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Virogenomics: the virus–host interaction revisited

Arno C Andeweg, Bart L Haagmans and Albert DME Osterhaus

Genomics tools allow us to assess gene expression 'genome wide' providing an unprecedented view on the host-side of the virus–host interaction. The success of the application of these tools crucially depends on our ability to reduce the total information load while increasing the information density of the data collected. In addition to the advanced data analysis algorithms, gene annotation-pathway databases, and theoretical models, specifically designed sets of complementary experiments are crucial in translating the collected genomics data into palatable knowledge. A better understanding of the molecular basis of virus–host interactions will support the rational design of improved and novel intervention strategies for viral infections.

Address
Department of Virology, Erasmus Medical Center, Rotterdam, The Netherlands

Corresponding author: Andeweg, Arno C (a.andeweg@erasmusmc.nl)

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Introduction

Till date the genome sequence of many virus species and their hosts is known and a range of novel tools has become available to study virus–host interactions at the molecular level. The advent of genomics tools provides us with an unprecedented view on the 'host-side' of this interaction. Together with advances in high-throughput technology, bioinformatics, and statistics this progress allows us to assess gene expression controlling the host response to viral infections in a genome wide fashion. With the latest generations of microarrays this can be achieved at the mRNA level with one single microarray. Microarray technology also extends to genotyping of the host (SNP analysis), and diagnostics by the identification of imprints in the host transcriptome characteristic for certain clinical conditions. In addition recent progress in the field of proteomics allows for the measurement of the expression levels of hundreds of proteins in a single biological sample, for example by mass spectrometry. Consequently, the 'genomics revolution' offers high-throughput tools to study the complex virus–host interaction with changing expression levels of many genes and gene pathways as a direct function of viral and host genome properties. The challenge is to translate and digest this avalanche of information into palatable knowledge.

The principle of microarray assisted mRNA profiling has first been described in 1987 using a collection of cDNA fragments spotted on filter paper [1,2]. Since then the technology has maturated considerably [3] and presently a range of high quality commercial microarray platforms is available either based on long DNA oligonucelotides (single probe per transcript) or short DNA oligonucleotides (multiple probes per transcript). Most of the available expression microarrays cover all genes or even all expressed exons of a certain organism [4]. In 1998, Zhu *et al*. first applied mRNA expression profiling to characterize the innate response to a viral infection (hCMV) *in vitro* [5]. Microarray technology has been more widely used in this field since 2001. Over the past years more than 200 papers reporting the results of mRNA expression studies of the host response to virus infection have been published, in which usually a restricted set of experimental design formats was applied. Here we review the recent developments in mRNA profiling of the innate antiviral response by highlighting *in vitro* studies that represent different experimental formats which allow optimal data analysis in 'virogenomics'.

Virogenomics: formats of experimental design

Basic design: single virus, single cell type

Most early studies aimed at the characterization of the transcriptional response in a single cell type to a single virus. Examples are the studies on influenza virus, HIV-1, HSV-1, and RSV [6–11]. In general these studies were limited in size and the results generally provided a global description of the (innate) antiviral response mainly expressed in terms of sets of either up-regulated or down-regulated genes that usually supported earlier observations. As it is difficult to interpret these results in isolation, Jenner and Young performed a meta-analysis on the data obtained from 32 studies that involved 77 different pathogen–host interactions [12]. They were able to define a common host-transcriptional response in addition to a set of specific subresponses. Obviously the studies of this nature are 'conservative' in design and will at best identify shared (sub) responses that are strong enough to be detected against the intrinsically high level of noise because of the diversity in infection model systems and microarray platforms that were used in the original studies.

Basic design: time course format

Most of the *in vitro* virogenomics studies, have used a time course design. As the host response to virus infection is dynamic a time course design is required when measuring the expression levels of many genes simultaneously without having pre-existing knowledge about the expression dynamics for most of the genes represented on the microarray. Piqueras *et al*. [13] elegantly demonstrated the power of the simple time course format. Using purified DCs from healthy donors it was shown that influenza virus triggers a 'coordinated chemokine production program' in three successive waves. This program allows for a coordinated mobilization of different immune effectors in response to viral infection: at different time points different sets of chemokine messengers are expressed that are associated with the attraction of neutrophils, CTLs, NK cells, memory T cells, and also naïve T and B lymphocytes. The format nicely revealed a gene expression time pattern associated with the role of DCs in orchestrating a mounting immune response.

