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Bhagirath Singh
University of Western Ontario Faculty of Medicine & Dentistry, bsingh@uwo.ca

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Review

Emergence of human immunoprofiling in health and disease

Bhagirath Singh[1,2,*], Kelly L. Summers[1,3], Gillian Barker[4], Eric Desjardins[4], Charles Weijer[4,5] and Joaquín Madrenas[6]

[1]Centre for Human Immunology, Department of Microbiology and Immunology; [2]Robarts Research Institute; [3]Lawson Health Research Institute; [4]Department of Philosophy, Rotman Institute of Philosophy; [5]Department of Medicine, University of Western Ontario, London, Ontario, Canada. [6]Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center, Torrance, CA 90277, USA.

ABSTRACT

The human immune system is critical for maintaining health and providing protection from infectious diseases and cancer. Major advances in our understanding of the immune system have largely emerged from studies using animal models such as mice. However, this mouse-centric research has also limited our ability to comprehend the human immune system and how it changes with age and disease state. The fact that we have yet to define what constitutes a normal human immune system has hampered our ability to diagnose, treat, and prevent many human diseases. Immunoprofiling that measures the frequency of human immune cells based upon their functional biomarkers is critical for immunotherapy. With major advances in flow cytometry, mass cytometry, and imaging technology it is now possible to rapidly characterize many types of immune cells for immunotherapy and for monitoring disease. In this article, we discuss recent progress in immunoprofiling of the human immune system and how this system changes with age, chronic diseases, and autoimmunity. We also discuss this in the historical context as it relates to the emergence of human immunology. New knowledge generated by immunoprofiling studies will allow better understanding and monitoring of immune cells and their application in clinical medicine.

*Corresponding author: bsingh@uwo.ca

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1. Introduction

The human immune system is a complex network of cells and proteins that are highly interactive, diverse, and adaptive. Humans have over 100 immune cell types that are phenotypically highly stable within an individual but variable between individuals [1, 2]. This variability in frequency and functional responses of immune cells is due to genetic polymorphism and to the fact that the composition of the immune system is largely determined by non-genetic environmental factors, which can vary substantially from one individual to another [3]. Given its complex nature, it is difficult to define standard baseline values for the components of the normal immune system and how it changes with the long list of endogenous conditions (including genetics, sex, age, inflammation, microbiome and autoimmunity) and exogenous factors with which it interacts (such as infectious agents, diet, allergens, transplants and medications) [4-10].

Immunoprofiling is a measure of the state of the immune system at a given time point. It provides a window into what immune cell types predict, induce, promote or prevent disease, and how the immune system responds to infections, vaccines,

or immunotherapy. As such, it contributes to our understanding of certain immunological baselines for it can be used to measure the impact of various therapies and the environmental influences on the immune system. Immunoprofiling of blood leukocytes provides a powerful tool for the monitoring of immune cells *in vivo* [11]. Monitoring of $CD4^+$ T cell counts has been the hallmark of disease progression and therapy in HIV infected patients over the past three decades. What these activities have shown is that, in general, immune responses are driven by interactions between different immune cell types having stimulatory or suppressive proprieties. Immunoprofiling offers an opportunity to better understand these dynamic cellular interactions at a functional level. The development of immunoprofiling has been importantly powered by new technologies, such as flow cytometry, mass cytometry, cellular and molecular imaging, and the analysis of large data sets of genomics, proteomics and metabolomics studies that have added new understanding of the nature of immune cells. This has also contributed to a better tracking of pathogenic cells and immune cells *in vivo* and improved assessment of human immune function and clinical outcomes during immunotherapy [12, 13]. The area of human immunoprofiling is thus emerging from the recent developments in systems biology and human immunology [14]. It has provided new direction to the application of immunology in clinical medicine, the most noteworthy being cancer immunotherapy. This is likely to impact the discovery of new immunological mechanisms involving cell-cell interactions, cell signaling and the elucidation of genetic and environmental influences in human health. At the molecular level, immunoprofiling can be used to measure the diversity of immune responses based on antigen receptor specificity. Next generation sequencing of DNA allows profiling of antigen receptors on B cells and T cells and this can be used in turn to determine how the diversity of the receptors changes during immune response to antigens, allergens, infections, vaccines or transplants [15]. This has potential to develop genetic biomarkers for comparing baseline immune diversity and how it changes during disease or therapy. In cancer immunotherapy immunoprofiling is being used to develop biomarkers that can be used to monitor treatments and detect remissions at an early stage. Therefore, immunoprofiling can be used at the molecular level to understand how immune cells target cancer cells and how immunotherapy helps in this process.

