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Emerging Viral Infections

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INTRODUCTION

Infectious viral diseases, both emerging and re-emerging, pose a continuous health threat and disease burden to humans. Since the 1980s, an increased frequency of infectious outbreaks in both humans and animals has been observed (Figure 97.1). Human mortality from recently emerged diseases ranges from about 300 people due to infection with H5N1 avian influenza A virus to tens of millions of people due to acquired immunodeficiency syndrome (AIDS). Emerging viral infections have had a large impact on livestock production as well, by causing direct mortality or because depopulation policies had to be implemented to protect safety of international trade and to control virus spread. Nearly all of the most important human pathogens are either zoonotic or originated as zoonoses before adapting to humans (Kuiken et al., 2005; Smith et al., 2009; Taylor et al., 2001; Woolhouse and Gowtage-Sequeria, 2005), and we are continuously bombarded by novel animal pathogens. Two of the most devastating pandemics in human history, HIV/AIDS and Spanish influenza, started by interspecies transmission of the causative agents (De Wit et al., 2008; Gao et al., 1999; Hirsch et al., 1989; Osterhaus, 2001). Recent outbreaks of infectious diseases, among which severe acute respiratory syndrome (SARS) coronavirus, and swine influenza A virus H1N1 2009 in humans, were also initiated by transmission from the animal host to humans, and have further highlighted this problem (Haagmans et al., 2009; Kuiken et al., 2003; Smith et al., 2009; Song et al., 2005; Tang et al., 2006). The apparently increased transmission of pathogens from animals to humans is related to a plethora of accelerating environmental and anthropogenic changes, such as increased mobility, and demographic changes, which alter the rate and nature of contacts between animals and humans. Environmental changes are thought to have played a role in the recent increased distribution of the arthropod vector Aedes aegypti, which led to large outbreaks of dengue fever in South America and Southeast Asia (Weaver and Reisen, 2010). Intense pig farming in areas where frugivorous bats are common is the probable cause of the introduction of Nipah virus from bats into pig populations in Malaysia, and subsequent transmission to humans (Chua, 2003). HIV-1 and HIV-2 originate from non-human primates (Chen et al., 1996; Keele et al., 2006; Marx et al., 1991), and central African bush hunters have been infected with simian foamy virus (Wolfe et al., 2004). Consequently, there is increased awareness that an ongoing systematic global effort to monitor for emerging and re-emerging pathogens in both animals and humans is needed (Haagmans et al., 2009; Kuiken et al., 2005; Osterhaus, 2001; Wolfe et al., 2007).

Surveillance of viral pathogens in animals should focus both on domestic animals and on wildlife, with key reservoir species that have previously been shown to represent an imminent health threat to humans. In humans, populations with either high exposure to wild or domestic animals, such as hunters, butchers, veterinarians, and zoo workers, or populations with high susceptibility, such as immunocompromized patients, would be key targets for monitoring novel viral pathogens. Collectively, these efforts would result in the identification
of the diversity of viruses to which humans are exposed, the identification of animal pathogens that may threaten us in the future, and, hopefully, their early detection in humans and subsequently their control (Haagmans et al., 2009; Kuiken et al., 2005; Wolfe et al., 2007). It may even be possible to predict which viruses are most likely to cross the species barrier and what changes may be required for the evolution of an animal pathogen into a human-specific pathogen. Taken together, a more effective and rational approach to the prevention of new viral epidemics in humans and animals is essential. Research efforts to mitigate the effects of infectious threats, focusing on improved surveillance and diagnostic capabilities, and also on the development of intervention strategies such as vaccines and antiviral agents, are crucial.

Genomics-based tools are a potential candidate to respond to these challenges. Recent advances in genomics-based tools have allowed more sophisticated understanding of interactions among genes and genetic pathways, the environment, and the host and its pathogens. Already, it is apparent that genomics tools have started to change the practice of medicine. For example, they have been used to assess prognosis and guide therapy in several forms of cancer, to stratify patients according to risk of long-QT syndrome, and to shed light on the response to certain drugs, such as antiepileptic agents (Priori et al., 2003; Siddiqui et al., 2003; Van de Vijver et al., 2002). Our understanding of how living organisms and systems within organisms interact with each other and respond to the environment all changes dramatically in this genomic era. Full benefit from genomics tools in combating viral diseases requires understanding the dynamics of infection with regard to viral diversity, evolution, and epidemiology, in combination with a better understanding of the pathogenesis of the infection and the molecular basis of the host response to infection.