Now that the costs involved in microarray experiments are decreasing, larger comparative virogenomics studies are performed with experimental formats that provide a better context for data analysis. These formats can be divided in those that are 'virus-oriented' and those that are 'host-oriented', depending on whether they target the virus or the responding host system in the interaction studied.

Virus-oriented design: multiple viruses, single cell type

An early example of this format is a study performed by Huang *et al*. in 2001 [6]. The transcriptional response of DCs to different pathogens, *Escherichia coli*, *Candida albicans*, and influenza virus, was monitored. Both a shared core response and pathogen-specific programs for each of these pathogens were observed showing that DCs sense diverse pathogens and elicit tailored pathogen-specific immune responses. In a gene expression profiling study of a similar design we observed the induction of tightly regulated responses in lung epithelial cells to a set of respiratory viruses that segregated with the phylogenetic origins of the viruses involved (manuscript in preparation). Two other studies used a similar comparative approach but more closely related pathogens: human coronavirus 229E (HCoV-229E) that is usually associated with common cold and the coronavirus that causes SARS (SARS-CoV). Tang *et al*. [14] compared the transcriptional response of these two viruses in a human epithelial cell line of liver origin (Huh7 cells) and Cheung *et al*. did the same with primary macrophages [15]. At two and four hours postinfection, much more perturbation of cellular gene transcription was observed after the infection of liver epithelial cells with SARS-CoV than with HCoV-229E. Predominantly genes associated with apoptosis, inflammation, stress response, and procoagulation were up-regulated. In contrast to HCoV-229E (and influenza A virus, that was also included in this study), SARS-CoV did induce chemokine messengers for, for example CXCL10 (IP10) and CCL2, but not for IFN-β being a key component of innate immunity upon infection of macrophages [15]. This profile could explain certain key features of the pathogenesis of SARS.

Virus-oriented design: manipulated viruses

In several mRNA expression profiling experiments the response induced by virus infection is compared to that induced by exposure to UV inactivated virus preparations, in order to identify replication-dependent and replication-independent changes in gene expression [11,7,16]. In general live virus infections induce more changes in gene expression. First of all this is because of the triggering of Toll-like receptors and similar pathogen-associated motif sensing receptor systems by, for example dsRNA molecules that are synthesized during viral replication. Exposure to nonreplicating antigen only induces relatively mild and short lasting responses. More and more mRNA expression profiling studies use molecularly cloned viruses in which genes are mutated, deleted, or inserted. With this approach the effect of well-defined modifications of the viral genome is evaluated in its natural context. Using this approach Geiss *et al*. [17] examined the 'downstream' effects of NS1 protein expression during infection with either wt influenza A virus or del NS1 mutant influenza viruses in a human lung epithelial cell line (A549). Deletion of the NS1 gene increased the number and magnitude of expression of cellular genes involved in the IFN, NF-κB, and other antiviral pathways. Interestingly, a recombinant influenza virus carrying the 1918 pandemic NS1 gene was more efficient at blocking the expression of IFN-regulated genes than a closely related (wt) influenza virus (A/WSN/33). This demonstrated the contribution of the NS1 gene to viral pathogenesis by enabling the virus to disarm antiviral defense systems.

Virus-oriented design: individually expressed viral genes

This format is reciprocal to the previous format: upon expression of an individual viral gene the transcriptional response is measured in order to identify the function of the viral protein. This format has been applied to genes of, for example lentiviruses, hepatitis viruses, and herpes viruses [18–20]. The results obtained with this approach are highly specific and can only be interpreted in the context of detailed virus-specific information. An inherent disadvantage of this approach is that the effect of viral gene expression is not evaluated in the context of virus replication.

