Immunity
Perspective

Vaccines and the Future of Human Immunology

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In this issue of *Immunity*, a collection of detailed reviews summarizes needs, opportunities, and roadblocks to the development of new vaccines, all in the context of our current knowledge and understanding of key aspects of immune function and microbial interactions with the host. This Perspective is designed to provide a broad overview that discusses our present limitations in designing effective novel vaccines for diseases that do not typically induce robust resistance in infected individuals and how the addition of a systems-level, multiplexed approach to the analysis of the human immune system can complement traditional highly focused research efforts to accelerate our progress toward this goal and the improvement of human health.

Introduction

Life is a constant battle to survive and reproduce in a particular ecological niche, in competition with other organisms that seek to occupy and thrive in the same environment. In many cases, such competition is not between free-dwelling species independently seeking adequate resources, but between predator and prey. For humans in particular, if we exclude intraspecies conflicts (would that this was the case in reality!), the real battle is between us as prey and the microbial and/or parasitic world as predators. Beyond the physical barriers of skin and mucous membranes, our ability to prevail in this battle is dependent on the proper functioning of our immune system.

In this special issue of *Immunity*, experts in many of the aspects of immune system organization and function relevant to achieving immune resistance to infection, as well as others with a deep knowledge of vaccinology, provide timely reviews of the state of knowledge in their respective fields (Palucka et al., 2010; Chen and Cerutti, 2010; Coffman et al., 2010; Good and Doolan, 2010; Kaufmann, 2010; Liu, 2010; McElrath and Haynes, 2010; Pulendran et al., 2010; Sette and Rappuoli, 2010). The information conveyed in these reviews is indeed impressive. Yet, at the same time, they are revealing in what they say about the limitations we still possess with respect to understanding the true correlates of immunity for infections involving HIV, *Mycobacterium. tuberculosis*, or *Plasmodium. falciparum* and about our capacity for rational development of effective vaccines against the wide range of organisms that still cause substantial morbidity and mortality around the globe.

In this perspective, I present a less detailed, more descriptive and prescriptive view of where we are now in understanding human immune function and where we as a community need to go to more effectively harness the immune system for improved human health.

The Past Is Prologue

The existence of acquired or active immunity was implicit in observations made long ago in human history, when it was recognized that individuals who survived an overt infection were most frequently resistant to that same disease in the future (Silverstein, 1999). However, the practical utility of this knowledge was not fully appreciated until Edward Jenner (and others who have received less attention) undertook the use of a less pathogenic form of a virulent organism to actively protect against infection. The science (or art) of vaccinology is indeed frequently considered to have begun with Jenner's use of cowpox as a vaccine against smallpox, based on his observation that milkmaids who suffered the former infection were typically resistant to the latter (Gross and Sepkowitz, 1998; Kennedy et al., 2009; Plotkin, 2009). Rather than rely on survival of natural infection, the paradigm was established that medical intervention could precipitate an immune (pathogen-resistant) state with minimal risk to the individual through administration of a (relatively) nontoxic or nonpathogenic counterpart of the agent or organism against which resistance was desired.

From this modest beginning in the 18th century, the practice of vaccinology has undergone tremendous development. Initially through largely empirical routes involving isolation and inactivation of the toxic products of some microorganisms (tetanus toxoid for example), the use of attenuated viruses (in the case of polio or smallpox), or the use of killed versions of various pathogens (for example, influenza), we have developed and put to use a large armamentarium of vaccines against bacterial and viral diseases (reviewed in Plotkin, 2009; Pulendran et al., 2010; Sette and Rappuoli, 2010). In conjunction with better sanitation, these vaccines have been responsible for a remarkable reduction in early mortality from infectious diseases —indeed, over just a few generations, the developed world has gone from having the death of a child due to infection be a commonplace event to a rarity. Smallpox has been eliminated as a disease and only small pockets of polio remain as a result of intensive worldwide vaccination drives (Henderson, 1999).

Until the past few decades, successful vaccines were almost exclusively against pathogens to which primary exposure induced long-lasting resistance in the surviving host. That is, we simply mimicked nature and induced the immune system to respond in a manner that observations of the natural history of disease showed were adequate to produce microbial resistance. More recently, the development of glyco-conjugate

vaccines has led to a marked reduction in diseases caused by organisms that typically colonize many of us on an ongoing basis and cause invasive disease in a fraction of the population, a prime example being *Haemophilus influenza* (Chandran et al., 2005; Rappuoli, 2001; Sette and Rappuoli, 2010). In other cases, prevaccination can prevent infection by agents that once present in the body are not usually eradicated by the immune system. For example, persistent infection and the promotion of cancer by certain papilloma viruses can now be prevented by using a virus-like particle protein-based vaccine (Gillison et al., 2008; Trimble and Frazer, 2009; zur Hausen, 2009).

