et al., 2011). The generation of the CC lines eliminated long-range linkage disequilibrium within this population, removing the risk of identifying spurious associations between phenotype and genotype that plague other resources (i.e. no population structure) (Collaborative Cross, 2012; Flint and Eskin, 2012). Furthermore, removal of long-range linkage disequilibrium breaks apart co-adapted gene complexes, resulting in the emergence of extreme phenotypes driven by epistatic interactions, such as in the development of novel models of spontaneous colitis (Rogala et al., 2014) and Ebola hemorrhagic disease (Rasmussen et al., 2014). Thus, the CC accurately mimics the complexity of genetic diversity seen within human populations and models how natural variants at loci, as opposed to the extreme abrogation that genetic knockouts create, lead to variations in phenotypes and disease outcome. The CC shows promise of bridging an important gap between mouse models and human disease, providing a useful resource for studying basic aspects of pathogenesis and serving as a platform for antiviral and vaccine development.

2.1 Host genetics impacts Influenza A Virus pathogenesis

Host genetics is believed to influence Influenza A virus (IAV) pathogenesis in humans, however these findings have either been correlative and unable to show direct causal relationships, or identify rare mutations found within the human population (Cheng et al., 2015; Ciancanelli et al., 2015; Horby et al., 2012; Lee et al., 2014). To better model the impact of genetic diversity in influencing viral pathogenesis during IAV infection, Ferris et al (Ferris et al., 2013) infected a panel of different incipient CC mice (the preCC) with IAV. A diverse range of phenotypic traits was observed, including emergent phenotypes not present within the infected founder lines, such as high viral replication with low weight loss and inflammation, as well as significant weight loss despite a lack of viral replication. Transcriptional profiling revealed that several phenotypic disease traits, including weight loss and airway inflammation, correlated with transcriptional networks corresponding to immune and inflammatory processes, suggesting a direct relationship between disease severity and genetic variation within the host response. A major strength of the CC model is the ability to track phenotypic traits back to specific genetic loci using quantitative trait locus (QTL) mapping. When combined with gene expression data (e.g. qPCR or RNAseq),
genetic markers in the genomic DNA (e.g. SNPs) can be correlated to RNA transcript levels, identifying expression QTLs (eQTLs) that provide a link between genomic sequence variation and the regulation of gene expression. In this paper, several QTLs correlated with disease traits following IAV infection, including Mx1, a known antiviral effector gene with potent activity against IAV. Genomic sequencing of Mx1 exons across the CC founder strains led to the discovery of a novel allelic variant that provided protection from virus-induced weight loss, but had reduced ability to inhibit viral replication. eQTL analysis revealed that several sets of transcripts related to inflammatory and immune processes were decreased in CC mice containing this novel Mx1 allelic variant, suggesting decreased levels of immunopathology as a potential explanation for the reduced weight loss following IAV infection. Several of the QTLs correlating to disease traits did not contain known antiviral effectors and may contain unidentified genetic factors that influence IAV infection outcome in humans. Importantly, this group identified “reactive” transcriptional networks that were dependent on genetic variants at specific loci, in particular describing three unique transcriptional profiles derived from different Mx1 variants. Further implementation of this model across pathogenic and nonpathogenic influenza strains has the potential to drive discovery of novel host determinants of IAV pathogenesis, including potential therapeutic targets.

