TRANSLATIONAL IMMUNOLOGY

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Mechanisms and Pharmacologic Approaches

Edited by
SENG-LAI TAN, Ph.D.

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Academic Press is an imprint of Elsevier

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225 Wyman Street, Waltham, MA 02451, USA
525 B Street, Suite 1800, San Diego, CA 92101–4495, USA
125 London Wall, London, EC2Y 5AS, UK
The Boulevard, Langford Lane, Kidlington, Oxford OX5 1GB, UK

British Library Cataloguing in Publication Data
A catalogue record for this book is available from the British Library

Library of Congress Cataloging-in-Publication Data
A catalog record for this book is available from the Library of Congress

For information on all Academic Press publications
visit our website at http://store.elsevier.com/

ISBN: 978-0-12-801577-3

Publisher: Janice Audet
Acquisition Editor: Linda Versteeg-Buschman
Editorial Project Manager: Halima Williams
Production Project Manager: Lucía Pérez
Designer: Greg Harris

Typeset by SPi

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PREFACE

Human disease is complex and is intricately intertwined with some level of deregulation of the immune system. Improved understanding of how our immune system functions to fend off foreign invaders and remove cancerous cells while maintaining immunological tolerance to self and promoting tissue homeostasis is central to the development of more effective therapies for combating a wide variety of human illnesses. This book was prepared to summarize and highlight some of the most important advances in human immunology, examples of clinical translations, new tools to analyze therapeutic targets, and new pharmacological approaches for the treatment of immune disorders. To that end, we have assembled a diverse group of highly regarded experts, as well as emerging thought leaders in the field, who have prepared 12 chapters covering different concepts, which could fundamentally change how we target the immune system for disease therapy.

This monograph is organized into three sections: Human Immunology, Emerging Pharmacological Targets, and New Approaches. In the first section, Kidd and Dudley emphasize the need to understand human immunology at the systems level. Also, they review how recent advances in high-throughput "omics" technologies and computational analysis techniques are improving disease understanding, and accelerating the discovery of better diagnostic markers and therapies (Chapter 1). Next, Walter and colleagues review our current understanding of monogenic primary immunodeficiency disease and its implications for mechanism-based targeted therapies (Chapter 2). Indeed, the advent of electronic personal health records is enabling our ability to phenotype large human populations. In Chapter 3, Warner and Denny discuss how an important translational bioinformatics tool known as phenome-wide association study (PheWAS) can be applied to link diseases or traits to a given genetic variant or biomarker. Not to be outdone, single-cell analysis has the power uncover the complexity inherent in heterogeneous populations of cells and reveal important functional insights. This is showcased in Chapter 4, wherein Lee discusses the application of RNA sequencing in a high-throughput manner to study the T cell receptor repertoire.

Immune cells have the ability to differentiate into functionally distinct effector and regulatory cell subsets, depending on the cytokines present within the microenvironment during an active immune response. Each specialized immune cell subset plays a critical role in fine-tuning our immune responses. This is perhaps best studied in the T lymphocyte population, which is covered in the second section of the book. In Chapter 5, Yang summarizes our current understanding of the T-helper 17 cells (Th17) as key effectors of autoimmune inflammatory diseases, and outlines strategies targeting the lineage of Th17 cells. The growing evidence of Th9 cells' role in contributing to human disease, thus another emerging target for drug development, is discussed by Awe and Kaplan (Chapter 6). Ueno reviews the critical role of T follicular helper cells (Tfh) in providing help to B cells, allowing for the production of high affinity antibodies (Chapter 7). Graca and colleagues summarize our current understanding of the role of regulatory T cell subsets (Treg) play in maintaining peripheral tolerance while preventing autoimmune diseases and limiting chronic inflammation (Chapter 8). In the same vein, a subset of B cells with regulatory functions (Breg) is also gaining traction in their own right as a potential target for therapeutic manipulation. Simon and Hillion cover this topic in Chapter 9.

In the final section of the book, readers are treated to state-of-the-art approaches for interventional immunology. Induction of antigen-specific immune tolerance is the desired goal for the treatment of autoimmune and allergic diseases, and protection of transplanted cells and tissues. Miller and colleagues review recent alternative methods of inducing tolerance for the treatment of allergic diseases (Chapter 10). Humanized mice are increasingly being utilized as a preclinical bridge between mouse studies and clinical trials. Greiner and colleagues discuss advances in the development of mice engrafted with functional human immune systems, and the utility of humanized mice for translating the next generation of cell based and immunomodulatory therapies into the clinic (Chapter 11). Finally, personalized or stratified medicine is recognized as a high priority goal for healthcare providers, pharmaceutical industries, and patients. Ponchel and colleagues discuss the role of immunological biomarkers in stratified medicine, as well as the challenges that needed to be overcome in order to establish their utility in routine clinical practice (Chapter 12).

While this book is not meant to be exhaustive, we hope it will provide readers an understanding of the rationale and mechanisms underlying some of the current and emerging pharmacologic approaches for translational immunology, as well as the gaps therein, and new ideas for better and safer therapeutic approaches.

Seng-Lai Tan, PhD
Editor

CHAPTER 1

Systems Immunology

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Translational Immunology
http://dx.doi.org/10.1016/B978-0-12-801577-3.00001-0

1. INTRODUCTION

From the earliest to the latest moments in life, the immune system stands ready to recognize and respond to threats that arise from external (eg, infectious agents) and internal (eg, malignant cells) sources. Potential threats are processed and then acted upon by molecules and cells organized in a distributed network that covers every organ system. The molecular and cellular agents of the immune system coordinate their interactions in time to protect the organism. In the process, the immune system evolves and adapts to an ever-changing environment, forming a complex system that shapes an individual's health.