These achievements, especially those in which immunity has been induced that is superior to that normally existing in the host population, led in recent years to the hope—indeed in some quarters the expectation—that academic and industrial scientists could and would rapidly generate effective vaccines to the many pathogens that remain major health issues. But these expectations were not based on a deep understanding of the history of vaccinology or the limits of our current understanding of both human immunity and its capacity to handle some types of infectious agents. The reality is that nearly all useful vaccines developed to date act through the production of antibodies, neutralizing in the case of viruses or toxins or opsonizing in the case of bacteria (see Ravanfar et al., 2009, as well as Pulendran et al., 2010 and Sette and Rappuoli, 2010). These vaccines are rather specific, and for pathogens with significant genetic diversity, our success has been limited mainly to those cases in which the most highly pathogenic strains of a virus or bacteria can be identified and in which these limited numbers of serotypes do not vary substantially over time. This permits multivalent vaccines to be devised that cover (most of) the spectrum of strains to which resistance is desired—this is the case for polio, pneumococcal vaccines, and many others. Influenza, which does show significant variation in neutralizing determinants over short time frames, is dealt with successfully by vaccination because we have developed an early warning system that allows seasonal manufacture of the specific vaccine needed for that year, and indeed, protection can be limited if there are multiple circulating strains in a given season (Fiore et al., 2009).

We have learned over many years what the relevant surrogate markers are for such antibody-mediated protection to genetically stable (or at least easily tracked) pathogens (Pulendran et al., 2010). A large body of data has illuminated the relationship between serum titers of antibodies of suitable specificity and affinity to useful host protection, even when that protection is mediated by these antibodies not in the bloodstream where they are measured, but at sites of pathogen invasion such as mucosal surfaces. For example, in classic studies of resistance to respiratory syncitial virus (RSV), Chanock and colleagues determined the amount of IgG in the serum that would lead to transudation of an amount of this antibody that was effective at neutralizing RSV on the lung epithelial surface and showed that achieving that level of serum IgG through passive transfer provided the expected protection against this virus (Prince et al., 1985a; Prince et al., 1985b; Prince et al., 1985c). Although for RSV, this insight has not led to a practical active vaccine for various reasons, it has allowed effective passive therapy and represents a highly useful method for evaluating pilot studies for the likely efficacy of other antibody-based vaccines before they are put through expensive and time-consuming phase III efficacy studies. It also can be used for postlicensing assessment of whether individuals are likely to be protected or not, based on minimal titers in the serum. A similar quantitative approach relating serum IgG antibody concentration to effective protection is commonly employed to determine whether or when boosting is needed for a host of widely used vaccines.

However, this quantitative antibody paradigm is problematic for diseases in which we do not know how much of what specificity of antibody of what isotype in what tissue site leads to protection, or even knowing this, how to generate such antibodies in adequate titer and to maintain such levels over many years. It is also an issue when the pathogen varies in the relevant target structures for such antibodies to such an extent that even a multivalent vaccine would not generate adequate coverage of the variants, or in cases in which the best neutralizing sites are shielded by protein folds or carbohydrates, all of which are the situation with HIV (Forsell et al., 2009; Kwong and Wilson, 2009; Schief et al., 2009).

But beyond this, there are many cases in which the humoral antibody response does not seem to be the effector arm of the immune system best able to protect against or eliminate particular pathogens, requiring us to develop an entirely new understanding of the relationship between cellular immunity and host protection akin to that which decades of work have yielded for antibody-based immunity. This is the case for mycobacteria, for some viruses, and most likely, for many parasites. The challenge is all the greater because many of these same pathogens are the very ones that do not typically produced robust resistance upon initial infection, limiting our ability to count on the natural response to guide us in how to make a protective vaccine or even to know if the immune system is capable of mediating such resistance under optimal conditions.

