Vaccine xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

New approaches to understanding the immune response to vaccination and infection

David Furman [a,b], Mark M. Davis [a,b,c,*]

[a] Institute for Immunity, Transplantation and Infection, School of Medicine, Stanford University, Stanford, CA, United States
[b] Department of Microbiology and Immunology, School of Medicine, Stanford University, Stanford, CA, United States
[c] Howard Hughes Medical Institute, School of Medicine, Stanford University, Stanford, CA, United States

ARTICLE INFO

Article history:
Available online xxx

Keywords:
Systems immunology
Immune profiling
High-throughput methods
Vaccinology
Computational immunology
Regularization
Feature selection
Elastic net
Human immunology

ABSTRACT

The immune system is a network of specialized cell types and tissues that communicates via cytokines and direct contact, to orchestrate specific types of defensive responses. Until recently, we could only study immune responses in a piecemeal, highly focused fashion, on major components like antibodies to the pathogen. But recent advances in technology and in our understanding of the many components of the system, innate and adaptive, have made possible a broader approach, where both the multiple responding cells and cytokines in the blood are measured. This systems immunology approach to a vaccine response or an infection gives us a more holistic picture of the different parts of the immune system that are mobilized and should allow us a much better understanding of the pathways and mechanisms of such responses, as well as to predict vaccine efficacy in different populations well in advance of efficacy studies. Here we summarize the different technologies and methods and discuss how they can inform us about the differences between diseases and vaccines, and how they can greatly accelerate vaccine development.

1. Introduction

The immune system is a complex adaptive system with emergent properties such as memory and self-regulation. Its complexity can be exemplified at the network level, lymphocyte receptor diversity, clonotype selection, cell migration, cell–cell interaction inside immunological tissues and long-distance communication via fluid dissemination throughout the body, homeostatic regulation and adaptation to changing environments. The net functionality of a healthy immune system likely depends upon the interaction between immune system components at the molecular level and considerable integration and regulation at the system level. Therefore, as in any complex system, it is likely that immune system function cannot be predicted from the behavior of any of its parts separately but rather, is context dependent and hard-wired in dynamic and functional networks involving hundreds of components. Nevertheless, our current knowledge of immunology has been built for many years using a relatively deterministic and reductionist approach, as was necessary given our relative ignorance of the many components that have only recently come to light. However, examples where the same cell or soluble cell product exert distinct and even opposing functions depending on the site of the immune reaction and the presence of other immune constituents are abundant. Thus, the robust functioning of the immune system likely relies on a highly complex multi-level interaction network, linking intracellular biochemical networks, intercellular communication networks and inter-organ cellular trafficking networks through space and time. In addition, for obvious ethical and practical reasons, we have utilized model antigens and largely relied on mouse models of health and disease, which while extremely useful for deciphering the cellular and molecular bases of many immune responses, are rarely predictive of human vaccine results. There are a number of possible explanations for this discrepancy, evolution being one, with an estimated 65 million years separating humans from mice [1] and second possibility being that mice are kept in a relatively sterile environment, whereas cage-free humans are exposed to a much broader range of pathogens over their (much longer) lifespan. A third factor is that the whole inbred mouse strains are homozygous at all their alleles whereas humans rarely if ever are.

Recent years have seen the emergence of 'systems biology' approaches that are now applicable to human studies [2,3]. Initially using gene expression analysis of white blood cells from

* Corresponding author at: 279 Campus Drive, B219 Beckman Center, Stanford, CA 94305, United States. Tel.: +1 650 725 4755; fax: +1 650 498 7771.
E-mail address: mmdavis@stanford.edu (M.M. Davis).

http://dx.doi.org/10.1016/j.vaccine.2015.06.117
0264-410X/

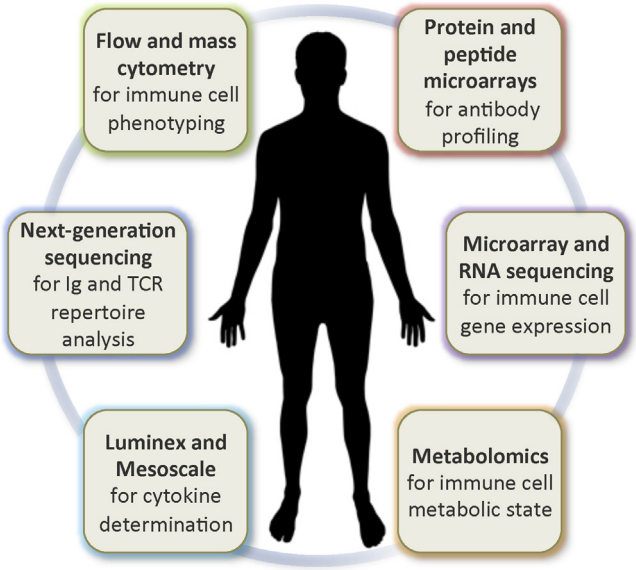

Fig. 1. Multi-level high-throughput analysis of the human immune system. Comprehensive immune profiling involves multiple technological platforms that allow us to capture and observe an important portion of the immune response. Peripheral blood is used to survey the perturbations in the immune system through a suite of available techniques including next-generation sequencing (NGS); gene and protein microarrays; multiparameter flow cytometry and mass cytometry (CyTOF); multiplex cytokine and chemokine analysis by Luminex and Mesoscale, and metabolomics that relies on major recent improvements in mass spectrometry now capable of resolving close to a thousand metabolites. This human-centered approach to immunology promise to improve our understanding of the immune response to vaccination and infection.