While technological advancements provided new research opportunities, any discussion of the clinical application of immunoprofiling will be incomplete without the historical and philosophical context of the emergence of human immunology [12, 14, 16, 17]. In the last century, the discovery of the complex adaptive nature of the immune system helped to motivate a shift in perspective that raised a number of issues with research that focused overwhelmingly on animal models, particularly genetically modified inbred mouse models. Significant differences exist between the human immune system and that of experimental mouse models, which have limited the translation of these studies to humans. Because of this shift, the technological developments mentioned above, and the recent elucidation of the human genome, human immunology is now embracing new opportunities that may lead to the improved translation of research results into clinical outcomes. In this article we discuss the recent progress in the field of immune profiling and focus on the characterization of immune cells through immunophenotyping during the course of disease or in response to therapy. These studies will also help to define what constitutes a healthy or normal immune system, and serve as a clinical benchmark to assess immune changes with age, disease state and treatment.

2. Human immunology – a developmental history

The field of human immunology has taken a leap forward in the past decade after being overshadowed by studies predominantly performed in mouse models of immunity over the past century. Both conceptual and technological advances have made this possible and we now have potential to better understand the immunological mechanisms and treatment of many human diseases given the genetic diversity and environmental influences in human populations.

In the mid-18^{th} century, immunology evolved as the study of mechanisms of protection against infection—mechanisms of immunity—with the

development of inoculation and vaccination as measures for preventing smallpox. The realization that an infectious disease could be prevented by such methods inaugurated a program of research that culminated with the work of Louis Pasteur and the generation of vaccines for many infectious diseases including fowl cholera, anthrax, and rabies. This early research, with its close connection to clinical practice, was followed by a period of intense basic research along two different but complementary lines: cellular components and molecular units, both probing the mechanisms underlying immune function. Indeed, the recent interest in human immunology suggests that immunology is returning to its historical and clinical roots.

For much of the last 50 years, immunology has focused on achieving a comprehensive mechanistic understanding of the molecular basis of immunity, aided by technological advances such as recombinant DNA and the application of molecular biology in developing and characterizing novel *in vitro* and *in vivo* models of immune function. Indeed, success in identifying molecular and cellular mechanisms of immunity has given immunology a high profile within medicine, offering potential mechanistic explanations for particular diseases and suggesting possibilities for therapeutic intervention and vaccine development. As a result, the discipline has grown beyond its descriptive focus of immunity [18] and now encompasses the study of disorders resulting from the loss of immune protection (e.g., primary and secondary immunodeficiency states), disorders associated with abnormal targeting of such mechanisms (e.g., allergy and autoimmune diseases), and therapeutic modulation of immune mechanisms (e.g., transplantation immunology and cancer immunotherapy). With this extended framework, immunology now reaches across disciplinary boundaries, demanding the integration of multiple disciplines including cell biology, biochemistry, anatomy, genetics, developmental biology, microbiology and infectious diseases, and epidemiology.

3. Complexity of the human immune system

Exploration of the conceptual landscape of immunology, its history, and its biological background suggests that the immune system is a Complex Adaptive System (CAS) without an overall master regulator. It is not a simple aggregate of the properties of the immune systems' components taken in isolation, but the result of complex and ongoing interactions between organisms and their environments. The immune system has emerged from the interactions among a large number of tightly integrated components in such a way as to enable the system to respond flexibly to a changing environment. In general, the immunity is modified in response to environmental stimuli of a different sort: the properties of certain kinds of molecules and antigens (e.g. vaccines) to which the organism is exposed *via* its immune receptors [19-21]. Ultimately, the primary selective pressure favouring the development of immunity is the need to resist infection [22].

Accumulating evidence shows that the history of antigen exposure, including exposure to microbiota residing in or on body sites, systematically shapes the development and function of an individual's immune system. Although immunological memory remains poorly understood at the cellular and molecular levels, some of its functional repercussions are well documented. We know for example that the immune system constantly builds a pool of memory lymphocytes based on microbial exposure and on immune interventions such as vaccines. Since the capacity of an organism to respond to pathogens depends on the repertoire of clones able to respond to antigens derived from those pathogens, the immune response depends on an individual's history of pathogen exposure. Thus, the physiology of an immune system depends not only on the present environment that it inhabits, but also on its history — both its individual developmental history and the evolutionary history of the population that it belongs to [23]. A further challenge to human immunology research results from the dynamic character of a CAS as it responds to a changing environment. The importance of both individual and evolutionary history of immune function implies primacy for the study of human immune systems *in vivo*.