A simple example in this area of limited knowledge of what to aim for with a vaccine based on cell-mediated immunity is provided by considering the $CD8^+$ cytotoxic T cell response, frequently targeted as a primary effector modality in HIV vaccines (Letvin, 2005). What state of differentiation of the mature effector cells is required for their optimal activity—is it the content of perforin or granzymes (Migueles et al., 2008), the ability to make cytokines or chemokines (Levy, 2003), or some other property or combination of properties that is most important? What state of differentiation at the time of infection (central memory, effector memory, and active effectors) is best for providing protection (Ahmed and Gray, 1996; Ehl et al., 1997) and how do we ensure that a vaccine induces enough specific cells in the correct condition? If memory cells are most important, how do we deliver a vaccine that favors their development over an acute effector response—if active effectors must be present at the time of infection, how do we provide the antigenic stimulation necessary to maintain these cells over long time intervals after vaccination without risk of depletion (Moskophidis et al., 1993), desensitization (Barber et al., 2006), or unacceptable levels of chronic tissue inflammation? How many of the proper type of cell are needed and how do we relate the number of antigen-specific cells measured in the peripheral blood to what is in either secondary lymphoid tissue or peripheral sites where these cells must perform as precursors or differentiated effectors, respectively

(Li et al., 2009)? What chemokine and integrin expression patterns are necessary to ensure proper homing of the ultimate effector cells to the tissue sites where their action is most relevant? Without such information, we are left with merely measuring what we can (and not necessarily what we should) after trying vaccine formulations whose design is not based on any deep insight into what the vaccine must yield to be effective and with only large and expensive field trials able to provide any hints on whether we are moving in the right direction.

These various complexities all funnel into a major bottleneck—our limited understanding of the human immune system and, hence, our capacity to optimally assess the state of the system and to manipulate it in predictable ways. As recounted in the reviews accompanying this perspective, we have a great deal of information at present about the nature of antigen presentation and regulation of T and B cell differentiation by various subsets of dendritic cells (Palucka et al., 2010); about the special nature of the mucosal immune system (Chen and Cerutti, 2010); the effects of diverse adjuvants on immune responses (Coffman et al., 2010); the mechanisms used by pathogens such as HIV (McElrath and Haynes, 2010), *M. tuberculosis* (Kaufmann, 2010), or *P. falciparum* (Good and Doolan, 2010) to evade immune control and how these limitations might be overcome by vaccination; the ability of viral and other vector platforms to help promote effective responses (Liu, 2010); and the utility of reverse vaccinology for identifying novel potential vaccine components (Sette and Rappuoli, 2010). Yet even with all this knowledge, there is a level of integrated understanding that is clearly still lacking. What signals best promote persistent high-titered antibody responses through production of long-lived plasma cells derived from activated B cells with the right specificity of isotype-switched, somatically mutated immunoglobulin loci? What are the antigen structures and form(s) of delivery that will focus the specificity of such antibody responses on relevant rather than distracting determinants of pathogen molecules? Which T cell subset in what differentiated state and in what numbers would be effective in protecting against those agents for which antibodies are not the most effective mode of resistance? In some cases, we know what response we need but not how to get it— in others we are still ignorant of even the right type of response or whether we have knowledge of the full range of effector modalities that can be drawn on for host defense, and hence, the best combination of responses to promote to achieve effective immunity after vaccination. Without such insight, it is exceedingly difficult to produce new generations of vaccines that are likely to be effective and safe against diseases in which natural resistance is not the norm.

If we are to move past these limitations and probe the limits of human immunity as a means of protection against a diverse array of pathogens, it is apparent that a concerted effort to better understand the operation of this system is required through a combination of continued detailed analysis and a new emphasis on systems level study, as so cogently discussed by Pulendran et al. (2010). We must add to the existing research portfolio of sharply focused studies of a small number of samples from only a few individuals more robust, highly multiplexed, in-depth analyses on larger populations and apply to these more complete data sets new computational and statistical tools for extracting biological insight. Fortunately, this need has been recognized and investigators and funding agencies are mobilizing in a major effort to make rapid progress in this arena, in large measure as an integrated community rather than as competitive independent investigators. The remainder of this perspective will discuss these emerging efforts, what they can contribute to our rapid acquisition of a better grasp of human immune function in health and disease, how the information from such investigations can be put to use in vaccine research, and some of the limitations of this new research direction.

Existing Paradigms and Accomplishments in Human Immunological Research

The statement above about our lack of adequate understanding of human immunity is not meant to imply that we do not know a great deal or that the many investigators who have worked in this arena have not made major strides forward in cataloging the cellular and molecular components of the human immune system, in dissecting how these elements interact to produce function, or in characterizing what aspects of the system show too little or too much activity in immunodeficiency or autoimmune states, respectively. Indeed, monoclonal antibodies were first produced against and used to phenotype human hematopoietic cells (Reinherz and Schlossman, 1980, 1982), much of the available data on signaling by the TCR was developed with human T cell tumor cells (Imboden et al., 1985; Weiss et al., 1984; Weiss et al., 1991), the initial cloning of cDNAs corresponding many of the surface proteins identified by the anti-lymphocyte and myeloid cell monoclonal antibodies that led to the CD nomenclature involved human molecules (Aruffo and Seed, 1987; Seed and Aruffo, 1987), and the relevance of many of the components identified in these studies to host defense is only really known from experiments of nature involving genetic lesions in the human population.