Throughout the twentieth century, researchers identified the building blocks that comprise the immune system and teased apart the molecular regulatory pathways that govern immunity. These findings led to advances in vaccines and the development of targeted therapeutics like monoclonal antibodies. By contrast, the twenty-first century has ushered in a transition where we are now moving from cataloging the immunological parts list to integrating these components into a whole. In connecting the dots between the elements in this complex system, we are constructing a more complete picture of how these myriad parts coordinate to protect the organism from danger, damage, and disease.

In this chapter, we describe the burgeoning field of systems immunology, which is a discipline that connects the dots between how cells and molecules interact to create functions that are greater than the sum of their parts. We discuss how the confluence of high-throughput technologies and large-scale data generation are providing new insights into disease mechanisms and offering new opportunities for better treatment and prevention of immune-mediated diseases.

2. IMMUNE SYSTEM IS A DISTRIBUTED AND DECENTRALIZED NETWORK THAT PROTECTS AGAINST DISEASE AND PROVIDES A READOUT ON HEALTH

The immune system comprises a complex network of molecules and cells that coordinate their actions to protect against disease and maintain health. Individuals are protected from disease *via* numerous regulatory pathways and mechanisms that are both distributed (ie, found in almost every anatomical location) and work by acting on multiple levels (eg, genes, proteins, cells, tissues, and organ systems). Health is maintained through repair mechanisms that remove infected or dead cells and supervise rebuilding damaged tissue. Both maintenance and protection are ongoing operations that the immune system performs throughout the course of an organism's lifetime. In this section, we examine the interactions among immunological agents—molecules and cells of the immune system—as well as the interface between the immune system and its environment to explore some of the exciting opportunities for new therapies.

2.1 Immune cells are distributed and specialized

The immune system comprises numerous molecular and cellular agents that are distributed throughout the body in a decentralized network. Some agents, eg, antibodies, granulocytes, lymphocytes, and monocytes, circulate throughout the blood and lymphatic systems, the immunological superhighways. Other agents, eg, cytokines, dendritic cells, and macrophages, are found in specific tissues, taking up residence to serve as sentinels against potential threats. All cellular agents of the immune system originate from hematopoietic stem cells in the bone marrow, which differentiate into specialized cells based on environmental cues that activate a particular genetic programming. Once activated, these genetic circuits refine and restrict the molecular arsenal of each leukocyte, turning pluripotent progenitors into distinct cell types that fulfill a specific functional niche (eg, dendritic cells process and present antigens, whereas CD8 + T cells recognize antigens and secrete cytotoxic enzymes). These diverse cell types form a network that together build an immunological wall to protect the host against innumerable threats.

Immune cells have two special properties that make them particularly good at sensing health and preventing disease. First, immune cells can recognize a vast repertoire of molecules and molecular patterns in their environment. In specialized cells, these patterns are encoded and stored in immunological memory so the system can mount a more rapid and vigorous response upon subsequent encounters. This mechanism for creating and storing immunological memories means the immune system can learn from and adapt to its environment. Second, immune cells integrate numerous environmental signals and decide on the most appropriate response based on the contextual cues. These responses setup complex intercellular signaling patterns and feedback loops that can convey a broad array of information between immune cells and their environment. Remarkably, this communication is robust and allows the immune system to disseminate information with high fidelity so cellular actions are coordinated and regulated.

Immune cell signaling is an area where systems approaches have become essential for capturing the full complexity of all the molecular interactions between immune cells and decoding how they influence immune system states. To date, 456 cell-signaling proteins (cytokines, chemokines, growth factors, and hormones) have been identified that can influence the immune system. These molecular messengers interact with one or more of the 306 distinct receptors present on the surface of the more than 450 different immune cell types that have been characterized. This large pool of signaling molecules, receptors, and cell types form a decentralized and distributed communication network that relays information amongst immune cells and regulates their function. To capture and organize the full set of possible interactions between cells and signaling proteins, researchers have applied systematic approaches that mine the scientific knowledge base to connect the dots between cells, cytokines, and function (Shen-Orr et al., 2009; Patil et al., 2010). These text-mining methods construct inter-cellular interaction maps, which can be linked with other systems approaches to connect up to the internal signaling networks responsible for controlling cellular states (Linding et al., 2007; Janes et al., 2008). Using these systematic approaches to analyze and improve our understanding of immune cell-signaling networks is critical because these networks bind the immune system together and connect it to other systems. This more complete picture of how the full communication system operates will help uncover the mechanisms underlying many different diseases (Osborn and Olefsky, 2012; O'Sullivan et al., 2007; O'Shea et al., 2011; Neurath, 2014; Griffin, 2013) and suggest strategies for better treatments (Cosgrove et al., 2010; O'Shea and Plenge, 2012; O'Shea et al., 2013; Feldmann, 2002).

2.2 Immune system connects to all aspects of health and disease

The features that make the immune system effective at recognizing, repelling, and removing danger also establish the immune system as a master integrator of information about health and disease. Moreover, the immune system connects to every other organ system and interfaces with the environment to provide protection and help maintain health (Fig. 1). The immune system plays a central role in allergy, autoimmunity, and infectious disease. Immune surveillance is key for recognizing and removing abnormal cells and many cancerous cells emerge and flourish by finding mechanisms to evade this surveillance. The immune system is highly active and requires continual renewal and regeneration of its cells. Many cancers arise from genetic abnormalities in immune cells that originate during growth and differentiation. Furthermore, dysregulation of inflammatory mechanisms and other immune-mediated processes have been associated with cardiovascular disease, metabolic disorders, and neurodegenerative diseases. This

