Analysis of TCR repertoires for tracking and evaluating distinct T cell subsets

Chen Lin

Department of Microbiology and Immunology, Medical College, Jinan University, Guangzhou 510632, China
Correspondence to: Chen Lin. Department of Microbiology and Immunology, Medical College, Jinan University, Guangzhou 510632, China. Email: tlinc@jnu.edu.cn.

In this article published online of Stem cell Investigation 2015, Li *et al*. reviewed recent studies on T-cell receptor (TCR) repertoire diversity recovery in patients with leukemia and autoimmune disease who have received hematopoietic stem cell transplantation (HSCT). TCR repertoire diversity analysis has been a common method for characterizing immune reconstitution after HSCT and analyzing the oligoclonal expansion of T cells during virus infection, graft-versus-leukemia (GVL) effects, and graft-versus-host disease (GVHD) (1).

Immune T cells play not only a crucial role in immune response against viral, bacterial and parasitic infections, but also in the induction of immune responses to some tumours. T cells exist with distinct phenotypic and functional characteristics. The two major T cell subsets are CD4+ helper T cells and CD8+ cytotoxic T cells (CTLs). Both the CD8+ and CD4+ sets use specific TCRs to recognize complexes of peptide (p) bound to major histocompatibility complex (MHC) class I (CD8) or class II (CD4) glycoproteins on the surface of antigen presenting cells or targeted cells. Following ligation to these pMHC complexes, T cells proliferate and differentiate, which initiate the production of cytokines (e.g., IL-2, IFN-γ, and TNF) and cytotoxic effector molecules to eliminate the antigens and, in the longer term, establish immune memory.

We now know that TCRs are similar to antibodies. The TCR includes the α, β, γ and δ chains, which form α/β or γ/δ heterodimeric chains expressed on mature T cell surfaces. T cells with α/β chains are called αβ T cells. A less common type of TCR is composed of TCR γ and δ chains, and these cells are called γδ T cells. Each TCR chain is encoded by multiple gene segments that are joined together during the maturation of T cells in the thymus.

The TCR antigen (Ag)-binding site is formed by three complementarity determining regions (CDRs). CDR1 and CDR2 are encoded by sequences of V genes alone, whereas CDR3 consists of rearranged sequences of V, D and J genes. Junctional diversity plus non-templated addition of N nucleotides generate new sequences that make up the variability in CDR3 regions. Therefore, TCR CDR3 represents the most variable TCR region that contacts the central residues of the bound peptide. Each clone of T cells expresses antigen receptors with a single specificity, thus, the antigen receptors of T cells are clonally distributed in a normal individual. The fundamental determinant of T cell diversity is the clonotypic TCR, which clonotype is defined by the CDR3 profiles within a particular TCRVβ (or TCRVα) testing profile. The clonotypic approach can be used to both track and define functional subsets within phenotypically distinct T cell populations.

The TCR repertoire analysis technique was established in different ways, including the determination of DNA sequences or using polymerase chain reaction (PCR)-based CDR3 size spectratyping approach. Although the spectratyping technique has been used extensively to measure CDR3 length since the early 1990s (1-5), the early TCR repertoire analyses did not specifically target pMHC (epitope)-specific T cells because of performing on total lymphocyte populations recovered during the course of immune responses. Lim *et al*. developed a procedure for detecting and monitoring T cell responses by combining T cell sorting using HLA-peptide complexes multimerized on magnetic beads together with the quantitative immunoscope approach (6). It was then possible to use the spectratyping approach with sorted, epitope-specific T cells. Shortfalls of this method are that the specific T cells was undetected

when the frequency of these T cells was too low and/or that specific T cells did not transcribe a significant amount of TCR β chain transcript.

An alternative approach is to sort single tetramer + Vβ + CD8⁺ T cells into the wells of 96-well plates, followed by cDNA synthesis and nested PCR to amplify the Vβ regions of interest. Kedzierska *et al*. has used this protocol to analyze the clonal characteristics of epitope-specific T cells. They showed that the real advantage of single-cell RT-PCR over bulk PCR analysis is for studies analyzing low-frequency T cell populations, such as distinct subsets of memory T cells (7).

Recently, novel techniques were developed to quantify TCR diversity, enabling quantification of T cell diversity at unprecedented resolution. van Heijst *et al*. combined 5' rapid amplification of complementary DNA ends PCR with deep sequencing to quantify TCR diversity in 28 recipients of allo-HSCT using a single oligonucleotide pair (8) and Woodsworth *et al*. described that high-throughput TCR sequencing (TCR-seq) using next generation sequencing platforms generate large numbers of short DNA sequences covering key regions of the TCR coding sequence. Although advances in next generation sequencing (NGS) technologies have been transformative for immune repertoire analysis, there are some limitations on TCR-seq studies. For example, many of these studies show disparities in TCR repertoire diversity among different groups of patients. The reason may be explained by HLA restriction. This requires investigators to make interpretations and decisions regarding where to set thresholds during data analysis (9).

In conclusion, analysis of TCR repertoires could characterize the features of the host T cell immune status in patients and normal individuals. In particularly, dynamic analysis of the TCR repertoires of T cells is valuable for estimating the immune reconstitution in patients after HSCT. At present, the different TCR analysis technology is available to evaluate the immune reconstitution in patients with different clinical situations. For improved immunotherapy protocols, a better understanding of the main determinants, consequences and constraints governing TCR repertoire diversity is not only important at the clonal level in T cells, but much more valuable in phenotypically distinct T cell populations.

Acknowledgements

Footnote

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