In this chapter, we describe how genomics may aid in mitigating viral threats, by highlighting potential roles and limitations of genomics approaches in surveillance and diagnostics, and development of vaccines and antivirals (Figure 97.2). As the clinical outcome of an infectious disease depends on properties of both the pathogen and the host, a distinction is made between viral genomics and host genomics.

**VIRAL GENOMICS**

Viral genomics can be defined as the study of viral genomes, functions of their individual genes, and interactions of all of the genes present in a viral genome. Most studies involving viral genomics focus on virus discovery and diversity analyses, which in turn positively influence the development and efficacy of surveillance tools, diagnostics, vaccines, and antivirals (Figure 97.2).
molecular assays for virus identification in clinical samples. Classical methods used for the identification of known viruses include cell culture-based assays, immunological assays, and molecular detection methods such as polymerase chain reaction (PCR). Although these diagnostic assays are successful, failure rates in determining the etiological cause of the disease can be significant (Granerod and Crowcroft, 2007; Hajjeh et al., 2002), at least in part owing to the limited detection of divergent viruses due to the high specificity of these assays. During the outbreak of pandemic H1N1 influenza virus in 2009, rapid influenza diagnostic tests (RIDTs) were widely used for their ability to quickly detect viral antigen in respiratory clinical specimens. In a comparative study by the Centers for Disease Control and Prevention (CDC), 65 clinical respiratory specimens, which previously tested positive for pandemic H1N1, and seasonal H1N1 and H3N2 influenza virus by reverse transcriptase (RT)-PCR, were tested using three different RIDTs (Table 97.1). The RT-PCR assay is more sensitive than antigen detection assays. Moreover, the sensitivity of the RIDTs also seemed to vary with different influenza virus strains (Centers for Disease Control and Prevention, 2009). The failure rates in determining the etiological cause for example in encephalitis, acute flaccid paralysis, and non A-E hepatitis were reportedly 30–85% (Granerod and Crowcroft, 2007), 12% (Saeed et al., 2007), and 18–62% (Chu et al., 2001), respectively. In a relatively large proportion of patients suffering from diarrhea (Finkbeiner et al., 2008) and acute respiratory illnesses (Juven et al., 2000), no pathogens could be detected, despite the use of a wide range of sensitive diagnostic assays. There are many clinical syndromes in which viruses are suspected to play a role, but for which current techniques fail to uncover an etiological agent, underlining the limitations of even the best current methodologies for diagnosis of viral infections. Overall, the fact that many acute and chronic diseases with a suspected infectious cause are still of unknown etiology may point to the presence of as-yet-unidentified viruses in the human population. Apparently, the virus-specific assays currently used in routine diagnostic settings are not equipped for the identification of new and previously unrecognized pathogens. Modern genomics-based laboratory techniques, such as generic PCRs, pan-viral microarrays, and sequence-independent amplification combined with high-throughput sequencing, will play an increasingly important role in virus identification and discovery.

With the development of PCR and sequencing techniques in the 1970s and 80s, we have seen an unprecedented increase in the identification and characterization of viral genomes. This led to the development of new species-specific diagnostic assays for viruses infecting animals and humans, which are...
The limitations of existing viral detection methodologies require additional labor-intensive procedures for final diagnosis. Setting discrimination between subtypes or genera of viruses in a single assay is relatively low, and in a diagnostic setting, the maximum number of detectable viruses is constantly evolving, the maximum number of detectable viruses for which no etiological agent has been identified. Also, viruses are highly divergent viruses may still elude identification. Also, implementation as a standard diagnostic assay in clinical settings would require the establishment of a reference library of hybridization signatures for hundreds of individual viral subtypes, training of skilled laboratory workers to read these “viral barcodes,” and the ability to create a platform in which the method is cost-effective and reproducible. These barriers may hamper large-scale clinical use of this method.

Knowing that the speed, accuracy, efficiency, and cost-effectiveness of DNA sequencing have improved continuously since its introduction, it is no longer unrealistic to sequence thousands of genes of a single individual with a suspected viral infection. Therefore, the next generations of sequencing technologies have certain important advantages over pan-viral PCR assays, especially in terms of broader-reaching and less biased detection of viruses and the possibility of subtyping viruses.

