The Top Five “Game Changers” in Vaccinology: Toward Rational and Directed Vaccine Development

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Abstract

Despite the tremendous success of the classical “isolate, inactivate, and inject” approach to vaccine development, new breakthroughs in vaccine research are increasingly reliant on novel approaches that incorporate cutting edge technology and advances in innate and adaptive immunology, microbiology, virology, pathogen biology, genetics, bioinformatics, and many other disciplines in order to: (1) deepen our understanding of the key biological processes that lead to protective immunity, (2) observe vaccine responses on a global, systems level, and (3) directly apply the new knowledge gained to the development of next-generation vaccines with improved safety profiles, enhanced efficacy, and even targeted utility in select populations. Here we highlight five key components foundational to vaccinomics efforts: applied immunogenomics, next generation sequencing and other cutting-edge “omics” technologies, advanced bioinformatics and analysis techniques, and finally, systems biology applied to immune profiling and vaccine responses. We believe these “game changers” will play a critical role in moving us toward the rational and directed development of new vaccines in the 21st century.

Introduction

PUBLIC HEALTH SERVICES including the availability of vaccines, has appropriately been based on a vision of population-wide access. Although encouraging and enabling wide-spread access to life-saving vaccines, this one size fits all approach has ignored person-to-person, and even population-to-population-based differences in vaccine outcomes. We believe that understanding these differences are key to future advancements in vaccinology. Furthermore, although empiric vaccine development has served us well in the past, conventional vaccinology is currently facing a number of difficult challenges that requires us to move beyond the historical paradigm of “isolate, inactivate, and inject.” Successful vaccines for some pathogens, particularly hypervariable viruses and complex bacteria and parasites such as HIV, malaria, and tuberculosis still elude us, whereas vaccines for other pathogens (influenza) require yearly reformulation and repeat immunization. In addition, many tropical diseases (leishmaniasis, schistosomiasis, amebiasis) have been neglected by vaccine research, and the threat of emerging pathogens must also be considered. In many cases we do not have well-defined correlates of protection, or even a detailed understanding of what immune pathways are protective, or how pathogens might interfere with immunity. Over the last several decades increased public scrutiny of vaccine safety has

raised the bar for what constitutes acceptable risk with serious implications for both vaccine design and for development costs. We believe that the emerging field of vaccinomics will address many of these current challenges and provide valuable knowledge to supplement current vaccine strategies and concepts, allowing us to enhance public health services and address vaccine response heterogeneity, thus serving both entire populations (through newer, and/or safer vaccines) and specific groups of individuals (through individualized medicine approaches).

We originally introduced the term “vaccinomics” to mean the application of immunogenetics and immunogenomics to the study of vaccine-induced immune responses (Poland et al., 2007). Subsequently, we have broadened the term to encompass the broader concept of understanding the mechanisms behind heterogeneity in host response to vaccination in order to rationally design new and effective vaccines (Haralambieva and Poland, 2010; Poland and Oberg, 2010; Poland et al., 2009). This expanded definition is now increasingly pertinent due to a suite of cutting edge techniques, approaches, and concepts such as: reverse immunology, systems biology, immune profiling, functional studies of genetic associations, comprehensive “omics” technologies, fully sequenced pathogen genomes, host–pathogen interaction studies, as well as advanced bioinformatics and computational modeling.

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Vaccinomics proposes the integrated use of modern immunologic techniques, existing biologic knowledge, and innovative ideas and approaches to circumvent current obstacles to vaccine development and thereby create safe and effective new vaccines. Inherent in this concept is the idea that both pathogen and host *heterogeneity* exists leading to inter-individual variability in vaccine response, and that this variability must be understood in order to develop new vaccine formulations that safely provide protective immunity. Here we identify and propose five components of “vaccinomics” that we think will provide the greatest return on our investment of vaccine research dollars.

