Molecular Immunology 116 (2019) 174–179

Contents lists available at ScienceDirect

Molecular Immunology

journal homepage: www.elsevier.com/locate/molimm

TRAV gene segments further away from the TRAJ gene segment cluster appear more commonly in human tumor and blood samples

Nagehan Pakasticali[a,b], Tommy Gill[a], Boris I. Chobrutskiy[a], Wei Lue Tong[a], Michael Ramsamooj[a], George Blanck[a,c,*]

[a] Department of Molecular Medicine, Morsani College of Medicine, University of South Florida, Tampa, Florida, 33612, United States
[b] Department of Basic Sciences, National University of Health Sciences, Pinellas Park, Florida, 33781, United States
[c] Department of Immunology, H. Lee Moffitt Cancer Center and Research Institute, Tampa, Florida, 33612, United States

ARTICLE INFO

Keywords:
Immune receptor recombinations
Genomic distance
V and J usage

ABSTRACT

We considered the possibility that the greater the distance between an immune receptor V and J, the more likely the V usage. Such a hypothesis is supported by results from mouse experiments. And, such a hypothesis is consistent with the fundamental nature of recombination and genomic distance: the further the distance, the greater the chance of a DNA break. Thus, we exploited the vast dataset of V and J recombination reads available for the human TRA gene, particularly from cancer and blood specimens, to assess the frequency of TRAV usage with respect to distance from the TRAJ cluster. Results indicated that, indeed, over the entire TRAV cluster, there is a greater chance of V usage the further the distance from the J cluster. These results do not address causation, and are not consistent for certain individual V gene segments, but the results do indicate that overall, the larger the distance between the V and J gene segment cluster, the more likely the appearance of at least a subset of TRAV segments, particularly among tumor infiltrating lymphocytes. With a similar approach, the distal TRAV gene segments were also found to be more commonly associated with a subset of distal TRAJ segments. These results have implications for restrictions on the apparent TRA repertoire in disease settings.

1. Introduction

There are several fundamental tenets of the adaptive immune system that explain the immune response to a large variety of foreign, pathological organisms; and represent an apparent explanation for the lack of tumor development in a healthy, immunologically robust individual. First, the mammalian genome contains a large number of related, but distinct, V, D, and J gene segments that are available for assembling a coding region for each of the seven immune receptor genes that contribute to T-cell and B-cell receptors, respectively. By having a variety of combinations of V's, D's, and J's for each immune receptor gene coding region, there is, apparently, the opportunity to have a diversity of receptor structures for binding to a variety of antigens representing the foreign pathological organisms or representing tumor non-self, *e.g.*, tumor mutant peptides. However, it is clear that distinct V and J regions, in the case of the T-cell receptor, have effective associations with distinct HLA types (Attaf et al., 2018; Callahan et al., 2018a, b; Cui et al., 2018; Di Sante et al., 2015; Faham et al., 2017; Hosie et al., 2017; Roca et al., 2019; Tong et al., 2018; Zeng et al., 2016), thereby raising the question of how much antigen binding diversity can be attributed to the V and J usage versus how much V and J usage is restricted by the allelic variants of the available HLA antigen presenting molecules?

Second, the recombination events bringing together the V's, D's and J's, to generate the immune receptor polypeptide coding regions are variable, leaving an unpredictable set of a few amino acids at the junction, termed the complementarity determining region-3 (CDR3). The CDR3 region is thought to have the most molecular contacts with antigen, in most cases (Reiser et al., 2002; Tsuchiya et al., 2018). Thus, the opportunity for diversity for recognition of foreign and tumor

Abbreviations: BLCA, bladder cancer; BRCA, breast invasive carcinoma; CDR3, complementarity determining region-3; CESC, cervical squamous cell carcinoma; ESCA, esophageal carcinoma; GBM, glioblastoma multiforme; HNSC, head and neck squamous cell carcinoma; KIRC, kidney renal clear cell carcinoma; KIRP, kidney renal papillary cell carcinoma; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; OV, ovarian serous cystadenocarcinoma; PAAD, pancreatic adenocarcinoma; PRAD, prostate adenocarcinoma; SKCM, skin cutaneous melanoma; STAD, stomach adenocarcinoma; TCR, T-cell receptor; TCGA, the cancer genome atlas; TILs, tumor infiltrating lymphocytes; UCEC, uterine corpus endometrial carcinoma; WXS, whole exome sequence