Table 97.1 Performance characteristics of RT-PCR versus rapid influenza diagnostic tests (RIDTs), point-of-care tests that detect influenza viral nucleoprotein antigen, in patients infected with pandemic H1N1 influenza virus 2009 (pH1N1), seasonal H1N1 virus (sH1N1), or seasonal H3N2 (sH3N2)

| RIDT       | Detection | Total no. of specimens positive by RIDT/ Total no. positive by RT-PCR | Sensitivity (%) |
|------------|-----------|-------------------------------------------------|-----------------|
| BinaxNow   | pH1N1     | 18/45                                           | 40              |
|            | sH1N1     | 3/5                                             | 60              |
|            | sH3N2     | 12/15                                           | 80              |
| Directigen EZ Flu | pH1N1 | 21/43                                           | 49              |
|            | sH1N1     | 3/4                                             | 75              |
|            | sH3N2     | 10/12                                           | 83              |
| QuickVue   | pH1N1     | 31/45                                           | 69              |
|            | sH1N1     | 4/5                                             | 80              |
|            | sH3N2     | 12/15                                           | 80              |

Table adapted from Centers for Disease Control and Prevention (2009).
per instrument run (Kuroda et al., 2010). The key to virus identification by sequence-independent amplification methods is increasing the levels of viral nucleic acids while reducing background nucleic acids. This is generally done via filtration, centrifugation, and enzymatic removal of non-particle protected nucleic acids (Delwart, 2007). Already, many new viruses have been identified using sequence-independent nucleic acid amplification techniques (Allander et al., 2005; Finkbeiner et al., 2009; Fouchier et al., 2004; Gaynor et al., 2007; Jones et al., 2005; Kapoor et al., 2008b, 2009; Palacios et al., 2008; Smits et al., 2010b; Van der Hoek et al., 2004; Van Leeuwen et al., 2010; Victoria et al., 2008). Viral genomic analyses of environmentally collected samples or unmanipulated biological samples, such as fecal material, showed a high diversity of viral communities (Breitbart et al., 2002, 2003; Finkbeiner et al., 2008; Venter et al., 2004). Before wide-scale random amplification of nucleic acids and sequencing becomes an accepted medical practice, however, a number of challenges need to be overcome, including development of high-throughput, cost-effective, and reproducible methods, bioinformatics to analyze the generated data, training of skilled laboratory workers to interpret the data, and linking the presence of virus in samples to disease (Delwart, 2007; Service, 2006; Ten Bosch and Grody, 2008). In case new viruses are identified, epidemiological studies and ultimately experimental infection studies in appropriate animal models are required, to prove that the identified virus is truly the causal agent of infection.

Modern genomics-based laboratory techniques will play an increasingly important role in the surveillance and identification of new and previously unrecognized pathogens in both animals and humans. This will provide new targets for development of virus discovery tools, specific diagnostic and surveillance assays, and vaccines and antivirals that can be used in the clinical setting. Although ongoing innovations in genomics-based tools would aid perfection of these techniques, allowing them to become standard in medical practice, implementation into routine diagnostic settings awaits several major technical breakthroughs and improvements in cost-effectiveness and analysis of results.

Virus Diversity

Genomics-based tools are not only useful for virus identification and characterization; they also allow investigation of patterns of virus evolution by in-depth sequencing of viral genomes in individual hosts. There is growing evidence that epidemiological inferences that are made on consensus sequences of populations of viruses within an individual hide information on generic and phenotypic diversity. Moreover, viral lineages identified in individual hosts are often highly divergent, suggesting that they did not evolve in the host via mutations and antigenic drift. Thus, it is clear that single individuals can be superinfected with multiple virus variants, which may have major consequences for development of vaccines and intervention strategies. At present, full genome sequences of each major human pathogen are available, and over 3000 viral genomes have been completed in recent years. With the advances in sequencing technology and bioinformatics, exponential growth is expected in sequence information of viruses and viral quasispecies in individual hosts. This increasing availability of viral genomic sequences allows a previously unattainable route to investigate phenomena such as viral pathogenesis, virus evolution, development of resistance to treatment, and vaccine development, as illustrated below.