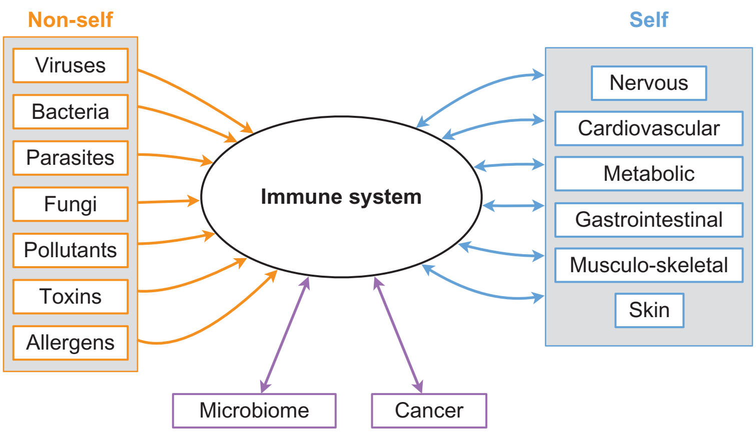

Figure 1 Immune system is a master integrator of information about health and disease. The immune system is a complex network of cells and molecules that integrate information from multiple sources to provide immunity and maintain health.

entrenched connection to every organ system is significant because it implies that immune system health is strongly tied to overall health. Maintaining proper immune function may be the best course of action for preserving health. As we will see later, immune monitoring provides a readout of an individual's well-being and offers more precise feedback on therapeutic interventions.

2.3 Commensal organisms

Investigations into the human microbiome are uncovering complex microbial ecosystems that engage with the immune system and altering how we think about health and disease (Human Microbiome Project, C, 2012a,b; Brown et al., 2013). For example, commensal bacteria play a critical role in both the development, education, and maintenance of proper immune function (Shen et al., 2014; Ahern et al., 2014).This area of research is developing rapidly and in tandem with systems immunology so much remains to be done. However, what's become clear is the microbiome shapes the immune system's response to the environment—diet, infection, and allergy—and influences how the immune system contributes to both the development and treatment of disease (eg, diabetes, cancer, and autoimmunity).

Recent investigations into this immune-microbiome interface are revealing novel regulatory interactions and control that will have implications for clinical medicine. Preliminary findings suggest the microbiome can affect both the development of cancer, as well as the immune response to cancer therapy (Iida et al., 2013; Zitvogel et al., 2015; Viaud et al., 2013). Other studies have shown that the microbiome plays a role in nutritional uptake and influences metabolic control over the immune system in ways that have interesting implications for understanding and treating metabolic disorders including type 2 diabetes (Henao-Mejia et al., 2012; Kau et al., 2011; Dastani et al., 2012). Collectively, these findings have interesting implications for immune homeostasis, allergy, and autoimmunity. These findings clearly need additional follow-up and consideration to determine what role they will play in the clinic. Given the complexity of the interactions between the immune system and the microbiome, systems approaches will be required to identify therapies that restore control from microbe–immune system imbalances.

2.4 Infectious diseases

The main cause of morbidity and mortality for most of human existence has been disease-causing microorganisms. Pathogens exploit transient openings in the immunological wall to invade their host and co-opt cellular machinery or resources for survival. This process causes havoc on the host and results in acute, or sometimes chronic, disease. Not surprisingly, every tissue in the body is susceptible to infection by some microorganism. Fortunately, the immune system's influence extends to every tissue in the body, and the immune system uses its molecular and cellular arsenal to counter pathogens by engaging and fending off these infectious agents wherever they are found. Thus, the immune system and infectious agents remain locked in perpetual battle for survival. While some microorganisms can be challenging to isolate, assaying the immune system yields clues about whether an infection has happened, what type of response was mounted, and whether the host will recover.

All infectious agents interact with the immune system and leave a mark that can be used help track their origin. These agents elicit specific immune responses, which can be helpful in predicting their virulence and identifying therapeutic options for treating the disease. For example, antibodies present in serum signal that the immune system encountered a pathogen at some point in the past. A recent study harnessed this property and showed how to comprehensively characterize the complete history of pathogen encounters by profiling human sera on microarrays displaying viral epitopes (Xu et al., 2015). The abundance of antibodies indicates how well protected an individual will be to future infections and whether that individual might need an immune boost to raise the circulating levels of a particular antibody. The presence of antigen-specific T cells signifies cellular immunity against a particular infectious agent. Further functional and phenotypic characterization of these T cells provides a readout on the strength of immunity. While systems approaches currently offer information on how the immune system responds to, or determines the components of protection against infection, later sections will explore real-time monitoring for immediate feedback on host response.

2.5 Allergic diseases

Asthma, atopy, and allergic diseases are common across all age groups, affecting around 8% of the population in the United States (17 million adults and 7 million children). These conditions are clinically characterized by inflammation of either the airways or skin, brought about by exposure to an allergen or other environmental trigger. In many cases, the environmental triggers of allergic diseases can be difficult to determine. Moreover, the immune response is complex as multiple cellular and molecular players can drive inflammation and allergic reactions. Systems immunology offers tools for building more accurate models of this complexity and suggesting novel treatments.

The primary treatment for atopic diseases is immune and inflammatory suppression with corticosteroids. While effective in the short-term, long-term topical glucocorticoid use leads to cutaneous atrophy, thus making the skin susceptible to damage and infection. One example of how systems immunology approaches can identify novel treatment options comes from a recent study to characterize the transcriptional response to topical glucocorticoids. This study found that combination therapy with steroids and the mTOR inhibitor that regulates development and damage responses (REDD1) could eliminate the adverse side effects while maintaining the therapeutic benefit of steroids (Baida et al., 2014).