* Corresponding author at: 12901 Bruce B. Downs Bd., Tampa, Florida, 33612, United States.
E-mail address: gblanck@health.usf.edu (G. Blanck).

https://doi.org/10.1016/j.molimm.2019.10.010
Received 25 June 2019; Received in revised form 17 September 2019; Accepted 13 October 2019
Available online 05 November 2019

antigen is enhanced by the junction amino acid sequences. In sum, the CDR3 can include germline amino acids representing the V gene segment, just 3′ to a conserved cysteine, or the CDR3 can represent a completely novel set of amino acids just 3′ of the cysteine. Thus, it is highly likely that distinct V's, D's and J's contribute to antigen binding specificity, *i.e.*, contribute to the preparedness or availability of in adaptive immune response.

The above considerations raise the question, what additional factors might govern (and restrict) V and J usage (Hernandez-Munain, 2015; Carico and Krangel, 2015; Choi and Feeney, 2014). Although considerable progress has been made with regard to basic mouse immunology, and in particular, chromatin factors that govern V and J availability for recombination, addressed further in **Discussion**, very little is known about how the availability, for recombination, of V, D or J gene segments is skewed or biased in a given human population or in disease setting or at different ages. Thus, we have taken advantage of a large set of TRA recombination reads, amassed from both tumor and blood samples, to assess the frequency of TRAV gene segment usage as a function of the distance of the V gene segment from the J gene segment cluster. Results indicate that limitations on V usage can be attributed to proximity to the J segment cluster.

2. Methods

The genomic data commons (GDC) web portal (https://portal.gdc.cancer.gov/) was queried for the cancer genome atlas (TCGA) whole exome sequence (WXS) files. Primary tumor and blood WXS files were downloaded to USF research computing, *via* dbGaP approval number 6300, using the GDC data transfer tool (version 1.3). TRA recombination read recovery was performed in two stages. The first stage used a collection of scripts in the following reference publications (Tong et al. (2018), 2017) and the second stage performed pairwise alignment of candidate reads to known V and J sequences. Known V and J sequences were obtained from The International Immunogenetics Information System (http://www.imgt.org/). The quantitative parameters for the pairwise alignment have been previously published (Chobrutskiy et al., 2018a; Zaman et al., 2018a, b). Briefly, the validation of a candidate TRA recombination read included a minimum number of nucleotides for matching to specific V and J gene segments and a minimum percentage match, with penalties in the algorithm for mismatches. Then, the entire TRA collection for a dataset for a group of cancers, or a specific cancer dataset, as indicated in **Results**, was assessed for the number of times each TRAV gene segment was present in the dataset, counting only once, any reads with precisely the same DNA sequence. The statistical analyses included t-tests and Pearson's correlation coefficients, with associated p-values, with results provided in **Results**, supporting online material (Tables S1–S9), and in the Figure legends.

3. Results

To assess a potential correlation between the frequency of the usage of TRAV gene segments and their distance from the TRAJ gene segment cluster, we analyzed V-J recombination read recoveries from a set of WXS files from TCGA. These files represented all of the primary tumor WXS files for the following cancer genome atlas (TCGA) cancer datasets: BLCA, BRCA, CESC, ESCA, KIRC, KIRP, LUAD, LUSC, OV, and PRAD. The TRA recombination reads were recovered, and have been benchmarked as representing tumor infiltrating T-cells, in the following refs. (Roca et al. (2019); Tong et al. (2018); Chobrutskiy et al. (2018a); Zaman et al., 2018a, b; Patel et al., 2019). The multiple cancer dataset collection totaled to 9304 productive TRA recombination reads, the majority of which represented TRAV gene segments in the distal half of the V gene segment cluster, with respect to the position of the TRAJ gene segment cluster (Fig. 1A, $p = 0.0172$; Table S1). All TRA recombination reads in this dataset of 9304 reads were validated using precisely the same parameters.