A variety of distinct methods have generated our existing body of knowledge of human immunity. Scientists and physicians in the 19th and early 20th century made the first major contributions to the field as a consequence of both natural history studies in individuals with various diseases and laboratory analysis of serum and tissues from infected or ill subjects. The work of Pasteur, von Pirquet, Schick, Portier and Richet, Bordet, Arthus, von Behring, Kitasato, and many others provided an initial picture of human immunity, including the antibody response to infection or vaccination, the effector activities of antibodies in vitro and in vivo, the nature of allergic and immunopathologic states, and the existence of responses characterized by mononuclear cell infiltrates, such as upon skin challenge of infected individuals with extracts of mycobacteria, along with the systematic capacity to provide protection of the host by passive and active immunotherapy methods (Silverstein, 1999).

However, in the late 20th century, much of the focus in immunological research shifted from humans to inbred mouse models. The distinction between T and B lymphocyte subpopulations of the small lymphocytes described by Gowans and their need for cooperation in antibody responses was made in mouse models (Miller, 1972), as were other major conceptual advances such as MHC restriction (Zinkernagel and Doherty, 1974) and thymic selection (Bevan and Fink, 1978; Zinkernagel, 1978). Important novel subsets of hematopoietic cells such as dendritic cells (Steinman and Cohn, 1973), NK T cells (reviewed in

Bendelac et al., 1997), and FoxP3$^+$ regulatory T cells (Fontenot et al., 2003; Hori et al., 2003) were first discovered and characterized in mouse models.

Human immune analysis moved along two paths during this time period. One especially productive direction was the analysis of the effects of genetic variation on response, in particular with respect to susceptibility to specific infectious diseases in the context of immunodeficiency. As more and more powerful tools became available to identify the genetic locus responsible for an immunodeficiency leading to the excess occurrence of specific infections, such studies have provided remarkable insight into which molecular players contribute to human host defense. The advances arising from such studies have been elegantly summarized in recent reviews (Alcaïs et al., 2009; Bustamante et al., 2008; Casanova et al., 2008), so I will only mention that the results range from the expected (IL-12-IL-12R interactions are critical for mycobacterial defense [Al-Muhsen and Casanova, 2008]) to the unexpected (the absence of the kinase IRAK-4, considered critical in Toll-like receptor signaling, has a minimal impact and only leads to enhanced susceptibility to a subset of pyogenic infections [Picard et al., 2007]). Other patient-based research has helped provide novel insights into apoptotic pathways (Chun and Lenardo, 2001) and the components of the signal transduction machinery downstream of the TCR (Su et al., 2005) or involved in CD4$^+$ effector T cell polarization (Milner et al., 2008).

Another path was the adoption of the "96-well plate" method to probe the cellular and molecular aspects of immune function. This method uses human cells in plastic and combines antibody and drug treatments in such in vitro cultures with functional read outs like proliferation or cytokine production along with dense cell phenotyping using flow cytometry. Several laboratories have been especially productive in using such methods and have advanced the field by first identifying and classifying subpopulations of memory T cells (Sallusto et al., 1999), work that was later replicated in the mouse (reviewed in Seder and Ahmed, 2003), by discerning specific phenotypic makers on subsets of effector T cells that closely correlate with polarization for cytokine production (Sallusto et al., 2000) or by relating memory B cell status to both specific and unspecific effects of vaccination on antibody titers (Bernasconi et al., 2002). Others have used molecular methods to examine the precursor and mature B cell repertoire for the existence of autoreactive B cell receptors on human cells and the impact of genetic variations that predispose to autoimmunity on the extent of repertoire trimming affecting such specificities (Tiller et al., 2007; Wardemann and Nussenzweig, 2007).

These few examples (highly selected from among a wealth of critical discoveries made by many investigators) make apparent the impact that even the ethically constrained studies that can be performed on humans or with human cells has had not only on our specific knowledge of the human immune system itself, but more broadly with respect to vertebrate immunity. Yet such efforts have not brought us to where we need to be to design effective vaccines, especially those requiring a response not readily engendered by natural infection. We have had only limited recent success with developing a vaccine for blood-stage malaria (Good and Doolan, 2010); BCG, despite its wide spread use, is not highly effective in preventing *M. tuberculosis* infection and reactivation disease (Liu, 2010; Kaufmann, 2010); for HIV, there is just a glimmer of success in the recent prime-boost Thai trial and the mechanism(s) of the modest effect seen is (are) not characterized (Rerks-Ngarm et al., 2009); we lack protective vaccines for a wide array of helminth infections, as well as for merging and re-emerging viral infections; and some vaccines candidates against several agents produce excess morbidity rather than protection, especially if infection occurs with a strain of the pathogen that differs from that used in the vaccine preparation (for example, with dengue [Webster et al., 2009]).

