Next-Generation Immune Repertoire Sequencing as a Clue to Elucidate the Landscape of Immune Modulation by Host–Gut Microbiome Interactions

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The human immune system is a fine network consisting of the innumerable numbers of functional cells that balance the immunity and tolerance against various endogenous and environmental challenges. Although advances in modern immunology have revealed a role of many unique immune cell subsets, technologies that enable us to capture the whole landscape of immune responses against specific antigens have been not available to date. Acquired immunity against various microorganisms including host microbiome is principally founded on T cell and B cell populations, each of which expresses antigen-specific receptors that define a unique clonotype. Over the past several years, high-throughput next-generation sequencing has been developed as a powerful tool to profile T- and B-cell receptor repertoires in a given individual at the single-cell level. Sophisticated immuno-bioinformatic analyses by use of this innovative methodology have already been implemented in clinical development of antibody engineering, vaccine design, and cellular immunotherapy. In this article, we aim to discuss the possible application of high-throughput immune receptor sequencing in the field of nutritional and intestinal immunology. Although there are still unsolved caveats, this emerging technology combined with single-cell transcriptomics/proteomics provides a critical tool to unveil the previously unrecognized principle of host–microbiome immune homeostasis. Accumulation of such knowledge will lead to the development of effective ways for personalized immune modulation through deeper understanding of the mechanisms by which the intestinal environment affects our immune ecosystem.

Keywords: next-generation immune repertoire sequencing, B-cell receptors, T-cell receptors, single-cell transcriptomics, human microbiome

INTRODUCTION

The jawed vertebrates have evolutionally acquired a unique immune system consisted of effector and regulator cells that can effectively respond or establish tolerance to millions of endoneous and environmental antigens in an epitope-specific manner (1). In this paradigm of the “adaptive” immune system, it is firmly believed that pre-existing repertoire of two types of lymphohematopoietic cells,
classic T cells and B cells, predominantly determine the mode and pattern of immune responses in a given individual. At the single-cell level, these cells express a single type of unique antigen-specific receptors: T-cell receptor (TCR) for T cells and B-cell receptor/immunoglobulin (BCR/Ig) for B cells that define a phenotypic clone or a clonotype of these immune cells. At the individual level, the estimated number of TCR and BCR/Ig clonotypes is from a few thousands to more than billions depending on the species of animal, which is believed to form the basis of the host ability to cope with innumerable immunologic threats. It is well-characterized that such huge diversity of antigen-specific receptors is created through somatic rearrangement of variable (V), diversity (D), and joining (J) (or V and J) gene segments located in TCR- or BCR/Ig-encoding loci and the concomitant incorporation of random nucleotide insertions and deletions. However, the underlying mechanism by which these repertoires are ontogenetically developed and shaped is largely unknown. Furthermore, until recently, there had been no available technologies that comprehensively identify each of TCR and BCR/Ig clonotypes constituting the whole adaptive immune cell repertoire.

During the past few decades, accumulative lines of evidence have indicated that the host microbiome and nutrients not only play key roles for balancing the host immunity in health and disease but have a tremendous influence on the generation and shaping of immune cell repertoire (2, 3). For instance, several groups have reported pivotal observations that alloimmune-mediated graft-versus-host reactions in the setting of hematopoietic cell transplantation and immune responses against malignant neoplasms triggered by immune checkpoint inhibition are associated with the abundance of distinct members of intestinal commensal flora (4–8). In this article, we highlight the recent advancement in high-throughput immune repertoire analysis by next-generation sequencing (NGS) and its possible application in future studies to elucidate previously unrecognized mechanisms of immune modulation by gut microbiota and oral nutrition.