Asthma is a complex condition where multiple therapeutic options are employed. These options include fast-acting bronchodilators that relieve airway constriction and enzyme inhibitors that target leukotrienes, inflammatory mediators produced by immune cells. The complex tissue architecture of the airways plus the myriad immune cells contributing to disease and range of therapeutic responses suggest that integrative approaches are needed to identify novel treatments for asthma (Bunyavanich and Schadt, 2015). These approaches have been useful for identifying some of the key pathways that drive allergic responses at the genetic and cellular levels (Sharma et al., 2014; Bunyavanich et al., 2015). The combination of systematic analyses (Bonnelykke et al., 2013) and well-characterized preclinical models (Kelada et al., 2014) provide platforms for discovery and testing that can take advantage of the highly connected nature of the immune system.

2.6 Autoimmune diseases

Autoimmunity represents a clinical condition where the immune system fails to discriminate between self and nonself. The occasional failure to distinguish friend from foe results in a coordinated attack by immunological agents on self-antigens. At a biochemical level, no structural difference exists between self and foreign antigens. In fact, the recognition of self-antigens plays an important part in the development, maintenance, and survival of B cells and T cells. Autoreactive immune cells can, and do, arise against any tissue, yet the prevalence of autoimmunity remains low because additional molecular, cellular, and contextual cues control immune responses to self-antigens. In order to capture and analyze the complex set of regulatory interactions that control autoimmunity, systems approaches offer a natural approach that can integrate high-throughput data collected from environmental, genetic, molecular, cellular, and microbiome measurements into models that can both explain various mechanisms by which autoimmunity arises and predict control points for treating these conditions.

The combination of genetic, environmental, and lifestyle factors that contribute to autoimmunity make these disorders complex diseases. Multiple genomic studies have identified hundreds of genetic loci that are associated with one or more autoimmune diseases (Visscher et al., 2012). While alleles in the human leukocyte antigen (HLA) genes contribute the most to disease susceptibility, a number of immune-related, but non-HLA genes have been linked to autoimmunity (Welter et al., 2014). Although genome-wide association studies (GWAS) permit an unbiased scan of the entire genome and provide strong association with particular regions to disease, understanding the causal drivers require fine mapping of genetic loci or functional studies that link the underlying genotypic variation with a phenotypic trait (Chen et al., 2014; Schadt et al., 2005). Functional data for these studies are often provided from high-throughput scans of all transcripts expressed in a particular sample. Transcriptional profiling of leukocytes collected from peripheral blood has been successfully applied to determine the molecular networks of many autoimmune diseases (Pascual et al., 2010), and the systems approaches have shown promise for characterizing autoimmune and autoinflammatory disorders (Banchereau et al., 2013). More work is required, yet these methods reveal the potential of systems immunology to advance our understanding of disease, identify better biomarkers, and improve drug development.

2.7 Inflammatory diseases

The inflammatory response represents a mechanism employed by the immune system to respond to infection or injury, and to orchestrate repair. The inflammatory response triggers multiple signaling processes and encourages cross talk among multiple cells and tissues. Multiple biomarkers can serve as surrogates for an inflammatory response (eg, C-reactive protein, IL-6, and TNF-alpha) and these molecules are measured clinically to assess whether an individual has chronic inflammation, which implies immune dysregulation and can indicate inflammatory disease. Almost all immune-mediated diseases contain an inflammatory component (McGonagle and McDermott, 2006) and inflammation is a major component of many noncanonical immunological diseases (eg, cardiovascular, obesity, type 2 diabetes, neurodegenerative, cancer). Whether inflammation is causal, or a by-product of other disease processes is not clear. Tracking inflammatory dysregulation and understanding the processes that might shift inflammation from a transient to chronic condition should be a top priority for systems immunology because of the problem's complexity and the societal benefit in identifying new therapies.

Inflammation is a protective response that causes collateral damage to normal healthy tissue in the process of preserving the host from injury and infection. From an evolutionary perspective, the trade-off between host damage and preservation has been optimized over a long timescale across multiple environments. Our current environment provides many immune stimuli that may be misaligned with the immune response traits evolution selected (Okin and Medzhitov, 2012). This suggested mismatch has been proposed as an explanation for why the inflammatory response is associated with so many modern diseases. To explore this evolutionary hypothesis further, one line of inquiry is to examine genetic conditions that lead to autoinflammatory disorders. Although monogenic autoinflammatory conditions are rare, they do shed light on mechanisms for immune dysregulation that influence inflammation and underlie common illnesses. In particular, the discovery of the proinflammatory molecule IL-1b, and its role in both autoinflammatory diseases and inflammasome activation, has made IL-1b antagonists potent therapies for inflammation, sepsis, and immunological disorders (Goldbach-Mansky and Kastner, 2009). However, more studies and follow-up are needed that utilize systems approaches to understand the broader therapeutic consequences and side effects, as well as to connect antiinflammatory therapies to other disorders associated with inflammation.

A strong argument for using systems approaches that consider evolution comes from the fact that the transcriptional response pattern of inflammation is shared across multiple disease models from mouse and rat (Wang et al., 2012b). The conserved immunological features reflect subcomponents of the inflammatory response involved in leukocyte activation, cytokine production, chemotaxis, and toll-like receptor (TLR) signaling. The gene expression profiles show similarity across diseases and the patterns are conserved across tissues and species. The shared biological subnetworks found in the different species and diseases tie together seemingly disparate processes, unifying them into a framework that helps explain molecular mechanisms of disease and identify potential therapies. This inflammatory signature also shows significant overlap with a macrophage-enriched metabolic network that has been found in both humans and mice.