Both hepatitis C virus (HCV) and human immunodeficiency virus (HIV) continue to exert a substantial disease burden on millions of chronically infected patients worldwide. These viruses are highly genetically diverse, both within individuals and globally (Rambaut et al., 2004; Simmonds, 2004; Simmonds et al., 2005). This has hampered and prohibited vaccine development and development of efficient antivirals. High levels of replication, and an error-prone RNA-dependent RNA polymerase and a reverse transcriptase enzyme in HCV and HIV, respectively, result in a cloud of individual quasispecies sequences in any infected person. Minor quasispecies variants that are resistant to antiviral actions of small molecule compounds are readily selected, also because they are often already present in the quasispecies swarm, and become dominant during therapy. A recent study examined treatment-naïve HCV-infected patients for pre-existing resistance mutations for 27 developmental antiviral compounds directed against nonstructural proteins NS3 (protease) and NS5B (RNA-dependent RNA polymerase). The results showed that 44.4% of genotype 1b patients had at least one mutation associated with resistance and 2.7% had mutations in both NS3 and NS5B (Gaudieri et al., 2009). Shimakami and coworkers (2009) commented that an even greater frequency of such mutations would undoubtedly be discovered with “deeper” sequencing methods. The risk of resistance can be diminished by using combinations of small antiviral compounds, although in vitro studies have already showed that double-resistant mutants can be selected, albeit often at an associated fitness cost (Mo et al., 2005). Similar observations have been made in HIV infections. Primary drug resistance mutations in treatment-naïve patients were observed in 5–10% of the patients (Gupta et al., 2008; Paredes and Clotet, 2010). Several primary resistance surveillance studies have demonstrated increases in detection of HIV-1 variants with higher primary resistance relative to standard population-based sequencing (Metzner et al., 2005; Paredes et al., 2007). If antiretroviral therapy is to remain successful, resistance-associated failure of therapy must be prevented by (1) identifying primary resistance, (2) tailoring antiretroviral therapy to ensure that all different compounds retain full antiviral activity, (3) ensuring long-term adherence by patients, (4) detecting failure of therapy early and identifying emerging resistance mutations, and (5) managing failure of therapy by switching to new antiretroviral regimens (Hirsch, 2008). Here, genomics-based tools aimed at resistance detection
could help design more effective personalized antiretroviral therapies.

Another example of how genomics-based approaches influence infectious viral disease prevention methods comes from influenza A virus evolution studies. Computational analyses of sequence data have changed our views on influenza A virus evolution. Genomics data have revealed that genome-wide interactions are a critical aspect of influenza virus evolution. This was shown by a global rise in resistance of influenza A viruses to adamantanes, one of the first-generation antivirals (Bright et al., 2005, 2006). Surprisingly, this rise in resistance was also observed in regions where adamantanes are rarely used. Apparently, the resistance mutation was linked to another beneficial mutation located elsewhere in the genome (Simonsen et al., 2007), resulting in much broader spread of influenza A viruses carrying the resistance mutation. Genomics-based studies also confirmed the importance of reassortment in influenza virus epidemiology and evolution, which sometimes results in generation of viruses with altered antigenic properties and in vaccine failure (Holmes, 2009; Holmes et al., 2005). The raw material for reassortment is provided by co-circulation of multiple and often diverse viral lineages from the same subtype in a given population (Holmes, 2009; Holmes et al., 2005; Nelson et al., 2008), and a global source population of human influenza viruses, located in Southeast Asia, ignites the seasonal influenza epidemics in the rest of the world (Rambaut et al., 2008; Russell et al., 2008). A global influenza surveillance network routinely characterizes genetic and antigenic properties of influenza A viruses, the latter mainly using the classical hemagglutination inhibition assay. This is a binding assay based on the ability of influenza viruses to agglutinate red blood cells with one of their surface proteins, hemagglutinin (HA), and the ability of antisera raised against the same or related strains to block this agglutination. In combination with sequence information on the immunogenic HA1 domain and epidemiological data, antigenic data are used to select strains for the vaccine against seasonal epidemic influenza (Smith et al., 2004). The fact that the source population of human influenza A viruses is known is crucial to vaccine development, as vaccines can fail due to a periodically more accelerated antigenic evolution than expected from genetic data (Smith et al., 2004). Knowing where these variants are generated and intensively surveying that geographic area will aid in formulating vaccine strains. Future genomics-based studies on influenza A virus variability, in combination with comparative pathology studies, may unravel, for example, whether there is a genetic basis for the observed differences in clinical presentation of influenza in humans.