ROLES OF GUT MICROBIOME IN SYSTEMIC HOMEOSTASIS OF ADAPTIVE IMMUNE REPERTOIRE

Gastrointestinal Tract as a Key Site for Systemic Immune Modulation

The gastrointestinal tract-associated lymphoid tissue is the largest immune compartment in the body. Therefore, it is quite reasonable to assume that the gut microbiome has a strong influence on the development and homeostasis of adaptive immune repertoire. In fact, the intestinal epithelium is an important anatomical site for the active interaction of the gut microbiome and various immune cells including antigen-presenting dendritic cells (2, 3, 9). For instance, the induction of gut-resident Foxp3+ regulatory T cells (Tregs), a key modulator of immune responses against dietary antigens and gastrointestinal commensal flora, has been shown to be causally dependent on the colonization of certain Clostridia that abundantly produce short-chain fatty acids (10, 11). Among those gut microbiota-derived short-chain fatty acids, butyrate is found to be a key factor for maintaining the integrity of CD326+ intestinal epithelial cells and mitigating graft-versus-host disease in a murine model of allogenic hematopoietic cell transplantation (4). However, homeostatic maintenance of intestinal Tregs appears to require not only the indigenous Clostridia species but flexible diversity of the host TCR repertoire. Transgenic mice genetically engineered to express a restricted TCRβ repertoire spontaneously developed severe colitis in association with hyperactivation of T helper 17 cells (Th17) and a striking decrease in a special subset of peripherally derived Tregs responsible for the recognition of intestinal microbiota (12). These “limited mice” showed no apparent alteration in the composition of commensal flora including segmented filamentous bacteria, a well-known inducer of Th17 cells in the small intestine in mice. Additionally, colonic inflammation observed in these mice is ameliorated by “total gut decontamination” by use of antibiotics cocktail, suggesting that TCR epitopes of effector Th17 cells are originated from gut microbiota rather than “self” antigens associated with autoimmunity. In this context, it is critically important to note that the use of broad-spectrum antibiotics disrupting anaerobic flora increases the risk for severe colonic graft-versus-host disease after allogenic hematopoietic cell transplantation in human patients as well as in mice models (5). Importantly, the colon lamina propria of carbapenem antibiotic-treated mice is characterized by high local levels of IL-23 and accumulation of effector CD4+ T cells concomitantly with reduced colonization of Clostridia and increased abundance of Akkermansia muciniphila, a unique bacterium that disrupts the intestinal epithelium junction by degrading luminal mucins as a source of carbohydrates and nitrogen.

More surprisingly, ongoing studies in patients with cancer highlight the crucial impact of gut microbiota on immune checkpoint immunotherapies using antibodies against programmed cell death protein 1 (PD-1) and its ligand (7, 8). Analysis of fecal samples from melanoma patients treated with anti-PD-1 immunotherapy revealed that the abundance of Ruminococcaceae in fecal microbiota is an indicator for good clinical responses, whereas that of Bacteroidales is a negative predictor (7). Another study including patients with advanced cancers showed that the prior use of antibiotics significantly compromised the clinical benefit of immune checkpoint inhibition, while the dominant gastrointestinal colonization of A. muciniphila was positively correlated with better responses after PD-1-based immunotherapy (8). Notably, A. muciniphila has been also shown to be associated with the development of autoimmunity against the central nervous system such as multiple sclerosis (13, 14), suggesting a unique immunodominant role of this particular mucin-degrading anaerobic microorganism.

It is also well known that “the first microbial gut colonizers” play an essential role for the development and shaping of the early immune system in neonates and infants (15). For instance, perinatal exposure to the Bisphenol A, a chemical found in daily consumed plastics such as the coating of food and drink packages, results in reduced frequencies of Th1/Th 17 cells in the intestinal mucosa and subsequently leads to an altered glucose sensitivity, a defective IgA secretion and a fall of Bifidobacteria in a mice model (16). The importance of “early colonizers” warrants the
have shown improved sensitivity compared with conventional hematopoietic cell transplantation (26, 27). These applications of human T-cell or B-cell neoplasms after chemotherapy or is a clinical analysis for quantitating minimal residual disease and is probably beginning to change our immune dysregulation (20–25). The introduction of this innovative approach has so far had a huge impact on basic and clinical immuno researches and is probably beginning to change our understanding of the immune system as a whole.

The most established use of immune repertoire deep sequencing is a clinical analysis for quantitating minimal residual disease of human T-cell or B-cell neoplasms after chemotherapy or hematopoietic cell transplantation (26, 27). These applications have shown improved sensitivity compared with conventional assays, such as CDR3-specific PCR and multicolor flow cytometry, thus will be useful for bedside decision-making of hematologic clinicians. Moreover, BCR/Ig-seq can be used for in vivo tracing of B cell dynamics after vaccination and cost-effective monoclonal antibody engineering by shortcuts of labor-intensive screening procedures (28).

In its simplest form, TCR-seq and BCR/Ig-seq comprise of three essential working processes: (i) PCR amplification of V-D-J for TRB, TRD and IgH or V-J for TRA, TRG, and IgL gene segments, (ii) massively parallel sequencing of the PCR amplicons, and (iii) alignment of NGS reads by use of sophisticated bioinformatic technologies. However, many technical caveats still exist in these approaches (29, 30). For example, limited sampling from peripheral blood or particular tissues/organs always raises the problem of “unseen clones.” In addition, the possibility of sequencing errors and amplification bias is theoretically unavoidable because it is inherent in PCR-based NGS platforms and methods for NGS library preparation. Also, the selection of the starting material, DNA or RNA, also significantly affects the quality of immune repertoire analyses. DNA-based approaches have an advantage in terms of sample preparation and storage but require complex PCRs using a multiplexed set of V and J segment-specific primers with the large reaction size because the template sequence for each TCR or BCR subunit loci exists as only single copy per cell. In contrast, RNA-based analyses, most commonly by using 5’-rapid amplification of cDNA ends, are capable of more comprehensive coverage and relatively unbiased amplification of the intended cDNA templates with a single pair of primers at the cost of the drawback that the read number of target amplicons is influenced by cell-to-cell variation in TCR or BCR mRNA expression levels. Finally, more serious limitation of these methodologies is a difficulty in exact pairing of variable region information (α and β units for αβTCR, γ and δ units for γδTCR, and immunoglobulin heavy and light chains for BCR/Ig) that determines antigen/epitope specificity of each clonotype.