As we've seen, systems approaches to immunological conditions can be applied across diseases to find common regulatory pathways or gene expression signatures that unify diverse conditions at the molecular, cellular, or phenotypic level. These approaches are also suited to focus on a single disease to understand how the different biological levels contribute to a complex disorder. As an example, we highlight a recent study that examined a complex inflammatory condition, inflammatory bowel disease (IBD) (Jostins et al., 2012). This study collected more than 75,000 cases and controls to develop quantitative models that linked genetics, gene expression profiling, evolutionary traits, and host–microbe interactions into the most comprehensive picture to date of inflammatory bowl disease. By combining these data, the authors showed what genetic elements are shared between the two major types of IBD (Crohn's disease and ulcerative colitis), as well as potential links to other inflammatory conditions and responses to infectious agents. Moreover, this type of comprehensive model can be combined with clinical trials data (Colombel et al., 2014; Sandborn et al., 2012) to better understand the connection between molecular profiles and clinical phenotypes in inflammatory diseases. More studies are needed; however, the initial findings from systems immunology approaches to study IBD, as well as the application to better understand other inflammatory diseases (International Multiple Sclerosis Genetics, 2013; International Multiple Sclerosis Genetics et al., 2013; Okada et al., 2014) show promise for stratifying patient cohorts and identifying new therapeutic targets or biomarkers.

2.8 Cancer

The paradigm of cancer is that normal cell processes are disrupted—through somatic mutation or environmental insults—such that cancerous cells are endowed with uncontrolled growth and the ability to invade and damage normal tissues. While certain cells are more or less susceptible to this transformation, cancer covers around 200 diseases that have been associated with almost every cell and tissue type in the human body. Although leukemias and lymphomas account for only ~10% of the estimated annual cancer deaths in adults,[1] the immune system plays a major role in cancer suppression and promotion

[1] US Cancer Statistics Working Group. United States Cancer Statistics: 1999–2011 Incidence and Mortality Web-based Report. Atlanta: US Department of Health and Human Services, Centers for Disease Control and Prevention and National Cancer Institute; 2014.

through the mechanisms of immunosurveillance and immunoediting (Schreiber et al., 2011), as well as protection against viruses associated with an increased risk of cancer (eg, human papillomavirus, Epstein–Barr virus, and Hepatitis C virus). While the intersection between cancer and immunology was postulated more than 100 years ago, evidence over the last decade has shown that infiltrating lymphocytes shape the tumor microenvironment and are associated with favorable prognosis in a variety of cancers (Gajewski et al., 2013; deLeeuw et al., 2012; Odunsi and Old, 2007; Shankaran et al., 2001; Dunn et al., 2002). Furthermore, these connections are revealing promising therapeutic avenues that leverage the immune system to identify and eliminate tumors through immunotherapy (Cancer Target et al., 2010; Gubin et al., 2014; Steinman and Mellman, 2004; Maude et al., 2014). An exciting extension of these approaches is to combine immunotherapy with small molecules to extend survival times even further and potentially drive remission to the point where we have effectively cured certain cancers (Vanneman and Dranoff, 2012). These intersections are where systems approaches can be effective for helping to advance our knowledge about the overlapping boundaries between cancer and immunology and use these connections to identify successful treatment strategies.

2.9 Metabolic diseases

Metabolic diseases such as obesity, insulin resistance, type 2 diabetes, and atherosclerosis have all been associated with a persistent state of inflammation. This chronic inflammatory state appears to be driven by aberrant signaling between the immune system and metabolic processes, both within immune cells and between immune cells and cells in adipose or other metabolic tissues (brain, liver, muscle, and pancreas). Uncovering these connections and how inflammatory processes mediate metabolic diseases is revealing exciting possibilities for targeting the immune system to treat many conditions that are related to obesity, metabolism, and inflammation.

All metabolic tissues contain resident immune cell populations that respond to molecular signals (eg, nutrients, cytokines, and hormones) and help maintain metabolic homeostasis (Lumeng and Saltiel, 2011). For example, macrophages interact with adipocytes to control lipid storage and release (Kosteli et al., 2010). Lipid metabolism and inflammation have been linked through multiple immune cells, signaling molecules, and receptors (Jin et al., 2013). Multiple proinflammatory inputs and pathways converge on genes that ultimately regulate nutrient sensing and metabolism. Drugs that target inflammation aim to interfere with the dysregulation that contributes to metabolic disease. For example, randomized trials on diabetic patients who were overweight or obese showed that modulating NKF-kB activity with salsalate improved glycemic control (Goldfine et al., 2008; Goldfine et al., 2010). In a separate study of obese individuals, blocking TNF-a with etanercept improved insulin sensitivity (Dominguez et al., 2005; Stanley et al., 2011). These examples are just the beginning of therapeutic applications to diseases associated with obesity.

Systems approaches that integrate genotypic, gene expression, and clinical data from human populations with an array of different metabolic diseases are revealing an underlying molecular network that not only is shared among these diverse disorders, but also appears to be causal for these diseases (Schadt, 2009). This molecular network highlights the power of how systems approaches can scan across multiple tissues, cell types, and diseases to identify genes that are significantly enriched in disease-specific transcriptional signatures. This network is highly enriched for genes associated with macrophages, which ties into one of the dominant immune cell types driving these metabolic diseases. Given the macrophage-specific nature of this network and its causal association to a diversity of metabolic traits, we refer to it as the macrophage-enriched metabolic network.

2.10 Neurological diseases

The nervous and immune systems are tightly connected through multiple cells, cytokines, and hormones that each system uses to regulate the other (Steinman, 2004). These complex interactions shape the development of each system, help maintain homeostasis, and when disrupted can contribute to disease pathogenesis (Boulanger and Shatz, 2004). As an example of these regulatory interactions, both autonomic and peripheral neurons are known to regulate inflammatory processes (Andersson and Tracey, 2012; Chiu et al., 2012) and recent evidence suggests that peripheral pain receptors are necessary for an IL-23-mediated inflammatory response in a mouse model of psoriasis (Riol-Blanco et al., 2014). This neural–immune connection is certainly more integrated than previously appreciated as indicated by other studies showing neurons sense and respond to bacterial products by influencing the immune response, even before immune cells respond to the pathogen (Chiu et al., 2013).