Applied Immunogenomics

For the purposes of vaccine development, the central issue is person-to-person and population-to-population variation in generating protective immune responses to vaccines. Much like pharmacogenomics has broadened our understanding of drug efficacy and toxicity, applied immunogenomics will allow us to understand the critical genetic and epigenetic determinants and pathways relevant to vaccine response and provide a framework upon which to build better, safer vaccines through “reverse engineering.” These studies must do more than simply report descriptive statistical associations between single nucleotide polymorphisms (SNPs) and immunologic phenotypes of interest. It is essential that these studies replicate and validate gene findings in independent cohorts in a mechanistic manner to ensure the scientific validity of the results, and have well-developed plans to understand the functional mechanisms driving differential vaccine response. To facilitate SNP prioritization for functional studies a number of groups have developed specialized algorithms, such as ASSIMILATOR, which compiles known data on given SNPs (Martin et al., 2011), or approaches based on sequence conservation such as MCS (McCauley et al., 2007), in order to interrogate and select the most appropriate SNPs for the expensive, downstream mechanistic studies that are needed. The introduction of human variants into knockout mice lacking the homologous murine gene may provide a powerful tool for the extension of a genome-wide association study (GWAS) results into functional studies, as has been done with LIPG variants associated with serum cholesterol levels (Edmondson et al., 2009). These types of studies are far more involved and expensive than a run of the mill GWAS, but the resulting knowledge can then be immediately applied to the directed, rather than empirical, nontargeted development of vaccine candidates (eliminating unnecessary antigens, reformulation to reduce side effects, judicious use of adjuvants to enhance immunogenicity or direct Th1/Th2 bias) to create individualized or population-subset specific vaccines. One example of an applied immunogenetics set of studies involves the OAS gene family and West Nile Virus (WNV). Initial reports indicated that polymorphisms in OASL were associated with differential susceptibility to WNV infection (Lucas et al., 2003; Mashimo et al., 2002; Yakub et al., 2005). One of the associated polymorphisms, rs10774671, was shown to be associated with reduced OAS activity in peripheral blood mononuclear cells (PBMCs) (Bonnevie-Nielsen et al., 2005). Lim et al. (2009) extended these studies by verifying that the OAS1 rs10774671 allele AA genotype was more common in WNV-infected individuals, and that this allele

was also associated with increased levels of WNV replication in primary human lymphoid tissue. The authors suggest that induction of OAS1 activity by therapeutic agents may be efficacious in controlling WNV infections. These results may also indicate that adjuvants stimulating strong OAS1 responses could enhance WNV vaccine efficacy. Furthermore, the OAS1 pathway may also be an important biomarker of vaccine efficacy as immune memory responses that are capable of triggering strong innate OAS1 activation upon reinfection likely provide greater protection against disease. We and others have recently expanded upon, and reviewed, key concepts of vaccinomics and personalized vaccinology (Janikashvili et al., 2010; Poland, 2007; Poland and Oberg, 2010; Poland et al., 2007, 2008, 2009; Yan, 2010). Such work immediately focuses attention on possible vaccine candidates for further study.

Next Generation Sequencing (NGS)

The advent of NGS methodologies has sparked a major change in how we approach scientific inquiry, and has been described as a technology limited only by our imagination (Metzker, 2010). Although initially used to provide incredibly large amounts of sequence data (measured in Gigabases per run), NGS has quickly evolved to providing researchers far more information (Horner et al., 2010; Marguerat et al., 2008; Morozova and Marra, 2008). mRNA-Seq and similar applications provide unprecedented sensitive transcriptomic datasets for gene expression analysis, identification of rare transcripts, alternate splicing, copy number variation, and sequence variation. ChIP-seq, and related methods allow global profiling of the epigenome. Perhaps most exciting in regard to this technology is that although costs are currently high, the price of whole genome sequencing is falling rapidly making personal genomics increasingly feasible. Liability and privacy issues aside, having a patient’s pharmacogenomics or vaccinomics data readily available in the medical records will, at some point in the near future we believe, allow a physician to avoid drug or vaccine prescriptions with a high likelihood of adverse reactions or to select a vaccine specifically designed to overcome given genetic restrictions and induce protective immunity (Dhiman et al., 2009).

“Omics” Technologies

The recent advent of high-throughput, “omics” technologies has had a profound impact on the ability to analyze complex systems. These methodologies provide quantitative measurements on a global scale and provide the ability to fully interrogate more than just the genome or transcriptome. More recent advances now allow the ability to comprehensively probe the proteome, metabolome, interactome, fluxome, lipidome, inflammasome, and other “omes.” These approaches have several distinct advantages: they allow for the analysis of biologic activity at many levels (DNA, RNA, protein, cellular location) and permit researchers to explore relationships between cellular systems and interconnections between measured analytes thereby treating the cell as an integrated system. The high-throughput omics data obtained in parallel from successive organizational hierarchies of cellular biology also help discern, in real time, the “system level” predictive value of an omics biomarker test, over and above the built-in molecular redundancies preserved in biology

during the course of human evolution. As a consequence, however, these technologies are expensive and produce vast amounts of data necessitating further expenditures to support the advanced statistical, bioinformatics, and computational infrastructure necessary to preserve and allow access to the data (Schadt et al., 2010). We think that these technologies, used appropriately, will continue to radically alter how scientists approach and frame fundamental biological questions. For example, recent studies of poxvirus gene expression have identified a number of novel genes involved in poxvirus infection, that might prove useful as targets for drug intervention (Alkhalil et al., 2010). Similar high-resolution analysis of virus and host transcriptomes using next generation sequencing has highlighted key pathways involved in poxvirus inhibition of antiviral pathways (Rubins et al., 2011; Yang et al., 2010). This work may logically lead to more detailed studies of the effect that these pathways have on viral infection, leading to the selection of vaccine adjuvants most appropriate for the target pathogen. Furthermore, one could easily imagine combining the previous datasets with proteomic data describing the antibody response to poxvirus infection/vaccination (Davies et al., 2008; Keasey et al., 2010) in order to uncover novel information regarding the role that viral/host gene expression has on eventual immune responses.