4. Normal human immune system

In light of the immune system's complex adaptive structure, it is important to develop conceptual

and investigative tools for studying the dynamic nature of the intact human immune system in its natural environment. In this context immunoprofiling offers a valuable tool to define the normal and an altered immune system and what "normal" should mean in the context of an immune system [16]. As Georges Canguilhem argued [24], the concepts of normality and pathology are essential to medicine and can be comprehended only in its context. The task of defining normality in a system such as the immune system is particularly challenging given its dynamic nature and the biological, social and environmental factors that may alter the status of an apparently normal or otherwise "healthy" immune system. Yet providing a precise specification of the normal immune system is an important step towards the definition of immune health determinants.

We need to understand two basic questions about the human immune system. First, what is a normal human immune system and how does it develop and decline with age? Second, how do disease states impact the immune system? Answer to these questions are prolegomena to expanding the horizon of the human immunology research and enhancing its impact on medical practice [25-27].

We believe that this can be done in a way analogous to the identification of individual and social risk factors. The complex adaptive structure of the immune system implies that what counts as normal will change from one environment to another, and indeed from one life-history to another even within the same environment. Thus, multiple alternative normal states for the immune system may be possible, depending on the environments (natural and cultural) in which it develops. In light of this historical and context sensitivity, we define a normal immune system as one that provides adequate immunity for a specific environment. This definition has important empirical and clinical ramifications. If we accept that what is "normal" should be determined by what is "adequate for the organism's health", then the induction of immune responses by vaccination or the presence of ongoing immune responses to environmental antigens or certain microbes should be viewed as compatible with normality. Our definition encompasses exposure to pathogens as a requirement for normal development of the immune system or to build normal immunity to minor frequent infections such as chickenpox and the common cold. Another implication of our definition is that one can discover different manifestations of immune system normality in a given context if there are diverse, yet adequate, responses to a given stimulus.

5. Immunoprofiling and human health

Immunoprofiling of cells in the normal immune system and their alteration during the course of disease progression or clinical treatment has become a cornerstone of immunotherapy. The selective use of biomarkers to simultaneously and rapidly measure distinct immune cell populations is critical for immunophenotyping and its application to disease monitoring and patient care [28, 29]. In this context we discuss the use of immunoprofiling in several health-related areas such as vaccination, aging, autoimmunity and chronic inflammatory diseases.

5.1. Immunophenotypic readout of immune responses to vaccination

In the last few years immune profiling has been successfully used in tracking immune responses in humans using systems biology approaches [17]. Using this approach, researchers have been able to identify early immune biomarkers that predict successful immune responses in humans vaccinated with the yellow fever vaccine [30], and provided correlates of successful vaccination with over 90% accuracy, thus measuring early vaccine efficiency. These studies have pointed to the development of rapid tests for determining whether a person can respond effectively to a vaccine and define the utility of phenotyping of immune cells in predicting vaccine efficacy [31, 32].

5.2. Age-related immunophenotypic changes

It is known that aging humans undergo dramatic changes in their immune system along with an increased susceptibility to infections and chronic diseases including cancer. Conventional approaches have identified several underlying factors such as reduced $CD8^+$ T cells and impaired dendritic cell function in the elderly. Similar changes are observed in patients with chronic diseases and cancer. However, given the diversity of the human population it is necessary to develop more universally applicable biomarkers that can be used to monitor the immune system and correlate to

disease risk. Immunoprofiling thus becomes an important platform for monitoring the age-related changes that occur in the human immune system [33]. Immunoprofiling done over 9 years in a human cohort gave an immune aging (IMM-AGE) score based on 33 cellular subsets, including $CD8^+$ T cell, monocytes, natural killer (NK) cells, B cells, and $CD4^+$ T cell subsets. [34]. This score, which enables tracking immune age in real time, was related to genetics, environmental and previous exposure to pathogens. Similarly, individual cytokine response was more significantly associated with the IMM-AGE score than with actual age of the subjects. This study suggests that the immune cell variation between individuals was greater than that within an individual over time. The high inter-individual immune cell variability is dependent on their baseline value. Moreover, there was significant immune-system dynamics in older subjects as compared to its stability in the younger subjects. It appears that immune cell homeostasis of young and older adults differs but over time they converge and move towards the adult phenotype as defined by an increased pool of memory lymphocytes with age [34].