Thus, virus diversity studies can have an impact on vaccine efficacy, antiviral efficacy, and resistance. The challenges for future virus diversity and evolution studies are to integrate viral genomics with data on clinical symptoms and signs, pathology, vaccine development, and antiviral resistance studies. Advances in genomics-based tools, in combination with increasing amounts and more in-depth analysis of individual samples from patients with mild to more severe illness, may allow a priori risk assessments regarding severity of disease, and may guide vaccine and therapy options. As mentioned previously, implementation of these methods into routine diagnostic settings will make it necessary to take large hurdles in the future.

**HOST GENOMICS**

It is common knowledge that immune status, age, nutritional status, and many other factors play an important role in the clinical outcome of a viral infection, underlining the importance of studying both host and pathogen parameters with genomics tools in the battle against viral infections. Following completion of the Human Genome Project, the investigation of underlying heritable mutations capable of inducing or increasing susceptibility to disease has become possible. But these mutations do not explain all patient-to-patient differences in pathology. In contrast to analyzing a patient’s DNA, gene expression profiling examines the expression of many different RNAs and/or proteins, and integrates the impact of DNA-based effects and a variety of other factors, including viral infections, that modulate cellular responses. Both analyses lead to a more complete understanding of the mechanisms contributing to pathology, and can drive development of new diagnostic and therapeutic strategies (Figure 97.2).

**Genome-wide Association Studies and Functional Screens**

The underlying mechanisms of many diseases and efficient treatments remain obscure. It also remains largely unknown how host factors influence the viral life cycle. Genomic research offers new opportunities to determine how diseases occur by characterizing molecular abnormalities underlying disease processes, and to identify host genes required for viral replication. The latter is done by performing genome-wide functional screens using small interfering RNAs (siRNAs), which interfere with the expression of a specific gene, in an infectious in vitro culture system. Genome-wide association studies (GWAS) are used to identify genes associated with disease. Surveys of genetic variation, with typically hundreds of thousands of single nucleotide polymorphisms (SNPs) in patients with and without a given disease, are performed to identify variant SNPs that are found significantly more commonly in patients with disease compared with the disease-free controls.

Many SNPs, associated with a wide range of diseases and characteristics, have been described (Cooper et al., 2008; Gudbjartsson et al., 2009; Liu et al., 2008; Miyagawa et al., 2008). For example, some 450 different heritable mutations involved in development of primary cardiomyopathies have been identified, and it was estimated that SNPs are responsible for up to 70% of hypertrophic cardiomyopathy (Margulies, 2008). Functional analysis of human pathogen-gene interactions is at an early stage, however, and many advances are anticipated, including the development of high-throughput screening methods to identify host genes that modulate viral infection and replication.
et al., 2009). Assays to screen for cardiomyopathy-causing mutations in affected patients are available, with 30–60% likelihood of detecting the genetic basis in a patient with hypertrophic and dilated cardiomyopathies (Margulies et al., 2009), which is likely to increase as more mutations are identified. Ultimately, these types of assays will be accessible to clinicians, who can identify patients and family members at increased risk for disease, and possibly prevent or modulate disease progression with disease-modifying therapies.

Despite the enormous impact of viral infections on human health, relatively few GWAS have been performed to successfully identify disease susceptibility variants in viral infections. Perhaps not surprisingly, most studies that have been done concern chronic, and often lifelong, infections, such as HIV and HCV. Recently, it was shown that several SNPs located near the IL28B gene were significantly more common in HCV patients who responded to interferon therapy than in non-responders (Ge et al., 2009; Suppiah et al., 2009; Tanaka et al., 2009), suggesting that there is a genetic basis for efficiency of anti-HCV therapy. Although diagnostic testing to identify likely responders to interferon therapy may be a future possibility, clinical decision-making will be difficult as the effect of the advantageous IL28B SNPs is not absolute, and no alternative to interferon therapy exists. Fellay and coworkers (2007) used a GWAS to determine why viral loads prior to onset of AIDS can differ up to five logs between individual patients. They identified polymorphisms associated with HLA loci B and C, and concluded that they were major determinants in the difference of circulating viral loads (Fellay et al., 2007). Moreover, their data underline the importance of carrying out similar studies for other chronic, and perhaps also acute, infectious diseases. After this initial study, several other implicated other loci that influence viral load and progression of HIV-1 infection to AIDS (Loeuillet et al., 2008; Pelak et al., 2010). Remarkably, CCR5, a gene critical for HIV infection, was not detected at all. This shows the difficulties imposed by strict statistical tests that, although they allow discovery of new genes, also guarantee that many true associations are missed in the process of avoiding false-positive associations (Winkler, 2008).