Bioinformatics and Data Analysis

As discussed above, “omics” technologies provide a wealth of data that require an appropriate computational infrastructure and advanced statistical and bioinformatics algorithms capable of organizing, visualizing, integrating, and interrogating these large datasets. It is becoming increasingly apparent that the ability to logically integrate diverse data types (transcriptomic and proteomic data, for example) is a powerful tool to gain novel insights into biological processes. With the ever increasing use of “omics” technologies, sophisticated integration techniques must likewise evolve. One common approach is the independent collection of different “omics” datasets, integration based on pathway analysis or interaction networks, and subsequent scoring/weighting of each pathway or network based on maximum correlation among all datasets. Variations of this approach have been used to integrate miRNA and mRNA expression levels (Cho et al., 2011), transcriptomic and proteomic datasets (Cheema et al., 2011), and lipidomic and transcriptomic data (Gupta et al., 2011). Excellent examples of the use of bioinformatics in vaccine design include: publicly available databases of user-supplied information merged with powerful analytical tools that allow investigators to explore systems level analysis of pertinent biological systems (Lynn et al., 2008; McGarvey et al., 2009), or the use of open reading frame, epitope prediction, and sequence conservation algorithms currently being used to develop broad-spectrum vaccines against *Streptococcus* (Maione et al., 2005) or *Meningococcus* B (Germain, 2010), as well as universal influenza vaccines (Ebrahimi and Tebianian, 2011).

Systems Biology and Immune Profiling

Systems biology is a top-down approach that seeks to use high-dimensional technologies to obtain a global understanding of biological processes. Inherent in this approach is the study of complex interactions between the myriad parts of

the biological system with the goal of gaining a deeper understanding of how the system is controlled. This type of integrated approach has increased our understanding of innate (Zak and Aderem, 2009) and adaptive (Germain, 2001) immune responses and is now being applied to study vaccine responses with encouraging results. For example, a systems biology approach combining genome-wide expression and yeast two-hybrid analysis not only confirmed interactions between viral proteins and components of the RIG pathways during influenza infection, but also identified novel viral genes regulating both interferon (IFN) production and viral replication (Shapira et al., 2009). Querec et al. (2009) have used similar profiling approaches to identify a novel signature predictive of neutralizing antibody responses (involving activation of TNFRSF17), and two separate predictive signatures (activation of complement C1QB or involvement of glucose metabolisms genes SLC16A5, SLC25A13, SLC39A11) of CD8⁺ T cells response to the yellow fever vaccine. Another group has found a distinct IFN response by blood neutrophils in tuberculosis patients (Berry et al., 2010) that may be useful in developing novel vaccine approaches. In order to fully capitalize on the full capabilities of systems biology we need to design iterative studies that include comprehensive immune response profiling, integrate and mine the resulting datasets for new knowledge, develop new hypotheses based on this increased understanding, and then test these hypotheses in mechanistic studies to validate our earlier findings (Pulendran et al., 2010). The knowledge gleaned from these repeated cycles of global discovery and validation must then be harnessed to develop the next generation of vaccines (Tisoncik et al., 2009).

Conclusions

Vaccines are one of the greatest medical success stories. They have saved the lives of hundreds of millions of human beings as well as reducing human suffering and disease related morbidity. In order to continue the success story we must take advantage of new advances in our scientific capabilities. We also need to bear in mind that there remains much (previously unknown) person-to-person and population differences in response (safety and effectiveness) to vaccine-based health interventions. For vaccines to be developed in a directed manner in the 21st century that stands the test of rational therapeutics, we need tools to discern the mechanisms of such markedly variable vaccine effects in human populations as well as individuals. Proper investment in research aimed at increasing our fundamental understanding of pathogen biology, vaccine response, and mechanisms responsible for the development of protective immunity along with support for the design, development, and production of next-generation vaccines can and will lead to success stories as important for mankind as the eradication of smallpox or control of polio.

Author Disclosure Statement

The authors declare that no conflicting financial interests exist.

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