Deep profiling of the mouse TCRβ CDR3 region in thymus and spleen

Cindy Desmarais1, Chris Carlson1,2, Harlan Robins1,2, Joe Blattman3, and Robert Livingston1

Adaptive TCR Technologies, Seattle, WA1, Fred Hutchinson Cancer Research Center, Seattle, WA2, Department of Immunology, University of Washington, Seattle, WA3

Introduction

To recognize a diverse and unpredictable universe of antigens, the adaptive immune system generates a remarkable breadth of diversity by combinatorial shuffling of T cell receptor (TCR) gene segments in somatic cells. The TCR signals an immune response by the lymphocyte when the TCR binds to an antigen displayed on the MHC of an infected cell. The TCR is a heterodimer composed of two chains, either the α chain or the β chain, the expression of which is determined during maturation of the naïve T cell in the thymus. Prior to exiting the thymus, the developmental pathway includes a number of selection events that commits the T cell to express either the α chain or the β chain by productively rearranging the TCR gene segments. The αβ-combined T cells then undergo additional selection events to screen out the thymocytes in which the TCR recognizes its cognate MHC ligand too avidly, thereby removing potential self-reacting T cells in a process termed negative selection. Conversely, during positive selection thymocyte survival and its developmental lineage are determined by the ability of the TCR to bind the MHC complex.

The CDR3 region was defined as the amino acid sequence from the CDR3 region of the mouse TCRβ locus. The CDR3 sequence was determined from the reading frame of the CDR3 region. Processed sequence data were deposited in the immunoSEQ secure proprietary relational database as a resource for the mouse immunology community.

Methods

To profile and compare the diversity of the murine TCRβ repertoire of thymus and spleen, we used ultra-high-throughput DNA sequencing with a proprietary multiplex PCR methodology to generate tens of millions of TCRB sequences. Total genomic DNA was extracted from the spleen and thymus of an adult female C57BL/6 mouse and subjected to the immunoSEQ TCRβ multiplex PCR assay targeting the CDR3 region of the mouse TCRβ locus. Millions of TCRB sequences were generated following a PCR amplification consisting of 36 forward V segment and 14 reverse J segment primers which targeted all possible somatic combinations of the rearranged TCRβ cell receptor locus in ~3.2 megabases of the genomic DNA, equivalent to approximately 10^4 haploid genomes. The forward and reverse PCR primers contain on the 5’ ends, the universal sequences compatible with the Illumina solid phase amplification. Following purification by silica solid phase extraction, the TCRβ CDR3 PCR library was quantified and loaded on an Illumina flow cell for sequencing on an Illumina Genome Analyzer GAIIx. Sequencing primers were anchored in a conserved J segment motif and used to collect 60 bases of sequence, sufficient to uniquely identify the V, D and J segments and span the length of the CDR3 region.

Results

Ultra-deep sequencing of the mouse TCRβ CDR3 regions in lymphocytes from spleen and thymus generated 17.1 million and 9.0 million sequences, respectively. The highest copy number CDR3 sequence is shared between thymus and spleen and is a public TCRβ clonotype, identified seven times in the NCBI non-redundant database of protein sequences.

Comparison of the distinct CDR3 sequence copy numbers between thymus and spleen revealed more high frequency sequences in spleen.

Conclusions

Ultra-deep sequencing generated 5.8 million thymus and 11.7 million spleen TCRβ CDR3 sequences from one million input genomes demonstrating greater than five-fold sampling of the input genomes.

The immunoSEQ mouse TCRβ assay can detect functional selection among millions of CDR3 sequences as demonstrated by the increased high frequency sequences and shorter CDR3 lengths in the spleen compared to the thymus.

Analysis of CDR3 sequences identified a high frequency CDR3 sequence shared between thymus and spleen and represented a public TCRβ clonotype.

Future directions

Develop additional immunoSEQ assays and analysis tools targeting the mouse TCRα, TCRδ, TCRγ and IGH loci to profile these components of the murine immune system.

Assemble a database of public TCRβ clonotypes observed in various mouse strains and environments as a resource for the mouse immunology community.

Apply the immunoSEQ mouse TCRβ assay to explore further questions of thymic selection and other applications of the mouse model such as vaccine development, dysfunction of the adaptive immune system, disease resistance, transplantation and immune reconstitution.

For additional information about immunoSEQ assays and the immunoSEQ Analyzer suite of bioinformatics applications at Adaptive TCR Technologies, visit our booth or contact us on the web at www.adaptivetcr.com and www.immunoseq.com.