Moving to the Future in Human Immunology

What are the limitations that are impeding progress? Various opinions on this topic have been offered, many recently focusing on the pervasive use of mouse models for the study of the immune system (Davis, 2008). In truth, inbred mice have performed admirably as an experimental model system for immunological investigation. The knowledge gained ranges across multiple biological scales, from details of molecular architecture to recent visualizations of dynamic cell behavior in living animals to whole organism responses to infection or vaccination. But to quote Hamlet—"Ay, there's the rub." Despite this wealth of immunological information, there is a growing realization that all this knowledge derived from mouse studies has not produced a proportional increase in our ability to understand and effectively treat human diseases with an immunological basis or to develop vaccine formulations that produce the right response in adequate magnitude. To be clear —there are many examples one can point to where mouse-derived information has proven to be important in better understanding the human condition and has even guided development of therapeutic approaches. But the translation of mouse findings to humans nevertheless is much less robust than one would like, raising two major questions: why is this and how can the problem be addressed?

On the first issue, there are two major viewpoints, nicely summarized in Davis's recent piece in this journal (Davis, 2008). One holds that the mouse cannot be considered a "small human," that evolution has produced an organism suited to its ecological niche that has a distinct physiology from that of humans and it is simply not possible with any great assurance to extrapolate from one species to another. The clear documentation of molecular differences in key components of host defense (Mestas and Hughes, 2004) (for example, the molecular nature of innate NK cell inhibitory receptors) or in the cellular distribution of orthologous gene products (for example, of TLR9) makes evident that there is at least some merit to this line of thinking.

The other view is that the way the mouse immune system is typically challenged, manipulated, or studied experimentally is so far afield from the conditions applicable to humans that the information obtained in mice has much less relevance than it could if the analyses had been done with more thought to their suitability for cross-species comparisons. For example, the route of antigen administration is typically subcutaneous or intraperitoneal in mice and intramuscular in humans; amounts of antigen and adjuvant are not adjusted on a weight or body surface basis and so on. In infectious disease models, the pathogen inoculum is often more than an order of magnitude higher than what is involved in a natural infection. In studies of

autoimmunity, the mouse genetic background and/or the immunization schedule is selected to obtain as close as possible to 100% penetrance of the disease, unlike many human clinical situations. There is no question that all these issues contribute to limiting effective translation of mouse results to the human situation and that better design of animal studies aimed at improving our understanding of human immunity would be a good thing.

But whatever the proportional contribution of these or other considerations to the difficulty of using mouse-derived information to inform our view of human immunology, the answer to the second question, ''What can we do about the problem?,'' has to be ''Learn how to better study and analyze the immune system of humans directly.'' Many in the field who have been ''mouse immunologists'' for years now agree with the viewpoint espoused in the call to arms by Davis for a much stronger, coordinated, and extensive effort to probe, quantitatively measure, and eventually manipulate human immune responses. But doing so is not a simple task for a multitude of reasons.

One major issue is that the analysis of the human immune system has largely been pursued piecemeal to date (one disease, one gene, or one gene product) and usually on a small scale. This is in part due to (1) the siloed nature of medical subspecialties that claim specific immunological diseases as their own based largely on organ system and not the underlying immune dysfunction at a cell or molecular level and (2) the dominance of the reductionist approach that has dominated biological inquiry both in experimental animals and in humans for decades. The upshot of this balkanization and microanalysis of human immunology is that our ability to ameliorate and/or cure many serious human autoimmune diseases remains limited, our insight into the likely shared pathophysiological basis of diseases with inflammation as a common denominator (arteriosclerotic cardiovascular disease, neurodegeneration, etc.) is restricted, and methods to manipulate the immune system to treat autoimmune diseases, fight malignancies, or, most relevant with respect to the present discussion, develop vaccines by rational means are still at an early stage of development.

A second problem is that even the best work is often done separately by many distinct laboratories using different protocols for nearly all of the tests, cell isolations, phenotyping, and functional measurements, making it extraordinarily difficult to compare data between studies and rendering optimal meta-analysis problematic. This is related to the third limitation, namely the small scale of many of the studies (a few or few dozen patients), sampled at limited times and analyzed with a modest number of tools of limited power. Unlike inbred or genetically modified mice, humans are individuals not only with a highly variable genotype but also with individual genetic imprinting, distinct commensal flora, and variable exposure to disease-modifying environmental factors, including divergent life styles. For these reasons, and because of the strong influence of environment and developmental history on immune function, analyses of modest scope impede our ability to draw broadly applicable, statistically reliable conclusions about the basis of disease or even normal human immune function other than in those rare cases of highly penetrant single-gene lesions causing immunodeficiency.