5.2.1. Cardiovascular diseases and immunophenotyping changes

Recently Alpert *et al*. [34] explored a link between inflammation and cardiovascular disease using immunoprofiling. This is a powerful example of the application of immunoprofiling to monitor chronic diseases that develop with age [34]. For this study, the authors explored the IMM-AGE score in the famous Framingham Heart Study cohort [35] and found that this score was strongly associated with cardiovascular disease. Therefore, IMM-AGE score can be used as a risk factor for the development of cardiovascular disease and appropriate subjects could therefore be potentially enrolled in clinical trials based upon their IMM-AGE score. Thus, immunoprofiling could serve as a useful tool in chronic disease prevention studies. There is a need to expand these studies to other chronic human diseases as well as in animal models to confirm their validity.

5.2.2. Age related mortality and immunophenotyping changes

Aging alters most physiological responses in a dramatic fashion, including the immune system. Although age-related changes in DNA methylation is a good predictor of overall mortality, recent studies [34] suggest that IMM-AGE score can predict mortality in older adults better than epigenetic clock data or chronological age. Using immunophenotyping the authors found changes in immune cells from their baseline values over a 9-year period in 135 subjects selected from the Framingham Heart studies [35]. These studies suggest that in older adults, the immune systems change over time and the IMM-AGE score can predict overall survival independent of age, gender, and cardiovascular diseases [34].

5.3. Immunophenotyping in cancer therapy

Immune cells are known to infiltrate tumors and there is good evidence that immunoprofiling can be used to predict a patient's anti-tumor response in cancer therapy [36]. Tumor infiltrating immune cells represent an important determinant of clinical responses in immunotherapy. The monitoring of anti-tumor immune responses by immunoprofiling during treatment with checkpoint inhibitor drugs such as anti-CTLA-4 and anti-PD-1 antibodies has become an essential tool in immunotherapy [37, 38]. This is important because only about 20%-30% cancer patients respond to checkpoint immunotherapy [37] and in some cases, it may even promote tumor growth [39]. Therefore, immunoprofiling of immune cells may be critical to determine their efficacy in cancer immunotherapy.

5.4. Immunophenotyping in autoimmune diseases

Autoimmune diseases are caused by the cells of the immune system. Monitoring and treating these diseases require a clear understanding of the type and function of immune cells involved in a particular disease. Significant progress has been made to determine the involvement of various immune cells in autoimmune diseases such as diabetes, lupus, arthritis, inflammatory bowel disease and multiple sclerosis [40]. However, the use of immunotherapy in autoimmune diseases remains a major challenge in clinical medicine due to the paucity of effective treatments to inhibit or regulate autoreactive effector immune cells in the disease process. Immunoprofiling of these cells is likely to facilitate development of new immunotherapies for autoimmune diseases. The use of biomarkers to define these immune cells has also become an important diagnostic step

in the classification and monitoring of the clinical course of an autoimmune disease [41, 42]. Use of immunophenotyping to monitor various mononuclear cell subsets, including memory B cells, effector T cells, and dendritic cells in autoimmune disease is likely to become a precision medicine tool in the diagnosis and follow-up of patients with autoimmune diseases.

Type 1 diabetes (T1D) is characterized by a change in the frequency and phenotype of immune cells during the development of the disease [43]. This is associated with humoral immune responses to islet b-cell autoantigens—particularly insulin, glutamic acid decarboxylase (GAD), islet antigen-2 (IA-2), and zinc transporter 8 (ZnT8). The titer of autoantibodies to these antigens in T1D increases with time. The immunophenotyping in this case is characterized by a more intense humoral autoimmune response to islet autoantigens. Patients with T1D also exhibited multiple immunophenotypic abnormalities in circulating B cells compared to healthy controls [44]. This is associated with decreased percentages of Fas receptor- positive mature B cells. Immunophenotypic analysis has shown that both CD4 and CD8 T cells are involved in T1D. These cells are kept in check by regulatory T (Treg) cells whose diminished Treg cell function appears to be involved in T1D development [45, 46]. It is, therefore, going to be critical to monitor the frequency of Treg cells in T1D patients using immunoprofiling in patients. The role of dendritic cells is also important in T1D as they influence the activation and function of T cells that are involved in the induction and progression of disease. In our studies [47], immunoprofiling of dendritic cells was done using 4-color flow cytometry of whole blood cells from type 1 and type 2 diabetic patients and control subjects. It was found that dendritic cell frequency in the diabetic state did not differ from nondiabetic control subjects but they were poor producers of IFN-α which may influence disease development.