Taken together, the early observational studies that viral or bacterial infection (eg, rabies, syphilis, influenza) trigger inflammatory responses in both the periphery and within the central nervous system, along with the recent attempts to tease out whether neural pathology is caused by the infectious agent or host response (Steinman, 2014) and the direct influence of sensory neurons on inflammation, the connections between the nervous and immune systems are clearly more complex and intertwined than we currently understand. Indeed, many neurological diseases have strong genetic components that can be linked to immune genes (Tan et al., 2014) or inflammatory processes (Zhang et al., 2013; Lucin and Wyss-Coray, 2009; Doty et al., 2014; Gonzalez et al., 2014). Determining how these processes are connected and can regulate one another is a perfect challenge for systems immunology.

2.11 Connections between the immune system and other organ systems

Clear cellular communication is essential in order for cells of the immune system to coordinate an appropriate and effective response. These responses require recruitment of the correct cells to the proper place at the right time. To achieve this level of orchestration immune cells secrete soluble factors known as cytokines and chemokines that can act on the secreting cells and other cells within their environment. In addition, immune cells must home to specific locations and adhere to the right tissues, both of which are achieved by surface markers and adhesion molecules. Together this collection of immune molecules make distributed communication amongst immune and nonimmune cells possible.

The HLA provides a striking example of a major molecular player in this distributed communication network. HLA molecules are derived from a family of genes that encode for a set of cell surface molecules that immune cells use to discriminate between self and nonself. Every cell in the human body expresses an HLA molecule, which provides one of the molecular signals immune cells rely on to determine whether another cell is foreign. HLA genes are among the most polymorphic in the genome. Given the thousands of allelic variants and the exquisite specificity of antigen recognition, one could argue the immune system has its own genome. These genes regulate development, perform surveillance, and respond to internal and external threats. The HLA genes, also known as our compatibility genes, resolve many puzzles about how the immune system distinguishes between self and nonself, explaining the molecular similarity needed for a successful organ transplantation, mechanisms for how the adaptive immune system recognizes viral and bacterial products, and how a related family of cell surface proteins found on NK cells (so-called killer cell immunoglobulin-like receptors (KIRs)) can recognize tumors. More importantly, these genes have provided a wellspring for some of the most important scientific questions in medicine and have played a critical role in helping scientists understand and treat disease.

The immune system sits at the boundaries between multiple organ systems, and between genetics and the environment. While immune cells are the quanta of the complex network, the system is multiscale in nature. Thousands of immune-related genes control cellular function, which in turn secrete proteins to control other cells, tissues, or organs. These different levels of organization are tightly regulated to perform critical tasks that are essential for survival. All of these interactions are dynamic and happen over multiple time lines. Reframing the immune system as a master integrator for understanding health permits new approaches to discover biological mechanisms that drive disease and identify therapeutic options that can restore balance to the immune system, and thus restore health.

3. HIGH-THROUGHPUT TECHNOLOGIES AND TECHNIQUES FOR SYSTEMS IMMUNOLOGY

The modern day origins of systems immunology can be traced back to technological innovations stemming from genomic sequencing projects that began in the 1990s. These projects articulated ambitious, yet clear goals for biology, which resulted in large-scale efforts amongst academia, industry, and government. Together, these ventures ushered in the era of high-throughput science where comprehensive sets of molecular interactions can now be examined in parallel from a single experiment and research questions are no longer limited to interrogating specific molecular interactions. The ability to collect comprehensive data on genomes initially, and then ultimately many "omes," has empowered researchers to construct a systems-view of immunology.

The ~3 billion nucleotide sequences in humans presented a challenge that was economically and temporally impractical for the technological capabilities at the Human Genome Project's onset. Thus, new technologies and techniques were invented and developed to address the experimental and computational needs for collecting and analyzing the orders-of-magnitude increase in information waiting to be unlocked in a complete genome. One consequence of these innovations is that high-throughput technologies now exist to probe multiple aspects of the immune system at unprecedented precision and scale. More importantly, high-throughput technologies have enabled the field of systems immunology by providing the data sets needed to synthesize how individual immune components coordinate their actions into a working system (Fig. 2). Below we discuss how various high-throughput technologies and complimentary computational techniques are driving systems immunology and permitting the transplantation of knowledge from immunology into clinical applications.