On the opposite end of the spectrum are the large clinical studies with thousands of patients conducted primarily to analyze the effect of drug treatments on various immune-related disease states or the efficacy of experimental vaccines. Some hints about the functioning of the human innate or adaptive immune system have come from these trials, but deep insight has been limited in part because only a small number of samples are collected and these are subjected to only a few assays. On the other hand, the sequencing of the human genome (Lander et al., 2001; Venter et al., 2001), the Hap-Map effort (http://hapmap.ncbi.nlm.nih.gov/), and the development of SNP arrays (Ragoussis, 2009) have together permitted genome-wide association studies in many immunological diseases (Lettre and Rioux, 2008). The information provided by many of these studies is robust and is slowly shedding light on the molecular and genetic pathway underlying physiologic and pathologic immune functions. However, because the effect of each individual polymorphism, mutation, or epigenetic variation is typically modest, the scientific community at large has been slow to undertake extensive tests of the physiologic significance as well as the mechanistic aspects of many of associations suggested by such studies.

Merely listing limitations with the field as it exists has less value than suggesting how to overcome these impediments. Fortunately, a convergence of technical developments and ''Aha!'' moments has begun to offer a new path forward toward this goal that builds on the important but still limited insights noted above. An increasing number of organizations and academic centers, including but not limited to the NIH extramural program (through such funding mechanisms as the Cooperative Centers for Translational Research and Biodefense (http://www.cctrhib.org/Other_CCTRHIBs.htm) and human immune profiling centers (http://grants.nih.gov/grants/guide/rfa-files/RFA-AI-09-040.html; Pulendran et al., 2010), individual academic centers (for example, Stanford's new Institute for Immunity, Transplantation and Infection [http://iti.stanford.edu/] or Emory's Vaccine Center [http://www.vaccines.emory.edu/index.php]), the NIH Intramural Program (http://www.nhlbi.nih.gov/resources/chi/index.htm), and a major effort at King's College in the UK (http://www.guysandstthomas.nhs.uk/news/newsarchive/newsarticles/20100331hird-study.aspx), have all recognized the need for large-scale, highly integrated, technologically driven programs to probe and measure human immune responses in normal individuals, those whose immune systems are intentionally perturbed in an ethical manner (most often through administration of a vaccine), and in individuals with immune-based diseases prior to and after therapeutic intervention (see Box 1). At NIH, in response to this recognition, leaders from many institutes and the office of the director acted in concert with intramural investigators to develop a trans-NIH research program, the Center for Human Immunology, Autoimmunity and Inflammation (CHI), which is designed to bridge the chasm between the rich world of basic immunology research at the NIH and the in-depth study of human immune diseases and inflammatory processes. The NIH program takes advantage of the enormous expert community in the intramural program whose members are involved in the study of basic immunology using the mouse model and also the many physician-scientists who have been pioneers in the direct investigation and clinical manipulation of the human immune system. The CHI will also have the unique advantage of the Clinical Research Center

Organization or Resource	Web address	Comments
Cooperative Centers for Translational Research and Biodefense	http://www.cctrhib.org/Other_CCTRHIBs.htm	NIAID-funded centers investigating human immune responses to pathogens
Human Immune Profiling Centers	http://grants.nih.gov/grants/guide/rfa-files/RFA-AI-09-040.html	NIAID-funded centers for measurement of human immune responses, primarily to vaccines
Stanford Institute for Immunity, Transplantation and Infection	http://iti.stanford.edu/	Stanford co-operative effort to analyze the human immune response
Emory Vaccine Center	http://www.vaccines.emory.edu/index.php	Emory center for studies of human immunity, especially to vaccines
Trans-NIH Center for Human Immunology, Autoimmunity, and Inflammation	http://www.nhlbi.nih.gov/resources/chi/	Trans-institute effort at NIH to study the normal and perturbed human immune system in depth
King's College Biomedical Research Centre	http://www.guysandstthomas.nhs.uk/healthprof/researchanddevelopment/biomedicalresearch/researchthemes/Infection-and-immunity/hird-immunity-project.aspx	UK effort to conduct large scale studies of human immunity
Immune Tolerance Network	http://www.immunetolerance.org/	Long-standing effort to analyze human immunity and tolerance, especially in the transplant setting
ImmGen Project	http://www.immgen.org/index_content.html	New co-operative effort for deep transcriptional profiling of 'all' immune cell types
Hap-Map	http://hapmap.ncbi.nlm.nih.gov/	International consortium constructing detailed maps of human genetic diversity
Human Genome Project	http://www.genome.gov/12513440#al-1	Pioneering rapid data release principles of the Genome Project that are being adopted by other large scale science efforts
Clinical Research Center	http://www.cc.nih.gov/ccc/crc/	Dedicated research hospital of the NIH, a national resource for conducting ethical research on patients