Host-oriented design: single virus, multiple cell types

Relatively few studies perform mRNA profiling in multiple cell types. The following studies clearly demonstrated the added value of this approach. Adamo *et al*. [21] performed mRNA profiling on rubella virus infected

primary human fetal fibroblasts and human adult lung fibroblasts. Although the gene expression levels of many functional gene categories were similarly perturbated, a marked difference between the two cell types was observed in genes associated with apoptosis (both for proapoptotic and antiapoptotic genes). Because fetal fibroblasts did not undergo apoptosis when infected with rubella virus it was postulated that this could promote fetal virus persistence. Another study by Sato *et al.*, monitored the transcriptional response of two cell lines to wild-type measles virus (MV) infection and to the infection of molecular cloned MV from which the V gene was deleted [22••]. Upon wt MV infection, most genes were differentially regulated in epithelial cells and IFN signaling was for instance not induced in the lymphoid cells. Surprisingly the same cell-type-specific response was observed upon infection with MV delta V. Because it was previously reported that the V protein of MV inhibited the IFN-signaling pathway, the results indicate that during MV infection, other viral factors may also act as IFN-antagonists. Furthermore this study demonstrated that during MV infection, host factors might counteract and/or overcome the inhibitory effect of IFN-antagonists like the V protein, depending on the specific cell type. These studies elegantly demonstrate the level of complexity and multidimensionality of virus–host interactions and what kind of specific experimental design and format may be required to address such complex interactions. In this case a combination of platforms has been fully exploited.

Host-oriented design: manipulated host cells

Another option to investigate the responding network of host genes is to directly manipulate the expression of selected (key) genes of the host cell during infection. This can be accomplished either by using genetically modified host cells (cells or cell-lines obtained from knock-out or transgenic animals), or by interfering with gene expression using, for example siRNA. For example, to identify the spectrum of host genes induced by the transcription factor NF-κB, Hela cells engineered to express a degradation-resistant mutant of IκBa under the control of an inducible promoter were used in two expression-profiling studies. This system allowed for a tight regulation of NF-κB-dependent gene expression. Tian *et al.* and O'Donnell *et al.* used this system to identify the NF-κB-dependent gene network in cells infected with RSV and a mammalian reovirus respectively [23•,24] and demonstrated that NF-κB mediated the induction of innate responses induced by these infections. Elco *et al.* performed mRNA profiling on a set of gene deletion cell lines infected with Sendai virus and demonstrated that TLR3 was dispensable for gene induction by this virus. By contrast, Jak1, NF-κB, and IRF-3 were essential for the induction of specific subsets of genes and IRF-3 could even suppress the expression of NF-κB-dependent genes in Sendai virus infected cells [25]. Fredericksen *et al.* showed in experiments with a similar design, that RIG-I and MDA5 are responsible for triggering downstream gene expression in response to West Nile virus infection by signaling through IPS-1 [26•]. Taken together, such experiments dissect and define the roles of individual genes and the interconnected networks in which they interact with at multiple levels. Although less specific, the studies in which host cells are preconditioned or treated with cytokines and/or other (immune) modulators may also help in dissecting interacting gene networks responding to virus infections [27,28].