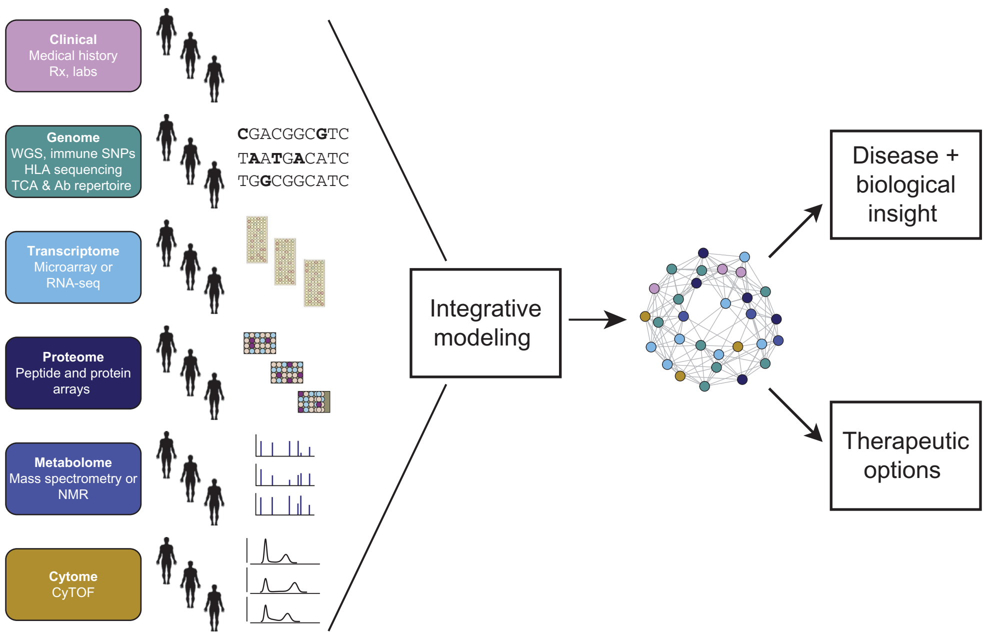

Figure 2 High-throughput technologies generate massive data that form the inputs for detailed and comprehensive models of immune system processes to guide insight into disease and suggest treatment options.

3.1 Genomic profiling of immune system genes

Large-scale sequencing efforts such as the 1000 Genomes Project (Genomes Project et al., 2012) have provided a complete annotation set for the ~6000 noncoding RNA (ncRNA) and the ~19,000 protein-coding genes in the human genome (Gray et al., 2015). Based on these annotations, the number of genetic elements that control immune function turns out to be larger than expected. Conservative estimates suggest that at least 10% of protein-coding genes regulate the immune system in some form, whereas less restrictive definitions of immune function increase this proportion to 40%. This allocation of genomic resources has implications for how we assess genetic control over the immune system, how we evaluate the immune system's contribution to disease, and what transcriptional products provide the best targets for therapies that modulate the immune system and immune-mediated diseases.

Humans harbor natural variation in their genetic code that confers protection and susceptibility to disease. However, most diseases lack a single causative variant. Systematic efforts to understand the specific gene variants that are more likely to be linked with disease conditions (eg, rheumatoid arthritis (RA) or IBD) or phenotypic traits (eg, longevity, lipid levels, or CD4+ to CD8+ ratios) take advantage of high-throughput screens that can interrogate > 100,000 single nucleotide polymorphisms (SNPs) in a single experiment. To connect genotype with phenotype, GWAS aim to identify SNPs in a gene or other regulatory element containing a causal variant that contributes to the disease or trait. GWAS are systematic approaches that take advantage of the natural genetic variation in large cohorts (eg, tens or hundreds of thousands of individuals). Since the introduction of GWAS in the middle of the last decade, the National Human Genome Research Institute (NHGRI) has compiled a catalog of published GWAS that as of this writing includes >1750 curated publications and over 11,900 SNPs that associate with disease, drug response, and phenotypic traits (Welter et al., 2014).

The immune system is shaped by genetic and environmental control. In the era prior to GWAS, clinical genetics identified a number of rare inherited disorders that increased susceptibility to multiple infectious agents. These primary immunodeficiencies gave rise to the dominant paradigm that alteration of a single immune gene increased susceptibility for a specific infectious disease (Picard et al., 2006). For example, individuals with mutations in the molecular circuit for IL-12/23 and IFN-g signaling have increased risk of mycobacterial disease, whereas those with inherited interleukin-1 receptor-associated kinase-4 (IRAK-4) deficiency are prone to pneumococcal disease (Casanova and Abel, 2007). While rare, these genetic alterations have provided insight into the genetic circuits that control immune response to infection.

The variation in an outbred population provides a natural experiment to determine the links between genotype and phenotype. Population studies in humans have revealed hundreds of genetic variants that control immune system function (Casanova et al., 2013;

Xavier and Rioux, 2008). For example, 23 SNPs have been found to regulate immune cell frequencies (Orru et al., 2013). At the time of publication, more than 300 genetic loci have been linked to one or more of the 80 autoimmune diseases (Visscher et al., 2012; Welter et al., 2014). A number of these loci can be mapped to shared biological pathways, indicating some sort of shared mechanism driving autoimmunity (Cho and Gregersen, 2011; Cotsapas et al., 2011; Voight and Cotsapas, 2012; Goris and Liston, 2012). By implementing a combination of systems approaches with whole-genome sequencing and high-resolution mapping on the loci described, we can tease apart the causal links and find the similarities that unify diseases processes.

Next-generation sequencing technologies are finding many applications to problems in immunology. One challenge is sorting out the tremendous repertoire diversity that provides exquisite antigen specificity. High-throughput technologies are revealing the breadth of both the T cell antigen receptor (TCR) (Robins et al., 2010) and antibody repertoires (Boyd et al., 2009), which have multiple applications for medicine in diagnosis and monitoring of clinical care (Boyd, 2013). These methodologies have also successfully been applied to identify immunogenetic risk factors for disease (Trowsdale, 2011), examine HLA polymorphism across population and pathogenic diversity (Prugnolle et al., 2005), and select immunodominant HLA-restricted T cell epitopes, eg, for vaccine or tetramer design (Newell et al., 2013). As these techniques are combined with other methodologies, they provide a more complete picture that connects receptor sequence and cell function (Han et al., 2014).