2.2. Modeling determinants of symptomatic West Nile Virus infection

The diversity of infection outcomes occurring during IAV infection is not unique, but instead a common feature with most human viral infections. Following infection with the neurotropic Flavivirus West Nile virus (WNV), 80% of cases present as asymptomatic with the remaining 20% of symptomatic cases ranging in severity from a mild febrile illness to severe encephalitis and death (Graham et al., 2015; Marion et al., 2009; Suthar et al., 2013b). In contrast to human disease, the predominant model for studying WNV infection has utilized C57BL/6J mice, where 100% of infected mice develop neuroinvasive disease (Daffis et al., 2008; Douglas et al., 2013; Suthar et al., 2010). Recently, Graham et al (Graham et al., 2015) challenged a cohort of F1 crosses of CC (CC-F1) mice with WNV and found that infection mirrored human disease phenotypes, with clinical scores stratifying mice into asymptomatic and symptomatic groups. The asymptomatic group could be further subcategorized based on the presence or absence of immune system involvement within the central nervous system (CNS), the former representing a previously unappreciated and emergent disease outcome. Analysis of innate immune responses revealed a correlation between sustained IFNβ and IFIT1 expression during symptomatic disease and confirmed a previously appreciated role for genetic diversity in the Oas1 gene in disease outcome (Bigham et al., 2011). Diverse adaptive immune responses were also observed, including elevated CNS CD4+ regulatory T cells in one of the CC lines exhibiting asymptomatic disease, further highlighting the complexity of host genetics and immune regulation. While the determinants of symptomatic WNV infection remain poorly understood, this work has identified CC mouse lines with divergent infection outcomes (asymptomatic and symptomatic) that can be further studied to define host novel factors contributing to WNV pathogenesis.
2.3. Development of an improved small animal pathogenesis model for Ebola Virus infection

Mouse-adapted Ebola virus (EBOV) is lethal in mice, but they fail to develop the hemorrhagic fever syndrome observed during human disease. The lack of a small animal model for EBOV hemorrhagic fever has been a major obstacle for advancing our understanding of EBOV pathogenesis and developing antiviral therapeutics. Recently, Heinz Feldmann and Michael Katze employed the CC to better understand the impact of host genetics on EBOV pathogenesis (Rasmussen et al., 2014). Using a mouse adapted EBOV, infection of CC-F1 cohorts resulted in diverse outcomes ranging from complete protection from lethal disease (“resistant”) to mice that succumbed, developing hemorrhagic fever-associated pathology prior to death (“susceptible”). The spectrum of disease phenotypes across the screened lines parallels observations in the human population during the 2014 EBOV outbreak in West Africa (Bah et al., 2015; Schieffelin et al., 2014), highlighting the potential of the CC to model human EBOV infection. Virologic comparison of a representative susceptible and resistant CC line revealed that, despite similar levels of EBOV genomic RNA, there was a significant increase in the production of infectious virus from the spleens and livers of susceptible mice. This was accompanied with widespread infection of hepatocytes in susceptible mice. In contrast, the livers of resistant mice exhibited restricted infection of endothelial and Kupffer cells. Transcriptional profiling of susceptible livers uncovered enrichment for genes and pathways correlating to vascular integrity, endothelial activation, and inflammation. In particular, a regulatory network centered on the endothelial kinase Tek, a regulator of coagulation, was found to correlate with disease severity: susceptible mice exhibited diminished expression of this Tek-centered network relative to naïve mice, while resistant mice had elevated expression. Interestingly, these CC-F1 animals shared one Tek allele with each other, while their other Tek alleles came from divergent subspecies, again highlighting how divergent phenotypic outcomes can often derive from breaking apart co-adapted gene networks from divergent mouse subspecies. Further studies are needed to define the relevance of Tek and other positively correlating genetic loci with disease severity during human EBOV infection. Nonetheless, this work exemplifies the utility of the CC genetics resource to identify novel platforms for new viral pathogenesis models and small animal models for developing therapeutics.

