Past and Future of the Molecular Characterization of the T Cell Repertoire: Some Highlights of Eli Sercarz's Contributions

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ABSTRACT: The contribution of Eli E. Sercarz to immunology and immunopathology has been remarkable and achieved many milestones in the understanding of the processes of the mechanisms fine-tuning immune responses. A part of his work was dedicated to the study of the deep complexity of the lymphocyte T cell repertoire and its importance during the physiologic development and disease, such as clonal heterogeneity of T cell responses. Starting from these studies, under his mentoring, we had the opportunity to implement the spectratyping method and apply it to human and experimental autoimmune diseases, obtaining intriguing results. The open question of this brief review is the possible role of this fine and complex technique, the immunoscope analysis, in the era of the big data and omics.

KEY WORDS: immunoscope, spectratyping, T cell receptor, repertoire, antigen specificity, next-generation sequencing

ABBREVIATIONS: CDR3, complementarity-determining region 3; **NGS**, next-generation sequencing; **TCR**, T cell receptor

I. INTRODUCTION

One of the main topics to which Eli Sercarz has given a profound contribution has been the study of the T cell repertoire, pioneering both methods and results achieved by the scientific community, sometimes many years later. Eli Sercarz was among the earliest researchers to apply spectratyping in human and experimental pathology. His work dissected the deep complexity of the lymphocyte T cell repertoire and its role during its physiologic development and in disease.[1] His main focus was the implications of T cell receptor recognition in tolerance mechanisms,[2,3] providing new insights into autoimmune processes such as clonal heterogeneity of T cell responses,[4,5] epitope spreading[6,7] and molecular mimicry.[8]

Here we will highlight Eli Sercarz's introduction of spectratyping, contributing to the deepening of molecular characterization of the T cell repertoire through immunoscope analysis[9] and applying it, in more than a decade of studies, to human[10] and experimental[3,11,12] autoimmune disorders, infectious diseases,[13] and cancer.[14,15] His contribution in the field of basic immunology and immunopathology increased knowledge and allowed a better understanding of T cell response through the study of specificity.

II. TCR DIVERSITY

T cells play an important role in immune response through stimulation of the TCR, a surface receptor responsible for recognizing endogenous and exogenous antigens. Most TCRs combine one α with a β chain.[16] The diversity of TCRs originates during early lymphoid differentiation from the random combination of variable (V) and joining (J) gene segments in the α-chain and V, diversity (D), and J gene segments in the β-chain germline gene. Specifically, random recombination of β chain gene segments generates the so-called complementarity determining region 3 (CDR3), which represents the loop with the most sequence diversity of the αβ TCR and accounts for the specificity and diversity of the receptor, allowing T cells to recognize many different antigens.[17]

III. IMMUNOSCOPE

Immunoscope, also known as CDR3 BV-BJ spectratyping, has been widely used to analyze CDR3 length polymorphisms.[18] This strategy is based on PCR technology that, through use of complementary constant (C), V, and J primers amplifies target cDNA corresponding to various rearranged transcripts with different CDR3 lengths from specific TCR V genes. Specifically, cDNA is subjected to PCR amplification using a common C b primer in combination with V primers.[19] V-C amplicons obtained from PCR are used as templates for subsequent run-off reactions, performed with a single-primer reaction for each J segment. After denaturation these products are resolved through capillary electrophoresis. Finally, analysis of spectratyping data is achieved by evaluation of the area and shape of CDR3 profile peaks to establish the degree of skewing and oligoclonality.[20]

IV. IMMUNOSCOPE IN OUR EXPERIENCE

Under the mentoring of Eli Sercarz, we first applied immunoscope in deep molecular characterization of the TCR repertoire in an experimental mouse model (SJL strain), detecting several myelin-specific (myelin-derived peptide, $PLP_{139-151}$) public clonotypes and examining their contribution to pre-immune and post-immune CD4+ and CD8+ responses.[12,18,21] These observations were the first to hint that pathogenic and nonpathogenic T cell repertoires specific to the same self-antigen could be different in their TCR use. Through the translation of these results to human pathology, we were later able to identify collagen-specific T cells involved in the pathogenesis of rheumatoid arthritis, distinct from those present in healthy controls, that are detected during active phases.[22] In addition, the presence of this pathogenic repertoire behaved as an early biomarker of responsiveness to treatment,[23] leading to the potential use TCR-repertoire analysis in the personalization of therapy.[24,25] Similar results have also been obtained in myasthenia gravis[26] and multiple sclerosis (manuscript in preparation). These observations together may form the basis for future use of methods able to evaluate directly the T cell compartment in the clinical assessment of immune responsiveness in autoimmunity and cancer immunotherapy. In fact, we also applied the immunoscope method in a mouse model of cancer, where we were able to show that Treg cell depletion combined with anti-rErbb2 vaccine blocks tumor progression by expanding latent pools of low-avidity CD8+ T cells characterized by a specific antitumoral TCR repertoire, while the dominant, high-avidity CD8+ repertoire is depleted by central tolerance.[19,27]