Another method developed to find HIV and HCV dependency factors is a siRNA genome-wide functional screen (Brass et al., 2008; Li et al., 2009). In this approach, siRNAs were used to knock out some 20,000 genes one by one in a cell line that supported HIV or HCV replication. In these assays, many HIV and HCV dependency factors that influence viral replication were identified. As these factors were not required for cell viability, they are excellent, genetically stable candidates for antiviral drugs. It is of note that 10 of the host genes needed for HIV replication are also important for HCV replication, which could be exploited in cases of co-infection (Li et al., 2009). In addition, several identified HCV dependency factors were found to be required for replication of two other members of the Flaviviridae family, West Nile virus and dengue virus (Krishnan et al., 2008), and for particle formation of multiple viruses, including Marburg, Ebola, and measles viruses (Murray et al., 2005; Shisheva, 2008). The siRNA screens are, however, limited to the identification of cellular genes. Moreover, the effects on disease of the identified gene products in the infected population remain unknown. Similar functional genomics experiments were performed to identify host cell factors that are temporarily dispensable for the host, but crucial for virus replication of a broad range of influenza A viruses, including highly pathogenic avian H5N1 and pandemic H1N1 viruses (Prusty et al., 2011). These host factors provide promising new antiviral targets that can block or alleviate the downstream effects of pathogen infection.

Overall, GWAS and genome-wide functional screens have been shown to elucidate biomarkers of disease risk, provide new biological insights into pathophysiological processes, clarify how known risk factors predispose to disease development, and discover host genes required for replication of viruses. To fully interpret the results of future studies, data should be incorporated from functional and expression experiments and virus-mediated changes in gene expression (Ge et al., 2008). The insights obtained from these types of studies may be used in development of intervention strategies, and hold promise for the personalization of medicine by linking individual genetic information to disease prevention and therapy response. It is highly interesting that different viruses share the need for certain host proteins for replication in vitro. Defining host factors and pathways upon which multiple viruses depend could aid in development of common intervention strategies applicable to a large number of viral infections.

**Gene Expression Profiling**

As the outcome of viral infections depends as much on viral as on host properties, scientists have used a wide array of methods to study how viruses interact with the host and the mechanisms by which the host protects itself against viruses. With the development of expression microarrays and other high-throughput genomics and proteomics techniques, our ability to characterize the key interactions between host and pathogen has greatly increased, and has unraveled possibilities for development of diagnostic assays and multi-targeted therapies designed to limit virus replication and mitigate immunopathology (Andeweg et al., 2008). Treatments may even be tailored to target host response pathways common to many virus infections, which could be used early in infection when an accurate diagnosis is not yet available. Although expression profiling experiments have been performed for many different viruses, including HIV, HCV, SARS coronavirus, influenza A virus, dengue, and West Nile, only a few examples will be discussed here to show the principal applications of genomics/proteomics-based tools to study infections with different pathogenic viruses.

Dengue virus infection is a global public health problem that causes 50–100 million cases of dengue fever (DF) yearly,
resulting in 500,000 clinical cases of the life-threatening dengue hemorrhagic fever syndrome (DHF). DF and DHF patients display a similar clinical picture early after infection. In endemic areas with outbreaks of dengue cases, the capacity of medical assistance is limited, and an early discrimination between DF patients and patients at risk of developing DHF would allow a more effective allocation of medical attention. A recent study using genomics-based tools on a well-characterized cohort of dengue patients aimed at obtaining insight into early immune mechanisms associated with dengue severity and identifying biomarkers to predict infection outcome. The results suggested that DF and DHF are extremes of a continuum of the same disease, and not two separate diseases (Nascimento et al., 2009; Sierra et al., 2007). A set of genes were identified that, upon quantification by PCR assays on the first medical visit of a dengue virus-infected patient, could be used to predict whether the patient will develop DHF symptoms a couple of days later (Nascimento et al., 2009).

Peripheral blood gene expression signatures have been identified that accurately distinguish individuals with symptomatic acute respiratory infection from uninfected individuals. In addition, these gene expression signatures distinguish individuals with respiratory viral infection from bacterial infection, and even distinguish between individuals with respiratory viral infections caused by human rhinovirus, influenza A virus, and respiratory syncytial virus (Zaas et al., 2009). Thus, gene expression profiling may serve as a useful diagnostic test for tailoring treatment in acute respiratory infections and could enhance traditional diagnostic assays.