3. Tissue level

3.1. Transcriptomics uncovers determinants of West Nile virus tissue tropism

WNV infects a broad range of cell types and tissues (Suthar et al., 2013b). The spleen is the primary permissive tissue during the visceral organ stage of WNV infection, while the liver is non-permissive to infection (Suthar et al., 2013a). RIG-I like receptor (RLR) signaling through MAVS and type I interferon (IFN) receptor (IFNAR) signaling are critical for protection during WNV infection (Lazear et al., 2011; Pinto et al., 2014; Suthar et al., 2013a; Suthar et al., 2010). To better understand the contributions of these pathways in dictating viral tropism, Suthar et al (Suthar et al., 2013a) infected WT, Mavs−/−, Ifnar−/−, and Mavs−/−Ifnar−/− double knock out mice with WNV and performed transcriptomics on permissive (spleen) and non-permissive (liver) tissues. Through an integrated approach, molecular signatures were defined for RLR and type I IFN signaling and used to dissect
their individual contributions to host antiviral immunity during WNV infection. Network analysis revealed that RLR and type I IFN signaling trigger strong antiviral immune responses that restrict viral replication within the liver. Infected livers were also selectively enriched for pathways associated with natural killer (NK) cell responses, uncovering a previously unappreciated protective role for NK cells during WNV infection. Biologic validation studies revealed expansion and activation of hepatic NK cells during WNV infection in a RLR and type I IFN signaling dependent manner, supporting a role for NK cell responses in restricting viral tropism within the liver. This study explored the antiviral landscape during WNV infection, uncovering critical roles for RLR and IFN signaling in dictating tissue permissiveness to infection and identifying a previously overlooked role for NK cells in dictating viral tropism within the liver.

3.2. Transcriptomics enhances our understanding of severe Influenza A virus infection

In immune competent individuals, infection with different strains of Influenza A virus (IAV) ranges in disease severity from mild (e.g. H3N2 seasonal strains) to severe (e.g. H5N1 and H7N9). The host factors that influence the development of these divergent disease outcomes are incompletely understood. To clarify the contributions of host immunity to disease severity during IAV infection, Ron Germain’s group employed a top-down approach analyzing whole tissues and isolated cell populations from C57BL/6 mice infected over a time-course with a non-lethal strain of IAV (Tx91) and PR8 at both sub-lethal and lethal doses (Brandes et al., 2013). Whole lung transcriptomics revealed similar activation of antiviral and type I IFN pathways under all infection conditions. In contrast, a unique molecular signature emerged during lethal PR8 infection that was characterized by an early and increased enrichment for a group of genes associated with pro-inflammatory signaling pathways (e.g. neutrophil chemotaxis, NFκB, IL-1, IL-6, and TNF signaling pathways). This “fatal molecular signature” was also associated with decreased enrichment for gene clusters associated with pulmonary homeostasis and repair, consistent with the enhanced lung pathology observed during severe infection. Integration of whole organ immune profiling and transcriptomics on sorted cell populations revealed that all subpopulations of cells analyzed within the lung microenvironment contributed to the induction of antiviral and type I IFN pathways. In contrast, neutrophils were uniquely enriched for the pro-inflammatory gene clusters found with a “fatal molecular signature”. Consistent with this observation, enhanced lung infiltration of neutrophils was observed during lethal infection and partial neutrophil depletion provided a dose-dependent enhancement in survival. An integrated analysis of transcriptomes between sorted cells and whole lung immunohistochemistry revealed that neutrophils serve as a predominate source of chemotactic signals that promote neutrophil influx. Virologic analysis found that despite similar rates of viral replication during lethal and non-lethal infection, the former was associated with enhanced viral spread within the lung. Taken together, this integrated systems biology approach revealed that lethal disease outcome was associated with enhanced viral spread, leading to an early pro-inflammatory response in the lung that acts on neutrophils to trigger an inflammatory feed-forward circuit to promote pathologic neutrophil infiltration and fatal pulmonary damage.

While mice serve as a useful IAV pathogenesis model, studies in less evolutionarily distant non-human primates (NHPs) are thought to more closely model human disease (Bouvier and
Lowen, 2010). To better understand host determinants of severe IAV infection, Kawaoka’s group infected rhesus macaques with clinical isolates of H5N1 that had caused mild or severe disease in human patients (Muramoto et al., 2014). While NHPs are a better model of human IAV pathogenesis, they are expensive and difficult to manipulate experimentally. To overcome these limitations and best capture the antiviral landscape, transcriptomics of bronchial brush samples was performed. Several modules, or sets of genes with similar co-expression patterns, were identified as being differentially expressed during mild and severe infection. The enrichment for a subset of these modules correlated directly with viral titers, identifying potential virus-dependent transcriptional networks. The gene sets comprising these modules were associated with immune-related processes, including inflammatory cytokine production and antiviral responses. Animals with severe disease outcomes were found to diverge from those with mild disease by having weak activation of these modules early during infection, followed by strong activation at later points during infection. These findings support a model where a lack of early viral control results in uncontrolled viral replication and immune activation.

