Preliminary analysis of spatial-temporal homogeneity and heterogeneity of TCR β chain CDR3 repertoires in BALB/c mice

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Abbreviations

TCR, T cell receptor; CDR3, complementarity determining region 3; V, Variable; D, Diversity; J, Joining; TRBV, T cell receptor beta variable; TRBD, T cell receptor beta diversity; TRBJ, T cell receptor beta joining;

HTS, high-throughput sequencing;

IMGT, the international ImMunoGeneTics information system;

aa, amino acid;G, Glycine; A, Alanine ; V, Valine ; L, Leucine ; I, Isoleucine ; P, Proline; F, Phenylalanine ; W, Tryptophan ; M, Methionine ; Y, Tyrosine ; S, Serine ; T, Threonine ; C, Cystine ; N, Asparagine ; Q, Glutamine ; D, Aspartic acid ; E, Glutamic acid ; K, Lysine ; R, Arginine ; H, Histidine;

T, Thymus; S, spleen; B, blood; L, liver; I, intestine

M1, one month mouse; M3, three months mouse; M5, five months mouse

LEC, lowly -expanded clone; MEC, medium-expanded clones; HEC, highly-expanded clones

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Disclosure

The authors have no conflicts to disclose concerning the work in this paper.

Authors' contributions

Xinsheng Yao designed the research and wrote the paper. Yuehong Li, Long Ma, Bin Shi, Xiaoyan He, did the experiment and analyzed the data, Teng Zhang and Suhong Sun help to analyze the data. All authors read and approved the final manuscript.

ABSTRACT

T-cell response and tolerance in non-lymph tissues (liver and small intestine) differ from lymph tissue response and tolerance as occurs in the spleen and thymus. However, the distribution and composition of the TCR repertoire in non-lymph tissues, and how they differ and associate with counterparts in lymph tissue, peripheral blood and non-lymph tissue is unclear. Thus, we studied these tissues in BALB/c mice at one-, three- and five-months-of-age. Genomic DNA were extracted from organs and multiple PCR amplification was performed for the TCT β chain CDR3 region, followed by high-throughput sequencing(HTS) of the CDR3 region. Spatial-temporal homogeneity and heterogeneity of TCR β chain CDR3 repertoires were analyzed and compared. Data show that total CDR3 repertoire diversity was the same across mouse ages and diversity of thymal CDR3 in the youngest mouse was significantly greater than the older mice. CDR3 intestinal diversity in the oldest mouse was greater than in the other two mice. CDR3 diversity in the thymus spleen and blood for all mice exceeded that of the livers and small intestine and CDR3 in the spleen, blood, and liver decreased with ageing. At all ages, lowly -expanded clone (LEC) was greatest in the thymus, followed by the spleen, blood, liver, and small intestines and highly-expanded clones (HEC) had the opposite trend. Liver medium-expanded clones (MEC) was the most abundant compared to other tissues at all animal ages. Overlapping CDR3 in total CDR3 sequences were greatest in the small intestine, and least in the thymus at all ages and overlapped CDR3 had the opposite pattern. The distribution of CDR3 repertoire length was normal, with a median of 14 amino acids in tissues of all mice but the youngest mouse intestinal distribution had a median of 12 amino acids. CDR3 repertoire amino acid usage was consistent among all mouse tissues and K, M, H, I were abnormally low. V, D, and J usage in the CDR3 repertoire were not different at any age nor were TRBV, TRBD, and TRBJ usage. Usage of the TRBV1, TRBV5, TRB13, TRBV19, and TRBV31 family was high frequency, and the TRBJ01-7(ORF) and TRBJ02-6 (P) family was used at low frequency. TRBJ02-7 usage was significantly higher compared to other TRBJ families. TRBD01 usage was significantly higher than TRBD02 usage. For n-insertions and v, j, d5, and d3 deletions, there were some differences among examined tissues. Thus, the composition and characteristics of the CDR3 repertoire are unique across different tissues at different ages in BALB/c mice. CDR3 repertoire composition was similar within the thymus, blood, spleen, and liver at all ages; and the intestinal composition was different from the thymus, spleen, blood, and liver. These data offer a novel method to explore source, differentiation, proliferation and response of distinct T cells in different tissues at different mouse ages.

Keywords: TCR β chain, CDR3 repertoire, High-throughput sequencing, Homogeneity, Heterogeneity