To individualize treatment in chronic hepatitis C virus-infected patients, unbiased proteomic profiling has been used to identify host predictors of virologic response to interferon-based therapy. A serum-based protein signature was identified that could accurately predict whether chronic HCV-infected patients would respond to standard-of-care interferon-based therapy prior to treatment (Patel et al., 2011). As interferon-based therapy is associated with significant side effects and only about half of treated subjects respond to the relatively expensive therapy, accurate prediction of antiviral efficacy is an important tool in the proper tailoring of individual treatment processes.

Gene expression profiling studies in simian immunodeficiency virus (SIV)-infected natural and non-natural hosts have provided a unique view of how host responses influence lentiviral infection outcome. Many non-human primate species in sub-Saharan Africa are infected with SIV. These natural reservoir hosts do not develop AIDS as a result of infection, and appear to live a normal lifespan. In contrast, non-natural hosts such as Asian pig-tailed macaques develop AIDS in a manner similar to HIV-infected humans (Sodora et al., 2009). During acute infection of natural and non-natural hosts, virus replication peaks within a few weeks after infection, and high viral loads remain during the chronic phase of infection. It long remained unclear how natural hosts could sustain high viral loads, without developing disease. Gene expression profiling in pathogenic and non-pathogenic SIV infection models revealed that a strong type I interferon response is initially induced in both models, but is resolved after peak viral load in non-pathogenic SIV infection models only (Lederer et al., 2009). The natural host also exhibited better preservation of overall cell homeostasis, lower levels of immune activation, no progressive depletion of CD4+ T cells, and preservation of mucosal Th17 cells (Bosinger et al., 2009; Favre et al., 2009; Jacquelin et al., 2009; Lederer et al., 2009; Sodora et al., 2009). These results suggest that active immune down-regulatory mechanisms, rather than intrinsically attenuated immune responses (Mandl et al., 2008), underlie the low level of immune activation and coexistence with SIV infection without progression to AIDS. Human long-term asymptomatic HIV-infected patients with high viral loads and low immune activation status have been described that seem to underscore the studies in natural reservoir non-human primate hosts (Choudhary et al., 2007). These data show that the supposedly longstanding evolutionary coadaptation between primate lentiviruses and natural reservoir hosts, which allowed coexistence of virus and host, may eventually also occur in humans (Choudhary et al., 2007; Sodora et al., 2009). Most importantly, the genomics-based studies in SIV-infected natural and non-natural hosts showed that future research should focus on identifying the mechanisms responsible for chronic immune activation, and should devise immunomodulatory strategies that prevent it.

The zoonotic transmission of SARS coronavirus caused an outbreak in 2003 of severe acute respiratory syndrome, a pneumonic disease in humans, with an overall mortality rate of ~10%. The clinical course and outcome of SARS coronavirus-associated disease were more favorable in children compared to adolescents and adults (Hon et al., 2003; Leung and Chiu, 2004; Wong et al., 2003); elderly patients had a poor prognosis, with mortality rates of up to 50% (Peiris et al., 2003, 2004). This predisposition to disease is also shown when aged and young cynomolgus macaques are experimentally infected with SARS coronavirus, with aged macaques showing significantly more lung pathology upon infection than young adult macaques (Smits et al., 2010a). Gene expression profiling studies revealed that aged macaques display a more prominent host response to infection than young adult macaques, with an increase in differential expression of genes associated with inflammation, whereas type I interferon expression is reduced. This observation triggered another experiment, in which aged macaques were therapeutically treated with type I interferon after infection, which reduced pathology and proinflammatory gene expression without affecting virus replication levels. As the clinical manifestations of SARS coronavirus-associated disease are highly similar to acute lung injury caused by multiple other pathogenic conditions, including sepsis, gastric acid aspiration, and pulmonary infections such as H5N1 avian influenza virus, in multiple host species, a conserved injury pathway was proposed (Imai et al., 2008). Assuming that there is such a conserved injury pathway that can be induced by multiple triggers, including pandemic influenza A viruses, modulation of
the host response by type I interferon provides a promising outlook for novel intervention strategies (Smits et al., 2010a). More recent studies in African green monkeys, however, indicate that distinct SARS coronavirus-induced acute lung injury pathways may exist in different host species, which will need to be taken into account when analyzing intervention strategies in these species (Smits et al., 2011).