From *in vitro* to *in vivo* host responses

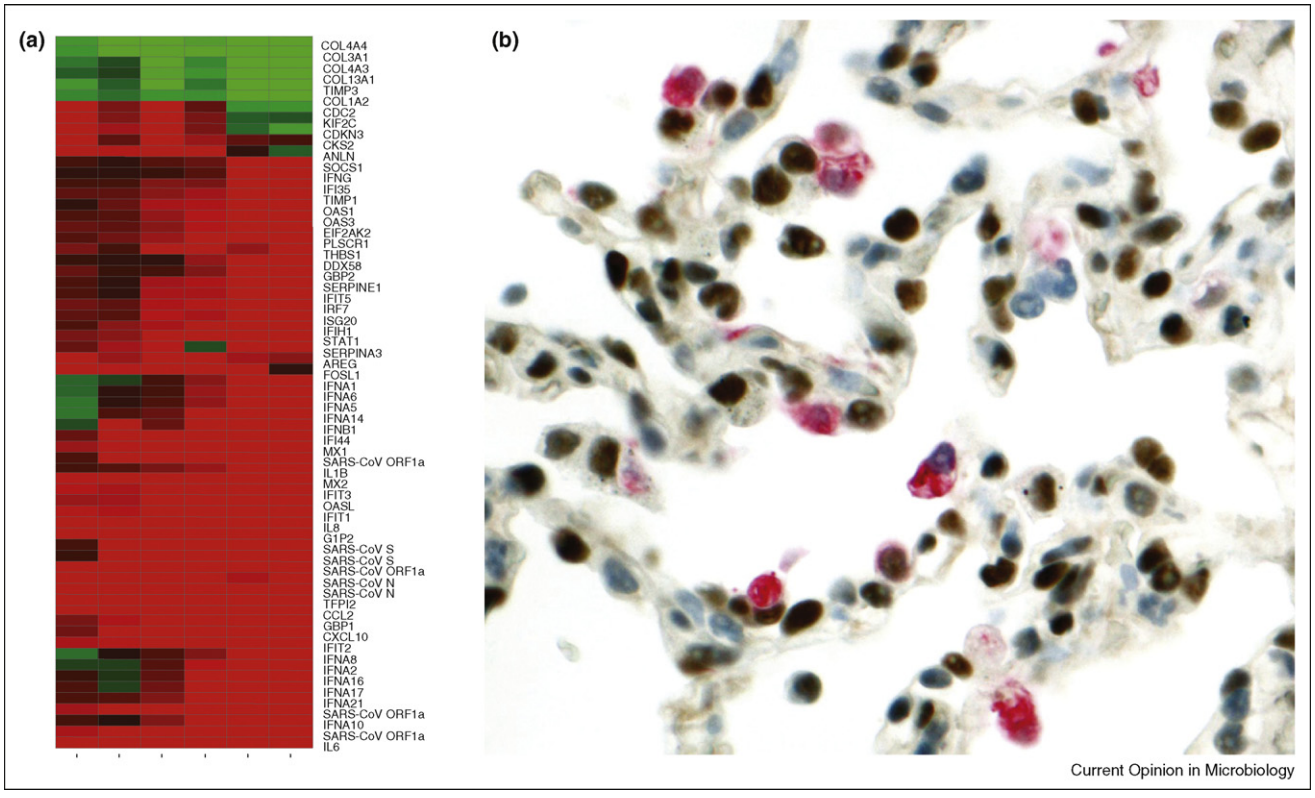
Using genomics tools for *in vivo* virus–host interaction studies adds another layer of complexity. Although eventually the most relevant information will come from this type of experiments, relatively few clues have been obtained so far with this format. However, some quite successful *in vivo* virogenomics experiments have recently been carried out in various species including nonhuman primates and also agriculturally relevant animals like chickens and cattle [29,30].

Host genomics analysis of the highly pathogenic 1918 influenza A virus infection in nonhuman primates indicated that atypical expression of the innate immune response may be a crucial determinant of the severity and outcome of infection [31]. From these overall gene expression patterns, detailed pathogenic pathways were however difficult to elucidate. Similarly, the analysis of host responses to SARS-CoV infection in the lungs of adolescent cynomolgus macaques revealed the induction of a strong innate immune response characterized by the stimulation of various cytokine and chemokine genes, including a wide range of type I interferons, interleukin (IL)-6, IL-8, and IP-10 (Figure 1A [32••]). Using immunohistochemistry, we revealed that these antiviral-signaling pathways, including the type 1 IFN-induced nuclear translocation of phosphorylated signal transducer and activator of transcription 1, were differentially regulated in infected and noninfected cells (Figure 1B). This suggests that, although SARS-CoV blocks IFN signaling in infected cells, locally produced IFNs are capable of activating noninfected cells and possibly can prevent the infection of these cells. It may be expected that *in vivo* virogenomics studies using series of genetically modified viruses in genetically modified host animals will ultimately be the best format to study virus–host interactions at the molecular level.