To overcome these impediments, our group has developed a novel high-throughput TCR repertoire sequencing method that combines RNA-based NGS and single-cell multiplex reverse transcriptase PCR assays for profiling TCR clonotypes with information regarding the CDR3 sequences of paired TCRα and TCRβ subunits (31–33). To perform relatively unbiased parallel sequencing, we installed adaptor ligation-mediated PCR for NGS library construction. With the help of this technology, we could comprehensively identify cytomegalovirus (CMV) pp65 antigen-specific paired TCR clonotypes of peripheral blood T cells obtained from HLA-A*02-positive healthy individuals. We found that HLA-A*02-restricted CMVpp65-specific CD8+ T-cells were extremely oligoclonal and consisted of a single or a few superdominant clones. When transduced into TCR-null Jurkat cells engineered to lack endogenous TCR by CRISPR-Cas9 system, these superdominant TCRs showed significantly higher affinities to HLA-A*02/CMVpp65 tetramers compared with other minor TCR clonotypes. Notably, such dominant TCR clonotypes were highly shared among different individuals and more enriched in stem memory T cells than in the central memory or effector memory T cell subpopulations. These observations may suggest that stem memory T-cell subset is a reservoir of highly functional and highly shared T cells responsible for protective immunity against chronically infected pathogens. Similarly, several studies using NGS-based TCR repertoire analysis have revealed that the sharing of TCR clonotypes among different individuals is a common phenomenon at least in rodents and humans (20, 34, 35). It is worthy of note that the TCR repertoire of zebrafish (Danio rerio), consisted of only a few thousands TCRα and TCRβ clonotypes per individual, also contains such “shared” fractions (Yasuko Honjo, Hiroyuki Sato, and Tatsuo Ichinohe, unpublished observations).

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Any opinions, findings, and conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the National Science Foundation.
that the vast majority of negatively selected TCRs are autoreactive and endowed with crossreactivities against multiple MHC haplotypes, while crossreactive TCRs are very infrequent among preselection TCRs (48). In this context, it is very intriguing that an excellent in silico study using proteome datasets has reported the extensive sharing of possible T-cell exposed peptide motifs between human proteome and gastrointestinal microbiome (49). Very recently, T cell epitopes of an integrase expressed by several species of Bacteroides were shown to be a mimotope of an established pancreatic β cell autoantigen in non-obese diabeticogenic mice (50). Monocolonization studies using integrase-transgenic Bacteroides in germ-free mice demonstrated that the recruitment of diabetogenic CD8+ T cells in a microbial epitope-dependent manner. Surprisingly, these crossreactive T cells consistently express an invariant TCRα chain and function to protect the host mice from experimental colitis, suggesting that TCR repertoire of effector and regulatory T cells might be inherently crossreactive. Accordingly, the classical dichotomy of immunologic “self” and “non-self” should be revised and redefined because it is difficult to clearly distinguish “genetic self” and “non-genetic/microbial self” (51). NGS-based high-throughput TCR repertoire analysis will confer a more clarified overview of the origin and composition of crossreactive TCR repertoire.

CONCLUSION AND PERSPECTIVES

During the past decade, rapid innovations in genomic and bioinformatic technologies in the interdisciplinary field of microbiology and immunology have radically changed the outlook of human host–microbiome interactions and their influences on human health and disease. In particular, advanced methodologies in high-resolution adaptive immune repertoire analysis will provide an essential clue to obtain deeper understanding of the ontogeny of our immune system with its microbiome fingerprints at the individual level. Given these backgrounds in mind, it is very attractive to identify and trace the members of adaptive immune cell repertoire by single-cell TCR-seq and BCR/Ig-seq in mono- or poly-colonized germ-free animal models. Such studies will reveal the potential full diversity of BCR/Ig-seq in mono- or poly-colonized germ-free animal models. The studies by the authors included in this manuscript were approved by the animal experiment committee and the ethical committee of Hiroshima University.

ETHICS STATEMENT

All authors listed have made substantial contributions to text and have approved the final manuscript for submission.

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Conflict of Interest Statement: RS is the chief executive director of Repertoire Genesis Incorporation. KK and HS are employees of Repertoire Genesis Incorporation. TS is the chief executive director of BITS Co. Ltd. No other authors have relevant conflicts of interest to declare.

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