Rheumatoid arthritis (RA) is a chronic autoimmune disease. In this disease, immunophenotyping has revealed a link between HLA-DRB1 and CXCR4 expression on memory $CD4^+$ T cells that are involved in the disease process [48]. In this study, the authors analyzed HLA-$DRB1^+$ RA patients by 24-subset immunophenotyping on peripheral blood mononuclear cells using flow cytometry. They found that the frequency of memory $CXCR4^+CD4^+$ T cells is linked to the expression of HLA-DR on B cells. Moreover, memory $CXCR4^+CD4^+$ T cells serves as an important biomarker for linkage between HLA-DRB1 genotype and disease activity in RA.

Immunophenotyping has also revealed that patients with systemic lupus erythematosus (SLE or lupus) can be classified into three subgroups based on the heterogeneity of T cells involved in the disease [49]. The level of Treg and follicular helper T (Tfh) cells were higher in SLE patients than in healthy controls. Cluster analysis from this study has shown that patients whose SLE was resistant to treatment was highest among the Tfh-dominant group.

Immunotherapy of multiple sclerosis (MS) has been monitored by immunoprofiling of immune cells in patients treated with interferon-β (IFN-β) and fingolimod. The study revealed significant alterations in their B cell subsets and an increase in B cell-activating factor (BAFF) following therapy [50]. This was unexpected as previous studies primarily focused on the role of T cells and not on B cells in MS [51]. Similar results were obtained in MS patients treated with anti-CD20 monoclonal antibodies that targeted B cells and modulated disease [52].

In Inflammatory Bowel Disease (IBD), a disease in which gut microbiota plays an important role, there appears to be a strong link between T cell subsets and disease induction as determined by immune profiling of T cells in the intestine [53, 54]. This study explored changes in intestinal T cells and found increased level of $CD4^+$ T cells, Tregs, and resident memory T (TRM) cells, and lower levels of $CD8^+$ T cells and $CD103^+$ T cells in patients compared to the controls. This suggests that the baseline level of $CD4^+$ Treg cells in IBD patients was strongly associated with IBD progression.

6. Significance of immunoprofiling data in health and disease management

The immune system impacts almost every disease in humans and immunophenotyping has potential to characterize individual's immune health by analysing the number of different types of

immune cells during the course of chronic diseases, vaccination or cell and organ transplantation. Healthcare providers can use immunophenotyping information not only to determine appropriate patient care, including better timing for clinical interventions, but also for managing personnel health and safety (e.g., by designing adequate vaccination programs for healthcare workers or other first responders during an infectious disease outbreak). The study by Alpert *et al*. [34] suggests that various clinical studies and drug trials for chronic and inflammatory diseases could benefit from the immune profiling studies using the patients' peripheral blood cells combined with genetic biomarkers. Chronic inflammatory immune-mediated diseases are a growing health burden around the globe and are affecting millions of people. These diseases pose a major challenge to healthcare providers and to all levels of governments as they require significant investments for treatment and prevention. A major research effort is needed to develop better understanding of the human immune system to harness the potential of the immune-mediated therapies and vaccines. The payoffs are likely to be very significant as is already evident from the new cancer immunotherapies where phenotyping of immune cells has become an essential step in monitoring disease remission. The human immunoprofiling platforms are likely to play an important role in both developing new knowledge of the complex human immune system and translation of the results into effective therapies (broad and personalized) to treat various clinical conditions.

Apart from public policy implications there are significant ethical challenges that impose a bias towards the use of experimental systems that make ex *vivo* use of human material (either from healthy volunteers or from patients) on which manipulations and perturbations can be freely applied. These concerns will need to be addressed as we develop powerful new large data sets of patient immune cells for specific therapy and for translation of their products for commercial clinical application.

7. Conclusion

The immune system is a Complex Adaptive System (CAS) which is strongly influenced by its ongoing interaction with the environment. Recent immunoprofiling studies outlined above have provide fascinating new insight into the human immune system and its promising role in health and disease. It is imperative to translate new basic biomedical research findings relating to the human immune system into clinical applications and outcomes. We demonstrated in this review that immunoprofiling offers an important new platform for analyzing immune cells in the evolution of human immunology as it emerges from the shadows of mouse centric studies [16]. Immunoprofiling studies outlined above point to the new opportunities that the area of human immunology research offers to clinical medicine going forward.

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CONFLICT OF INTEREST STATEMENT

CW receives consulting income from Eli Lilly & Company, Canada. The other authors have no conflicts of interest to declare.

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