Box 1. Selected Resources for the Global Study of Human Immunity

(http://www.cc.nih.gov/ccc/crc/), which helps provide intramural investigators with an ability to do clinical studies expeditiously and with fewer constraints than in typical academic hospitals. Academic centers with similarly rich groupings of basic and clinical immunology investigators are also banding together internally to pursue similar large-scale analyses of humans and human material, as detailed in the accompanying review on systems immunology (Pulendran et al., 2010).

A superb basic and clinical research infrastructure and a substantial cohort of expert investigators are not enough, however. Over the past few years the explosion of methods and instruments for assessing biological systems with increasing precision and breadth, in concert with the genetic resources provided by the Human Genome Project and it successors, has opened the door to an entirely new way to characterize and explore human immunity. The old standby technology of flow cytometry has moved from considering a four-color experiment as state of the art to routine use of nine to ten parameters and the potential for near routine use of up to 15 or more measurements to provide insight into not just cell phenotype and subset identity (Chattopadhyay et al., 2008), but state of activation, intracellular signaling status (through phosphoflow [Schulz et al., 2007]), and effector activity. Indeed, novel instrumentation with mass spectrometry to detect isotopic rather than fluorescent labels promises to increase the N-dimensionality of "flow" studies to >50 parameters in the next year or two (Bandura et al., 2009). Multiplexed cytokine assays allow nearly the entire known universe of such mediators to be measured at one time with high precision and great sensitivity in serum, sweat, other bodily fluids, or cellular supernatants. Microarray technology and next-generation sequencing have opened the door to obtaining complete determinations of the

transcriptional state (and miRNA status) of immune cells in human samples, most often blood, but because of the great sensitivity of these methods, even the few cells present in biopsy material. The combination of flow separation and these array or sequencing methods will allow a finely resolved analysis of transcripts in specific cell types, helping to make construction of gene regulatory networks from such data more practical and greatly enhancing the resolving power of the method with respect to distinguishing normal from perturbed states when these may only involve a minor hematological subset. Mass spectrometry is making rapid advances in precision and coverage and can be applied to both qualitative and quantitative tasks, including protein identification and cataloguing, post-translational modification discovery, and metabolic studies (Anderson et al., 2009; Choudhary and Mann, 2010; Gstaiger and Aebersold, 2009; Schiess et al., 2009). These tools can be combined with other rapidly emerging methods for analysis of antigen-specific cells using multiplex tetramer technology (Hadrup et al., 2009; Newell et al., 2009), for repertoire analysis using advanced sequencing tools (Freeman et al., 2009), for complete analysis of the genome and epigenome, for assessment of microbiome diversity (Grice et al., 2009; Hamady and Knight, 2009), and with new imaging methods for localizing immune cells or their products within tissues, to develop a remarkably deep and broad picture of the "normal" immune status in an individual and to assess the changes induced by infection, cancer, autoimmunity, inflammatory diseases, and, of course, vaccination.

This ability to collect massive amounts of data because of this growing capacity to interrogate the system with unbiased global methods that do not require specific hypotheses but instead are "hypothesis generating" necessitates a major change in how such data are handled. Rather than using biological intuition or simple graphs, charts, and textbook statistical analyses, it will be necessary to apply a sophisticated raft of informatics tools to extract the greatest insight from these large data sets. Indeed, experts in the emerging fields of bioinformatics and computational systems biomedicine are needed not only to help guide post hoc interpretation of results but also to help plan the nature and extent of the data gathered in the first place, to ensure that it will be possible to draw reliable and significant conclusions from the time, effort, and expense such extensive studies entail. We are already beginning to see the value and power of applying such computational approaches to systematically collected, large-scale transcriptional data sets in assessing human immune status (Chaussabel et al., 2008; Gaucher et al., 2008; Querec et al., 2009), and methods for using multiple data types to construct computationally useful models of organism-level physiology are emerging (Sieberts and Schadt, 2007). A detailed and insightful description of how such systems approaches, especially those based on RNA expression profiling, can be used to uncover the factors that control the nature and extent of human immune responses to vaccines is presented in the review by Pulendran et al. (2010).