3.2 Transcriptional profiling of immune cells

Transcriptional profiling measures the functional and dynamic landscape of gene regulation and the cell-specific programming that drives immune cell function. When performed at a whole-genome level, these approaches provide an unbiased examination of how transcript changes correlate with diverse states of the immune system. For example, immune cell subsets have specific transcriptional signatures, and systems immunology approaches have associated these expression patterns with a range of immunological conditions such as autoimmunity (Boisson et al., 2012; Chaussabel et al., 2008; Pascual et al., 2010), response to vaccination (Nakaya et al., 2011; Querec et al., 2009; Furman et al., 2013; Banchereau et al., 2014; Obermoser et al., 2013), and infectious disease (Berry et al., 2010; Cliff et al., 2013; Bloom et al., 2012; Law et al., 2013). Moreover, these molecular signatures have identified therapeutic strategies that might be helpful for patients with rheumatological diseases (Chiche et al., 2013) and lymphomas (Hummel et al., 2006). Although critics of high-throughput biology have suggested that little has come from an approach that measures everything and figures it out later, two clear facts have surfaced that would not have been identified from other means. The first is that multiple pathways drive immune cell responses, and these pathways offer new understanding of disease as well as therapeutic targets. The second is that comprehensive and unbiased profiling of the entire transcriptional landscape has provided data to generate complete regulatory networks, which serve as wiring diagrams for explaining the immune response to a diverse range of immune perturbations. However, the real power of these networks is they are (i) hypothesis-generating tools that lead to verifiable experimental designs and (ii) predictive models for exploring our knowledge of biology and testing out multiple therapeutic leads prior to moving toward more costly experimental validations.

Studies on genome-wide patterns of mRNA expression in immune cells have identified drivers of the immune response to antigens, cytokines, small molecules, and other environmental perturbations. For example, high-throughput technologies have identified cell-specific signatures and the genetic regulatory programs that govern lineage differentiation and pathogen response (Fairfax et al., 2012; Zhang et al., 2012; Shalek et al., 2013; Yosef et al., 2013). To understand the complex and adaptive nature of the immune system, these transcriptional profiles can be connected to SNP data to determine immune regulatory networks and causal drivers of disease. This approach has been recently demonstrated for complex diseases such as IBD, lupus, and type 1 diabetes (T1D) (Jostins et al., 2012; Westra et al., 2013).

3.3 High-throughput technologies to profile immune cells

Immune cells are the functional building blocks of the immune system and they offer readouts for the organism's state of health. High-throughput techniques provide the capability to examine millions of cells at the resolution of the single-cell level. New technologies such as mass cytometry have more than doubled the number of molecular probes that can be used to interrogate a single cell. This technological advance has vastly expanded the number of distinct cell types and functional states that can be examined in an experiment. This scale of experimental inquiry is unprecedented in the history of immunology and has great promise to answer many long-standing questions in immunology such as: what is the diversity of the immune repertoire, how many different cell types does the immune system need, and what are the functional states for different immune cells. The frontiers for systems immunology will be to examine time series data from various immune responses to construct the interaction maps associated with different states and how these states evolve. One key question that needs to be addressed is what immune cell subsets associate with different physiological states so the cells can serve as immunological indicators of health and disease states.

Over the past 40 years, cytometric measurements by flow have progressed by probing a steadily increasing number of cell surface proteins. Currently, single-cell mass cytometry (CyTOF) technology can measure >40 surface markers or intracellular proteins simultaneously, providing an unprecedented number of possible parameters to characterize distinct cell types (Bendall et al., 2011, 2012). Given the large number of markers, automated strategies are now required to process the high-dimensional information and identify cell types based on the extent of similarity among a specified set of markers.

These data-driven approaches select the combinations of markers that identify distinct cell types and can be used to classify differences between cohorts (Amir el et al., 2013; Bruggner et al., 2014), profile drug perturbation responses (Bodenmiller et al., 2012), or map lineage development (Bendall et al., 2014). The computational tools for these advanced cytometric technologies are rapidly evolving, yet areas for which there are unmet needs include (i) techniques to integrate cytometric with other high-throughput data (eg, proteomic, genome-wide profiling, or genetic data), (ii) automated annotation calls for cell subsets based on the complete marker sets, and (iii) statistical methods for data transformation and normalization amongst large numbers of samples.

4. CONTROLLING THE IMMUNE SYSTEM TO TREAT DISEASE

4.1 Vaccination to control infectious disease

The field of immunology has made unparalleled contributions to human health through vaccination. In the United States, vaccination has decreased the annual morbidity, as compared to figures from the twentieth century, by orders of magnitude, driving the numbers to zero for smallpox, diphtheria, and polio (Roush et al., 2007; Centers for Disease Control and Prevention, 2011). Global numbers in developed countries follow similar trends. To date, global vaccination efforts have resulted in the eradication of smallpox, with realistic estimates to eradicate polio by 2018. Despite these vaccine successes, humans are still plagued by many viruses (eg, HIV, hepatitis C), bacteria (eg, mycobacterium tuberculosis (TB)), and parasites (eg, malaria) for which we have poor or nonexistent vaccines. These pathogens are responsible for more than five million deaths per year and continue to challenge effective vaccine efforts because of their complex interplay with the immune system. Thus, multiple opportunities exist for applying systems immunology to direct the development of biologics or cellular therapies that can be used as prophylactic vaccines.

Systems immunology offers an entirely new approach to investigate how the immune system interacts with pathogens. Instead of simply scaling-up targeted approaches, high-throughput technologies, and by extension the system-wide analytical approaches they enable, augment what's possible. For example, data from whole-genome transcriptional profiling permit the construction of regulatory networks that can be used to infer the activities of transcription factors, RNA-binding proteins, and micro-RNAs from the transcriptome patterns. Such inferences are critical for identifying and understanding links between molecular responses induced by vaccine perturbations and physiological or phenotypic responses that correspond to protection.

One uncertainty in vaccine development is whether a new vaccine will provoke a protective immune response. To reduce this uncertainty, systems analyses can identify the molecular signatures associated with immune responses to vaccines, and then apply supervised learning algorithms to determine what molecular features in the signature can be used to predict high- versus low-antibody titers to the pathogen, which is a clinical