vaccinated subjects [4,5], this approach has now been extended to cover not only gene expression, but the basic components of the entire immune system, namely the hundreds of cell types and subsets, and many of the cytokines and chemokines that they communicate with [6] (Fig. 1). Here it should be noted that the "quanta" of the immune system are the many specialized cells that act relatively autonomously. T lymphocytes for example, can detect even a single molecule of a peptide antigen bound to an MHC molecule [7–9] and then act on that information by releasing cytokines in the case of $CD4^+$ cells [10]. Advances in DNA sequencing technology also make it possible to analyze the immunoglobulin (Ig) and T-cell receptor (TCR) repertoires responding to vaccines in great detail [11–13] and to do this on a single cell level as well [14,15]. It is also possible to obtain the exact DNA sequence of the whole genome in single individuals, the information about the genes that are expressed in a particular cell state, and the composition of hundreds of different metabolites from different tissues that provide extremely valuable information about a particular metabolic status in health and disease states (Fig. 1). This ability to generate high-throughput and high bandwidth data has co-evolved with technological advances in informatics to enable the generation of integrative models of the human immune response. This systems biology approach applied to infection and vaccination using people as a model is accelerating and will continue to accelerate our understanding of how the immune system works in humans, representing a necessary step to advance our basic understanding of immune system dysregulation and to enable future translational applications based on these basic findings. In this review paper, we discuss some of the most important technological advances to probe the immune system and the computational tools used to extract relevant biological information that can be used to gain mechanistic insights and/or to identify immune biomarkers.

2. Genes expressed in immune cells prior to (predictive of outcome) or in response to immune perturbation (microarray and RNA sequencing)

One of the most successful technologies originated from available genetic information generated by the Human Genome Project, is the gene expression microarray. Because this technology generates large amounts of expression data for a relatively low price, microarrays have gained extreme popularity during the last decade. The technology consists in hybridizing a nucleic acid sample (target) typically onto a glass surface containing microscopic spots with multiple identical strands of DNA that are printed by a robot, each of it representing one gene probe. Probe-target hybridization is typically quantified by detection of fluorophore-, silver-, or chemiluminescence-labeled targets to determine relative abundance of nucleic acid sequences in the target [16]. DNA microarrays allow for determination of genome-wide expression profiles and thus, are ideally suited for generating hypotheses to gene function that can help to identify appropriate targets for vaccine and therapeutic intervention. DNA microarrays have been used to systematically identify tumor antigens for tumor vaccine design [17]; to identify gene profiles or "signatures" in patients with bacterial pneumonia [18,19] and bacterial sepsis both in adults [20] and children [21]; in rhinovirus, respiratory syncytial virus, and influenza A infections [22]; malaria [23] and dengue virus infections [24]; in HIV patients [25,26]; as well as in different vaccination regimes such as influenza [27–30], yellow fever (YF) and meningococcus [31] vaccines. The use of this technique is accelerating our understanding of the bases of the host immune response to pathogenic insults and is also extending to the genetic characterization of genetically diverse infectious pathogens associated with a given disease.

3. Cells and biomarker proteins expressed in immune cells prior to (predictive of outcome) or in response to immune perturbation (flow cytometry, CyTOF)

3.1. Flow cytometry

Flow cytometry is probably the most commonly used technology in immunology research. It uses antibodies coupled with fluorophores to detect specific proteins expressed intracellularly and on the cell surface. It has been widely used for many decades to monitor immune responses to vaccination and infection in bulk cell populations as well as to track the phenotypic and functional characteristics of antigen-specific cells, but it has also been largely applied in routine clinical settings for the diagnosis, prognosis and monitoring of disease. For example, it helps in the diagnosis and staging of patients with a hematological diseases [32]; for the detection of minimal residual disease (disease beyond the limit of morphological detection using conventional microscopy) [33]; for stem cell enumeration during immunosuppressive therapies [34]; in solid organ transplantation to evaluate T cell cross-match [35]; to monitor changes in cell populations after cardiopulmonary bypass surgery for the prediction of infections in risk patients [36]; in HIV for the determination of $CD4^+$ T cell counts [37]; to predict hemolytic disease [38]; in primary [39] and secondary [40] immunodeficiencies; and largely used in blood transfusion [41].

The immunogenicity of vaccination and infection, and direct monitoring of the innate and adaptive immune responses can be measured by different methodologies. Flow cytometry can be used to analyze the contribution of innate immunity to vaccine efficacy and disease pathogenesis [42]. For T cells, intracellular cytokine staining (ICS) assays have proven to be useful to measure T-cell immunogenicity and there are numerous examples in the literature

Please cite this article in press as: Furman D, Davis MM. New approaches to understanding the immune response to vaccination and infection. Vaccine (2015), http://dx.doi.org/10.1016/j.vaccine.2015.06.117