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Research paper

Standardized analysis for the quantification of Vβ CDR3 T-cell receptor diversity

S. Alice Long [a,*], Jahan Khalili [b], Jimiane Ashe [a], Ron Berenson [a], Christophe Ferrand [c], Mark Bonyhadi [a]

[a] *Xcyte Therapies, Inc., Seattle, WA, United States*
[b] *MD Anderson, Houston, TX, United States*
[c] *Laboratoire de Thérapeutique Immuno-Moléculaire, Bensancon Cedex, France*

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Abstract

Assessment of the diversity of the T-cell receptor (TCR) repertoire is often determined by measuring the frequency and distribution of individually rearranged TCRs in a population of T cells. Spectratyping is a common method used to measure TCR repertoire diversity, which examines genetic variation in the third complementarity-determining region (CDR3) region of the TCR Vβ chain using RT-PCR length-distribution analysis. A variety of methods are currently used to analyze spectratype data including subjective visual measures, qualitative counting measures, and semi-quantitative measures that compare the original data to a standard, control data set. Two major limitations exist for most of these approaches: data files become very wieldy and difficult to manage, and current analytic methods generate data which are difficult to compare between laboratories and across different platforms. Here, we introduce a highly efficient method of analysis that is based upon a normal theoretical Gaussian distribution observed in cord blood and recent thymic emigrants. Using this analysis method, we demonstrate that PBMC obtained from patients with various diseases have skewed TCR repertoire profiles. Upon *in vitro* activation with anti-CD3 and anti-CD28 coated beads (Xcyte™ Dynabeads®) TCR diversity was restored. Moreover, changes in the TCR repertoire were dynamic *in vivo*. We demonstrate that use of this streamlined method of analysis in concert with a flexible software package makes quantitative assessment of TCR repertoire diversity straightforward and reproducible, enabling reliable comparisons of diversity values between laboratories and over-time to further collaborative efforts. Analysis of TCR repertoire by such an approach may be valuable in the clinical setting, both for prognostic potential and measuring clinical responses to therapy.

Keywords: T-cell receptor; Spectratyping; T-cell repertoire; Cellular immunity

1. Introduction

One measure of the status of an individual's immune capabilities is the diversity of their T-cell receptor (TCR) repertoire (Nikolich-Zugich et al., 2004). The TCR repertoire originates from naïve T cells that have recently migrated from the thymus as well as a circulating pool of antigen-experienced peripheral T cells. This

Abbreviations: TCR, T-Cell Receptor; PBMC, Peripheral Blood Mononuclear Cells; HIV, Human Immunodeficiency Virus; RA, Rheumatoid Arthritis; SLE, Systemic Lupus Erythematosus; MM, Multiple Myeloma; CLL, Chronic Lymphocytic Leukemia.

* Corresponding author. 1201 Ninth Avenue, Seattle, WA 98101-2795, United States. Tel.: +1 206 223 8813; fax: +1 206 223 7543.
E-mail address: along@benaroyaresearch.org (S.A. Long).

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diverse population of T cells is generated in the thymus through assembly of a constant (C) and variable (V) domain of the α and β chains of the TCR via random genetic rearrangement (Davis and Bjorkman, 1988). The V domain itself consists of recombined genetic elements from each of the variable (V), diversity (D), and joining (J) gene families. The linkage points where each of the V, D, and J regions combine are termed hypervariable regions. The third hypervariable region between the V and J region of the β chain, also called the third complementarity-determining region (CDR3), is unique in that it is key in determining binding of the TCR to peptide/MHC complexes, thereby, defining the specificity of a T cell. Further diversification also occurs within the CDR3 by imprecise joining of the V and J regions along with random additions and deletions of nucleotides at linkage points between each region.

The breadth of the antigenic diversity of the TCR repertoire is most commonly measured by assessing genetic diversity in the CDR3 region using length distribution of RT-PCR products encompassing the V, D, J, and C junctional regions of the TCR Vβ chain (Nikolich-Zugich et al., 2004; Pannetier et al., 1995). This technique is termed spectratyping. Although there is consistency in the molecular methods used to generate TCR Vβ spectratype data, a wide range of analysis methods exist to describe and quantify TCR diversity. One of the least sensitive, but more commonly used, methods is visual scoring, which characterizes TCR Vβ diversity as oligoclonal or polyclonal (Ferrand et al., 2000; Bour et al., 1999; Kook et al., 2002). This method may be used to accurately assess predominance of individual peaks, but it is not sensitive enough to interpret subtle changes in the TCR repertoire normalization. To quantify the complexity of the TCR repertoire, Bomberger et al. (1998) developed a method in which all peaks greater than 10% of the total peak area are enumerated. If there are greater than 6 peaks, then TCR distribution for that individual Vβ is considered normal. An average of all measured TCR Vβ peak counts is referred to as a "Diversity score". Others have developed various peak area calculations including peak counts for peaks greater than a determined percentage of the maximal peak area relative mean fluorescence divided by the peak area (Dumont-Girard et al., 1998), and combinations of the analyses described above (Lu et al., 2004). All of these methods of analysis require time-consuming manual handling of the data or apply subjective values to quantify TCR diversity.

Some methods have been developed to systematically and objectively quantify TCR Vβ diversity. Of these, the Gorochov analysis method is the most commonly used quantitative method of analysis (Manfras et al., 2004; Gorochov et al., 1998). For this analysis, peak areas in each Vβ are compared against the distribution of a normal standard that is determined by averaging values obtained from the $CD4^+$ T cell component of five normal tissues. The difference between the experimental peak areas and the normal values within each Vβ are averaged and reported as a Gorochov value. Data retrieval from Excel™ spreadsheets generated by Genscan™ are expedited using the software package, ISEApeaks™, which extracts raw data and performs data smoothing according to expected three nucleotide variation in product sizes. These data may be further manipulated using Immunoscope™, which automatically calculates peak area and the Gorochov value (Collette et al., 2003). Alternative quantitative methods of analysis include the Diversity system that compares experimental values against the distribution profile of cord blood (Peggs et al., 2003b) and TC Landscape™ that integrates data measuring genetic diversity with data measuring Vβ surface expression (Pilch et al., 2002).

Although each of the analysis methods described above has advantages when used alone, all current quantitative methods compare experimental values against a data set generated within the specific laboratory performing the analysis. This limits accessibility and comparability of data. Moreover, existing programs designed to analyze TCR Vβ diversity export numerous tables containing fragment length and the corresponding peak area into separate modules in order to interpret the raw data. Thus, manipulation and interpretation of the data generated by the currently used methods are both cumbersome and time-consuming.

Here, we introduce a novel analysis facilitated by the use of the software, REPERTOIRE™, which provides a comparative method in which experimental values are retrieved in a rapid and reproducible manner. These values are then compared against a universal standard value based upon an expected normal Gaussian distribution. Using REPERTOIRE™, perturbance of the TCR Vβ repertoire may be accurately and consistently measured across laboratories. The utility of analyses performed by REPERTOIRE™ was demonstrated by comparing PBMC isolated from healthy donors and patients diagnosed with different autoimmune diseases, several cancers, or persistent viral infections. Commonly, PBMC isolated from these individuals contain a limited diversity of TCRs that may be reflective of disease progression and poor overall health. Upon *in vitro* activation with anti-CD3 and anti-CD28 beads (Xcyte™ Dynabeads®) TCR diversity was restored. This new approach for TCR repertoire analysis may