Through the use of systems biology approaches spanning the tissue to cell levels, these papers establish a pathogenic role for excessive inflammation as a determinant of severe IAV infection in both murine and NHP pathogenesis models. This model is further reinforced by similar transcriptomic studies using the reconstructed 1918 IAV, highlighting the power of systems biology to illuminate the antiviral landscape (Cilloniz et al., 2009; Kash et al., 2006). Finally, recent transcriptional profiling of monocyte derived dendritic cells following infection with seasonal or pandemic strains of IAV uncovered a molecular signature that was found to be enriched in blood samples from individuals with symptomatic, but not asymptomatic IAV infection (Hartmann et al., 2015). This further highlights the utility of systems biology approaches to identify disease specific molecular markers of clinical relevance.

### 3.3. Transcriptomics defines human antiviral immunity to Dengue Virus

Dengue virus (DENV) is a mosquito borne Flavivirus that is responsible for nearly 100 million infections worldwide. While monocytes and dendritic cells are early target cells of viral replication (Balsitis et al., 2009; Cerny et al., 2014), very little is known about the early innate immune response following human DENV infection. Given the inherent lack of manipulability in human subjects, mechanistic studies remain difficult to perform. Recently, Marcin et al (Kwissa et al., 2014) overcame these limitations through the use of whole blood transcriptomics, defining the antiviral landscape during acute DENV infection in humans. PBMCs from patients acutely infected with DENV displayed distinct transcriptional profiles when compared to healthy DENV seronegative controls. Gene expression patterns segregated into distinct clusters that correlated with viral load, but were independent of disease severity. Pathway analysis of top ranked genes correlating with high viral load revealed enrichment for inflammatory and innate immune pathways, suggesting viral load directly impacts innate immune signaling. Conversely, top ranked genes correlating with low viral load revealed enrichment for stress-response related genes, including XBP-1 and associated target genes, as well as genes related to plasmablast differentiation. At the cell level, transcriptional and immune profiling revealed an expansion of CD14+CD16+...
intermediate monocytes during acute DENV infection. Ex vivo DENV infection of monocytes isolated from healthy human donors promoted the generation of CD14+CD16+ intermediate monocytes with a potent ability to drive plasmablast differentiation and antibody production through the secretion of BAFF, APRIL, and IL-10. Given the prominent plasmablast expansion observed during human DENV infection (Wrammert et al., 2012), these results implicate an intriguing role for CD14+CD16+ intermediate monocytes in the development of humoral immunity against DENV.

4. Cell level

4.1. Single cell transcriptomics

High-throughput genomic technologies have been invaluable tools for studying antiviral responses in recent years. While these approaches have provided novel insights into viral pathogenesis and immune signaling pathways, they have been limited to measurements from whole tissues or bulk cell populations, and thus have masked gene expression differences between infected and bystander cells. Conventional approaches to overcome this limitation have included comparison of responses between cells infected at low and high MOIs, using recombinant viruses expressing a reporter gene (e.g. fluorescent protein) to identify or enrich for infected cells, or enriching for RNA from virally infected cells (Konopka et al., 2007). Despite their historical utility, interpretations from these studies are confounded by the introduction of experimental artifacts (e.g. use of modified viruses, infection of cells with high virus doses). Recent technological advances in microfluidics and nucleic acid amplification technologies now allow for high-resolution gene expression analysis at the single cell level using qPCR (e.g. Fluidigm BioMark HD) and RNAseq (e.g. Illumina HiSeq 2000) (Trombetta et al., 2014). Additionally, the development of novel approaches, including droplet-based technologies (i.e. Drop-seq) (Macosko et al., 2015), has increase the ease and affordability of generating single cell sequencing libraries. While these technologies have recently been utilized to characterize the heterogeneity in gene expression within individual cells in the context of embryonic development (Shin et al., 2015), cancer (Miyamoto et al., 2015), and immunology (Lee et al., 2014; Meredith et al., 2015; Shalek et al., 2013; Shalek et al., 2014), these approaches have remained underutilized in the study of virus-host interactions.