Making a more than incremental advance in human immunology will also require changing the usual way the field does business in a sociological sense. A much greater degree of cooperation and integration among laboratory and clinical investigators across diverse subspecialties will be needed and enterprises capable of large-scale data collection with a high degree of reliability and quality will be essential, as will the integration into these efforts of computational experts that operate in a fully coordinated manner with the physicians and biologists, rather than being consulted after the fact. A new attitude toward rapid data dissemination and sharing akin to the procedures followed by the Human Genome Project (http://www.genome.gov/12513440#al-1) will play a big role in moving the field forward at the most rapid rate and producing the fastest translation of these new data into clinical benefits for patients. Some of these concepts and practices have already been put in place by the large ongoing efforts of the Immune Tolerance Network (http://www.immunetolerance.org/), but additional transparency in data access, among other improvements, will make this and other such efforts even more valuable to the entire research community. The rapid data release and public access polices of the ImmGen project for deep molecular phenotyping of immune cells (http://www.immgen.org/index_content.html) is another emerging example of how extensive consorted efforts can provide major benefits to the entire field, not just the few investigators actually funded for and involved in the data gathering, and one hopes that the ImmGen program will rapidly move from mouse to human cells in its analyses. Discussions are underway among the centers planning or in the early stages of efforts to conduct systems-level analysis of the human immune system to share SOPs, make data sets as compatible and as comparable as possible, draft data release guidelines, share technology developments, and aggregate findings to allow large meta-analyses that will be especially valuable in linking genetic variation to immune behavior, whether with respect to vaccine efficacy, autoimmune disease propensities, or therapeutic responses. An initial goal of the NIH CHI and most other centers involved in this new approach to the study of human immune function is to provide an in-depth description of the normal human "immunome," which will provide the entire community of investigators a reference point for assessment of the disturbed state of the system in diseased individuals and for relating the perturbations induced by various therapeutic interventions (including vaccines) to overall system function.

Concluding Remarks

Some will be uneasy at best and dismissive at worst with respect to the emphasis placed here on the promise of new "big science" efforts in the field of human immunology. A key point to be made is that the global, extensively multiplexed, omic-scale analysis of the human immune system that underlies the approach just discussed complements but in no way replaces insightful, focused studies of the components and fine-grained behavior of the immune system. The systems approach is not designed to reveal the details of intracellular signaling pathways in specific cells, although some of its technologies, like multiplex phosphoflow, can contribute to such studies. It is not optimally set up to discover a new type of cell if such a cell is only revealed by a new surface marker to which an antibody is not available and thus not included in the complex staining panels used for flow analysis, although improved computational methods for identifying cell subpopulations with unique combinations of staining achieved with large numbers of known markers can potentially identify such a cell type without the new antibody.

Existing technologies are not optimally suited to addressing how two cell types communicate with one another, identifying the counterligand of a novel immune receptor, or providing high-resolution descriptions of either the positioning of various inflammatory cells in specific tissue sites or the pathology of the involved tissue, although imaging methods may permit such studies in the future as part of the large panel of assays done in a systems-level enterprise.

The essential point is that "systems analysis" is really the new "physiology." There is a renewed interest in understanding the integrated functioning of the immune system in humans and not just obtaining descriptions of a few of the parts and their individual roles or nearest neighbor interactions, as important as such knowledge is. There is a desire to use deep, extensive, and quantitative measurements of as many aspects of the integrated system as possible in a concerted effort to discern the origins of disease and to provide insights into how to manipulate the system for improved human health, including through effective vaccination (Germain, 2001). Optimal use and interpretation of such global studies require the specific knowledge derived from conventional investigations as fundamental building blocks, and the detailed studies require systems-level efforts to put the focused information they produce into a broader context that provides a deeper mechanistic understanding of how the various parts of the system work together to provide protection in health or fail to do so in disease. Proponents of this strategy certainly do not wish to overpromise, an issue discussed in some detail in Pulendran et al. (2010). Nonetheless, it seems that only with such a more complete and integrated understanding of immune function can we hope to develop drugs and vaccines that work the way we want with limited toxicity and do so in the most efficient manner. The reviews in this issue provide insightful snapshots of the state of the art for many specific aspects of immunity, host-pathogen interaction, and vaccine development—the hope for the future is that such knowledge will be blended together with the large-scale efforts and systems approaches that are the heart of this Perspective so that the next time the subject is reviewed in these pages, a more holistic understanding of human immune function will be evident and substantial progress to a new generation of effective vaccines will have been made based on this new insight.

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