Microarray expression profiling studies have also been used to determine why newly emerging zoonotic influenza virus infections often cause more severe disease than the seasonal epidemic influenza A viruses. By using genetically engineered influenza A viruses, it was shown that the NS1 protein derived from the 1918 Spanish H1N1 pandemic influenza virus blocked expression of the host antiviral response more efficiently than the NS1 protein from an established seasonal influenza virus (Geiss et al., 2002). Other studies in mice and macaques with genetically engineered influenza viruses containing gene segments from the 1918 H1N1 virus or the pathogenic avian H5N1 influenza A virus suggest that highly pathogenic influenza viruses induce severe disease through aberrant and persistent activation of proinflammatory cytokine and chemokine responses (Baskin et al., 2009; Kash et al., 2004, 2006; Kobasa et al., 2007). In ferrets, it was shown that infection with H5N1 influenza A virus induced severe disease, with strong expression of CXCL10, among others. Treatment with an antagonist of the CXCL10 receptor reduced the severity of symptoms and viral titers compared to control animals (Cameron et al., 2008). These experiments show that comparison of different viruses and strains, with respect to their impact on host gene expression, could lead to development of therapeutic interventions, but also could aid in predicting the pathogenicity of new or past virus strains by using high-throughput profiling techniques (Geiss et al., 2002).

Overall, gene expression profiling studies of viral infections provide insight into pathophysiologic processes, elucidate biomarkers of disease risk, and allow knowledge-guided development of host response-modulating therapeutic treatments. Future efforts should focus on comparing and contrasting the outcomes of infection with closely related viruses in different host species, to delineate the various host pathways and viral traits that cause the differences in disease outcome. Integration of genomics/proteomics data obtained for different viruses and different hosts could potentially elucidate common host pathways involved in development of pathology, and allow rational design of diagnostic assays and host-modulating antipathology strategies applicable to a large number of viral infections.

**FUTURE CHALLENGES**

It is clear that genomics-based tools have obtained a firm foothold in many scientific disciplines. In combating viral infections, either established or newly emerging, genomics-based tools are predominantly applied to identification and diagnosis of viral infections, virus diversity studies, GWAS, genome-wide functional screens, and host gene expression profiling. These studies are delivering new targets of both viral and host origin for the development of diagnostic and surveillance assays, vaccines, and therapeutics (Figure 97.2). From a corporate perspective, large populations of patients could benefit from any vaccine or therapeutic agent that becomes financially of interest to produce. True personalized treatment for single individuals will be more difficult to accomplish. Thus, identifying biomarkers, assays, and host-modifying treatments, that work for and/or against a broad spectrum of different viruses, are of utmost importance. Comparative studies into dynamics of infection with regard to viral diversity, evolution, and epidemiology, in combination with the molecular basis of the host response to infection and pathogenesis, may enable the rational design of multipotent biological response modifiers that will mitigate the effects of viral diseases. By focusing on broad-acting intervention strategies and surveillance for new viral pathogens, we may be better prepared for any outbreak of newly emerging infectious disease.

To realize the full potential of genomics-based tools and data in a clinical setting, several major obstacles need to be overcome. For one, methods need to be cost-effective and in a high-throughput format. Although it has been contemplated that within two decades it will be possible to sequence anyone’s entire genome for less than $1000, this may be complicated by ethical, legal, social, and societal issues. Existing DNA sequence and/or method intellectual property claims may have an impact on development of multi-gene diagnostic tests (Chandrasekharan and Cook-Deegan, 2009). In addition, although seemingly far-fetched at present, privacy measures regarding genetic information need to be enacted and signed into law, to prevent abuse of such data by, for example, insurers or employers, which may one day become a realistic situation. Secondly, a large investment in bioinformatics tools, databases and data management is required, to make optimal use of the generated data. Thirdly, clinical diagnosis of disease will largely change when use of genomics-based tools becomes standard operating procedure, and molecular diagnosticians will need to gain experience in interpreting the data generated by these radically different and rapidly evolving techniques. However, as with so many other molecular biology tools that have found their way into the clinical setting, there is no reason why genomics-based tools could not transition from the research setting into the clinic in the future, despite the hurdles that need to be overcome (Ten Bosch and Grody, 2008).

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