In a rather unexpected turn, analysis of the TCR repertoire has led us to examine the ability of the infectious environment to modify directly T cell trafficking ability[28] including the ability to infiltrate target organs and promote self-specific proinflammatory polarization as well as expansion of ag-specific FoxP3+ Tregs.[29]

V. IMMUNOSCOPE VERSUS NEXT-GENERATION SEQUENCING

Positive aspects of immunoscope include no requirement for cloning before sequencing, ability to conduct multiple reactions in one lane, and performing sequence reading using specific primers for each reaction. Finally, the single capillary instrument allows automatization of all steps of sequencing.[30] However, although this technique is still used in studying the TCR repertoire, it has important drawbacks. The main constraints are the lack of a full-repertoire perspective, the limited number of TCR sequences,[17] and the potential loss of clonal aberrations or false-positive results, besides unavoidable bias due to the kinetics of hybridization during PCR amplification among primers and target *TR* genes.

More sensitive approaches have recently been developed to investigate immune repertoires using next-generation sequencing (NGS), which has improved knowledge of TCR biology and reduced costs and time, achieving unbiased and quantitative amplification of all TCR genes, including unknown variants (Fig. 1). To totally nullify the PCR, we needed to wait for the third and fourth generations of sequencing; however, library construction protocols can introduce bias in sample composition, often elongating sequencing time.[31,32] Nevertheless, the analysis of data obtained is time-consuming and

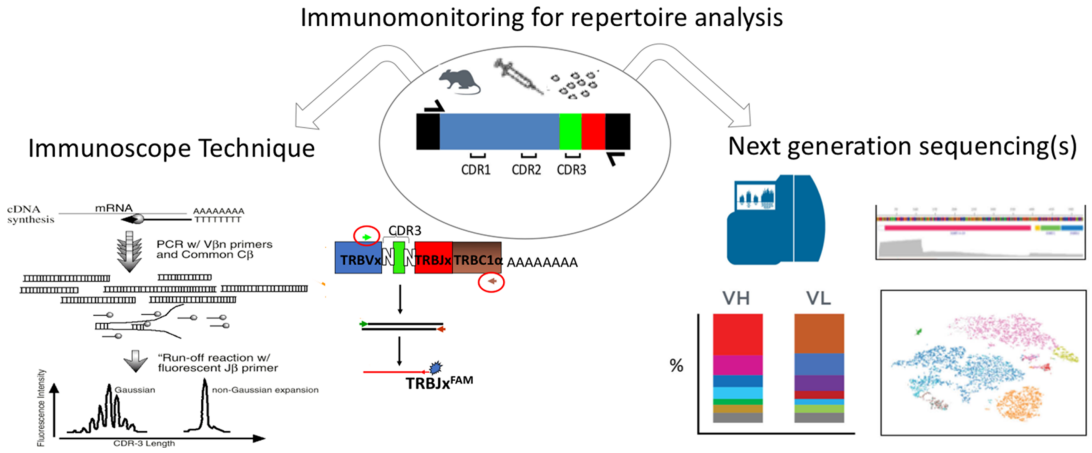

FIG. 1: Molecular chracterizaion of T cell receptor: Immunoscope workflow (left) compared with automatization and detailed outcome of next-generation sequencing methods (right).

TABLE 1: Comparison of immunoscope and next-generation sequencing

Next-generation sequencing method	Cost	Run time	read length	PCR bias	Sensitivity	Library	> 10^6 clones in lane	TCR clones
CDR3 spectratyping	Low	Short	Short	Yes	Low	No	No	Single
NGS I	Medium	Very long	Short	Yes	Low	Yes	Yes	Multiple
NGS II	Medium	Long	Short	Yes	High	Yes	Yes	Multiple
NGS III	High	Short	Long	No	High	Yes	Yes	Multiple
NGS IV	Very high	Short	Long	No	High	Yes	Yes	Multiple

complex, so that thus far it cannot be easily transferred to peripheral blood in a clinical setting.

For these reasons, it is still difficult to define one method as the gold standard, as all of them have advantages and disadvantages (Table 1). CDR3 spectratyping is a good choice when interrogating a limited number of samples with known antigenic targets and when studying single-clone antigen-specific TCR. Despite its drawbacks, it remains a good technique to investigate the TCR repertoire,[17] especially in immune-mediated disorders,[33] considering its affordability and its simple experimental setting and data analysis. A valuable approach may be a first analysis of a limited number of samples by immunoscope, providing the definition of public TCRs that are of clinical interest (e.g., pathogenic or protective clones in autoimmune diseases, proinflammatory versus inhibitory T cell public clones in cancer immunotherapy), followed by focused NGS that may at this point be easily applied to clinical management. Of much interest, when examining patients using the two technologies, we found that the same sequences were highlighted by both (manuscript in preparation). Therefore, in the omics and big data era, immunoscope offers a comprehensive and immediate view of the diversity of the entire T cell compartment and should flank NGS rather than be completely replaced by it.

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