Conclusions and future outlook

With the advent of novel genomics tools, the studies addressing virus–host interaction at the molecular level have entered a new era. Microarray-assisted transcriptional profiling has provided us with a wealth of information about the role and function of host genes and gene-interacting

Figure 1



Host responses to SARS-CoV infection in the lungs of adolescent cynomolgus macaques. A selection of genes, involved in the immune response, cell cycle, or lung repair processes, that showed an absolute fold change >5 and $P < 0.0001$ in at least two of the six animals was made **(a)**. The detection of phosphorylated STAT1 in lung of SARS-CoV-infected macaques using immunohistochemistry shows abundant presence of phosphorylated STAT1 (brown) in lungs of SARS-CoV-infected macaques at day 1 postinfection, but not in SARS-CoV-infected cells (red) **(b)**. Taken from: A de Lang *et al*. [32••].

networks in virus–host interactions. The complex and dynamic nature of these interactions involving large numbers of genes turns genomics studies in this field into a huge information processing and data management challenge. There are several key areas that need to be specifically addressed to benefit optimally from the genomics technologies that have become available over the past decade; the most important areas are listed below.

Experimental design

Careful design of complementary sets of experiments using different formats of virus–host interaction, each focusing on slightly different aspects should reduce the total information load while increasing the information density of the data collected. Figure 2 summarizes the experimental design formats discussed in this paper.

Technology

The standardization of protocols and the technology platforms should support further integration of data analysis between individual experiments, and platforms. Relatively low levels of interplatform variation [33] among the microarrays platforms that are currently used open new opportunities for meta-analysis of separately generated data sets.

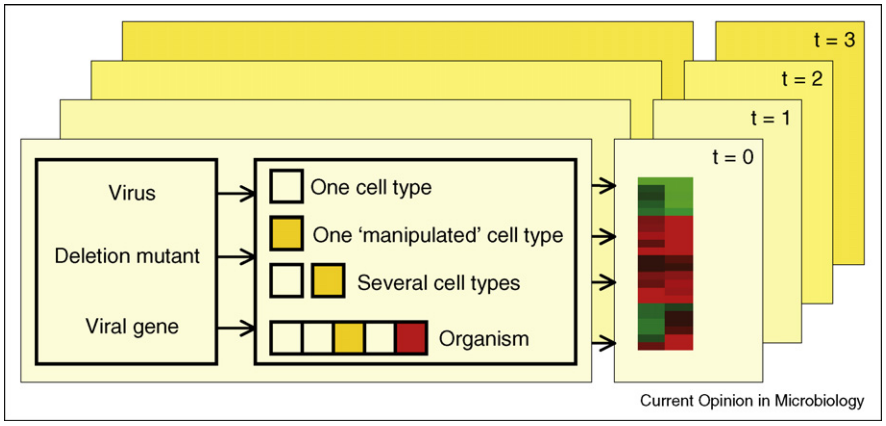
Data analysis and data management

The field of bioinformatics and statistics in the arena of transcriptomics and proteomics has developed alongside with the development of genomics tools. Uniform approaches to identify differentially expressed genes, gene-interacting networks, and pathways support integrated data analysis. Adherence of data reporting to the MIAME standard needs to be more enforced [34].

Gene annotation

Information on gene function especially regarding uniform definitions of biological pathways and processes is a notorious bottleneck in data analysis. Global analysis of mRNA expression profiles for example generally starts with a gene-enrichment type of analysis to test for over-representation of particular pathways or functions on the basis of data produced by the Gene Ontology Consortium [35] or that is available through (collections of) other data bases

Figure 2



Schematic drawing of experimental design formats applied in transcriptional profiling studies of the virus–host interaction using different viruses and different cell types at different timepoints.

[36,37]. It is encouraging to note that the Gene Ontology Consortium recently launched a program to improve the functional and pathway annotation of especially immune response related genes [38]. The more uniform and detailed information becomes available about the role of individual genes, the more the genomics field will benefit from the available advanced analysis algorithms.

Modeling gene-interacting networks

Development and implementation of mathematical and other models based on currently known and newly identified gene-interacting networks is pivotal to improve to data interpretation.

In conclusion, the success of the application of genomics tools like microarrays in studies on the complex and highly dynamic virus–host interaction crucially depends on our ability to discard most of the data that have been collected in an intelligent and appropriately selective way. This will lead to a better understanding of the molecular basis of virus–host interactions, which will support the rational design of improved and novel intervention strategies for viral infections.

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