4.2. Single cell RNAseq reveals bimodal expression of immune response genes in dendritic cells

Previous measures of antiviral responses within bulk DC populations have overlooked the heterogeneity present within this seemingly homogenous cell population. To determine the contributions of individual DCs to the overall antiviral landscape, Aviv Regev’s group treated bone marrow derived DCs (BMDCs) with prototypical pathogen associated molecular patterns, strong stimulators of DC activation, and performed RNAseq on isolated single cells (Shalek et al., 2013; Shalek et al., 2014). Surprisingly, transcriptomes from individual cells revealed bimodal transcript expression for subsets of genes involved in immune responses. While some of the divergence in cellular gene expression could be correlated to the developmental stage of the BMDCs, the remaining differences correlated with differential activation of *Irf7* and *Stat2* dependent regulatory networks. When observed
over a 6 hour period, a small subset of “early responder cells” activated antiviral signaling and type I IFN production within the first hours of stimulation. The remaining cells were “late responders” and required cell-to-cell communication and type I IFN-signaling to activate antiviral responses, suggesting activation by type I IFN produced by “early responder cells”. While these findings highlight the individual contributions of single cells to the antiviral landscape, further work is needed to extend these findings to in vivo viral infection, using freshly isolated cells from infected tissues. The bimodal expression patterns of transcripts raises the question of whether genes that are lowly expressed at the population level are highly expressed within a small subset of cells and may play a greater importance than previously appreciated.

4.3. Single cell analysis reveals subversion of type I IFN production in infected and bystander cells during rotavirus infection

Rotavirus (RV) causes severe diarrheal disease following infection of absorptive villous enterocytes within the small intestine, replicating to high titers despite an intact type I IFN response (Sen et al., 2012). While RV is known to suppress the type I IFN response, the exact mechanisms of viral evasion during in vivo infection remain unclear. To clarify how rotavirus evades host immunity, Harry Greenberg’s group analyzed viral and host gene expression in single enterocytes isolated from naïve or murine rotavirus infected mice. Striking transcriptional heterogeneity existed between cells, which could be segregated into “enterocyte\textsuperscript{lo}” and “enterocyte\textsuperscript{hi}” populations based on low or high expression of enterocyte related transcripts, respectively. The enterocyte\textsuperscript{hi} population from naïve mice displayed high levels of type I IFN transcripts, revealing that a small subset of enterocytes are responsible for maintaining homeostatic levels of type I IFN within the healthy gut. Rotavirus preferentially infected the enterocyte\textsuperscript{hi} population and resulted in decreased type I IFN expression, while failing to trigger type I IFN production in the enterocyte\textsuperscript{lo} population, consistent with the ability of rotavirus to antagonize type I IFN induction. Despite diminished type I IFN transcription within enterocytes, analysis of gene expression within the bulk intestine and sorted cell populations revealed type I IFN transcription is induced during RV infection by hematopoietic cells. Inhibition of NFκB, but not IRF-3, dependent transcription occurred following RV infection, suggesting that RV inhibits NFκB activation to prevent type I IFN production in infected enterocytes. To put these findings into context of the whole organism, Stat1\textsuperscript{−/−} mice, which are deficient in type I and II IFN signaling, were infected with murine or simian RV, a heterologous strain whose efficient replication requires a type I IFN signaling deficiency. Heterologous RV infection triggered stronger NFκB activation within the bulk intestine as compared to murine RV, despite equivalent activation of IRF-3 dependent responses. This correlated with higher type I IFN transcription within the intestines of heterologous RV infected mice, further suggesting that murine RV inhibits NFκB signaling to prevent type I IFN induction and promote viral replication. Thus, single cell transcriptional profiling uncovers a model where murine RV antagonizes NFκB mediated transcription of type I IFN in infected enterocytes, allowing for viral replication despite type I IFN production by intestinal hematopoietic cells.
4.4. Outlook for the coming age of single cell analysis

Further work is needed to better understand antiviral signaling within infected and bystander cells in the context of other relevant human viral infections using primary cells. Implementation of single cell analysis has the potential to identify novel mediators of the host response that may have low transcript expression within infected cells and have been overlooked in whole tissue or bulk cell analyses. Moving forward, it will be critical to determine the sensitivity of primer sets designed to detect viral RNA and replication intermediates to ensure reliable discrimination between infected and bystander cells. To maintain a holistic view, single cell approaches will require integration with bulk cell, whole tissue, and organism level analyses, thus providing a more complete view of the antiviral landscape at each level of complexity. The recent development of a droplet-based single cell RNAseq platform (i.e. Drop-seq) will be an invaluable tool for bridging tissue and cellular level analyses. For example, Drop-seq could be applied to profile the host response within specific cell populations of a virally infected spleen at single cell resolution. A comparison with transcriptional profiling of sorted bulk cell populations and whole spleens would reveal the cell specific contributions to developing the antiviral landscape, while highlighting nuances to the response that may be lost within bulk measurements. Finally, existing platforms for analyzing protein expression at single cell resolution (Yu et al., 2014), along with recent technological advances in single cell ChIP-Seq (Rotem et al., 2015), will allow for integrated analyses of individual cells at the epigenetic, transcript, and protein levels.

5. Conclusion

Conventional approaches have been instrumental to our understanding of virus-host interactions, but new holistic approaches, such as systems biology, are necessary to appreciate the entire scope of the antiviral response. Moving forward, integration of systems biology technologies is required to comprehensively chart the antiviral landscape, combining findings from transcriptomic, proteomic, metabolomic, epigenetic, and single cell analyses into predictive models of the antiviral response. The importance of systems integration was recently highlighted by the observation of notable discordance between transcript and protein levels in peptide-stimulated antigen specific CD8+ T cells (Hukelmann et al., 2015). Despite the utility of systems biology approaches, a notable challenge has been the extraction of meaningful information from the extensive data sets that are generated. The implementation of current and novel computation methods are required to identify and prioritize lists of top candidate molecules and signaling pathways to help drive hypothesis generation. A critical next step is experimental testing and refinement of predictive models, a too often overlooked process referred to as biologic validation. Further application of systems biology approaches will allow for the dissection of antiviral responses within traditionally difficult to study cell populations, such as rare primary cells and human clinical samples, allowing for a much needed enhanced understanding of cell type specific antiviral responses. Finally, systems biology approaches have the potential to redefine the field of personalized medicine, allowing treatment and vaccination strategies to be designed with host genetics in mind (Leroy and Qiang, 2012). In summary, the continued implementation of systems biology approaches will be essential for improving our understanding of viral...
pathogenesis and antiviral immunity, revealing novel targets for therapeutic and vaccination strategies.

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## Highlights

- Conventional approaches overlook the complexity of the antiviral response
- Systems biology approaches provide a comprehensive and unbiased analysis
- The Collaborative Cross studies how host genetics influences antiviral immunity
- Transcriptomics is a powerful tool to study tissue and cellular antiviral responses
- Single cell analysis allows for discrimination between bystander and infected cells
Figure 1.
Systems biology: a tool for charting the antiviral landscape. Systems biology can be employed to unravel the antiviral response at the organism (A), tissue (B), and cellular (C) levels of complexity. At the organism level (A), viral infection of a panel of collaborative cross mice probes the influence of genetic diversity on antiviral responses, identifying correlates of disease severity and improved small animal infection models. At both the tissue (B) and cellular levels (C), systems biology approaches can be utilized to uncover novel aspects of the host antiviral response through an iterative process of experimentation and computational modeling. Finally, emerging breakthroughs in single cell transcriptomics (C) can differentiate between infected and bystander cells, uncovering previously overlooked cell-specific differences in the antiviral response.