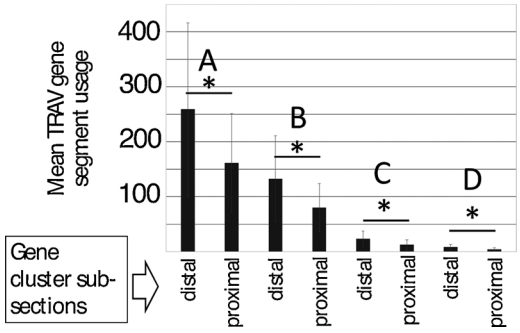

Fig. 1. Average TRAV usage representing either the distal or proximal TRAV gene segment sub-regions, with respect to the TRAJ segment region. The distal and proximal sub-regions were defined by numerically dividing the functional V gene segments in half. (A) Use of TRAV-J recombination read recovery algorithm-1 (Table S6), as described in **Results**, with a total number of 9304 TRAV-J recombination reads recovered from the pan-cancer WXS file set. (B) Use of TRAV-J recombination read recovery algorithm-2, as described in **Results**, with a total number of 4324 TRAV-J recombination reads recovered from the pan-cancer WXS file set. (C) Use of TRAV-J recombination read recovery algorithm-1, to recover 776 productive TRAV-J recombination reads from the metastatic SKCM WXS file set. (D) Use of TRAV-J recombination read recovery algorithm-1, to recover 252 unproductive TRAV-J recombination reads from the metastatic SKCM WXS file set. The asterisks indicate statistical significance in the differences in the TRAV gene segment usage, as indicated by the TRA recombination read recoveries that represent the distal or proximal portions of the TRA gene segment region, with respect to the TRAJ gene segment region. Specific p-values for the t-tests are indicated in **Results** and in the SOM.

The pan-cancer assessment of the preceding paragraph was repeated for another collection of TRA recombination reads obtained using a different search algorithm for the processing of the WXS files for recovery of the recombination reads. In the first approach, in the preceding paragraph, here termed algorithm-1, TRAV and J's were identified, *i.e.*, on a single read, by allowing a match of the read to the V sequences through the 3′ terminal end of the germline V gene segment. If N-diversity nucleotides of the CDR3 region interfered with this match, the match attempt was shifted one nucleotide 5′ and a match to germline V sequences was re-attempted (but without changing any standards related to the number of nucleotides and match-percent needed for TRA recombination read validation). This shifting approach was applied through the conserved cysteine in the V gene segment, just 5′ of the CDR3. In the subsequent search algorithm, here termed algorithm-2, all sequences 3′ of the indicated cysteine were ignored (*i.e.*, from the beginning of the matching algorithm), and V gene segment identification, for each TRA recombination read, required that the minimum match standards be applied exclusively on the 5′ side of the cysteine codon that represents the start of the CDR3. An important practical distinction between these two algorithms is that, in algorithm-1, there is the prospect of recovering more immune receptor recombination reads because the germline nucleotides 3′ to the cysteine codon can be exploited, if present. In algorithm-2, the recombination read recoveries are reduced, with a smaller nucleotide stretch available in the read for matching. Thus, in the case of search algorithm-2, 4324 reads were recovered, and the recovery of these TRA recombination reads has been benchmarked, as representing T-cell infiltrates in a series of refs. (Callahan et al. (2018a),b; Tong et al. (2017); Clark et al. (2019); Kinskey et al., 2018; Mai et al., 2018; Samy et al., 2017; Tu et al., 2017a,b; Tu et al., 2018), distinct from the references representing the benchmarking of algorithm-1.

In the case of the algorithm-2, we combined the TRA read recoveries from the following primary tumor, TCGA datasets: BLCA, BRCA, GBM, HNSC, KIRC, PAAD, PRAD, SKCM, STAD, and UCEC. Again, we assessed the TRAV gene segment usage representing the distal and proximal halves of the TRAV gene segment cluster, with respect to the TRAJ

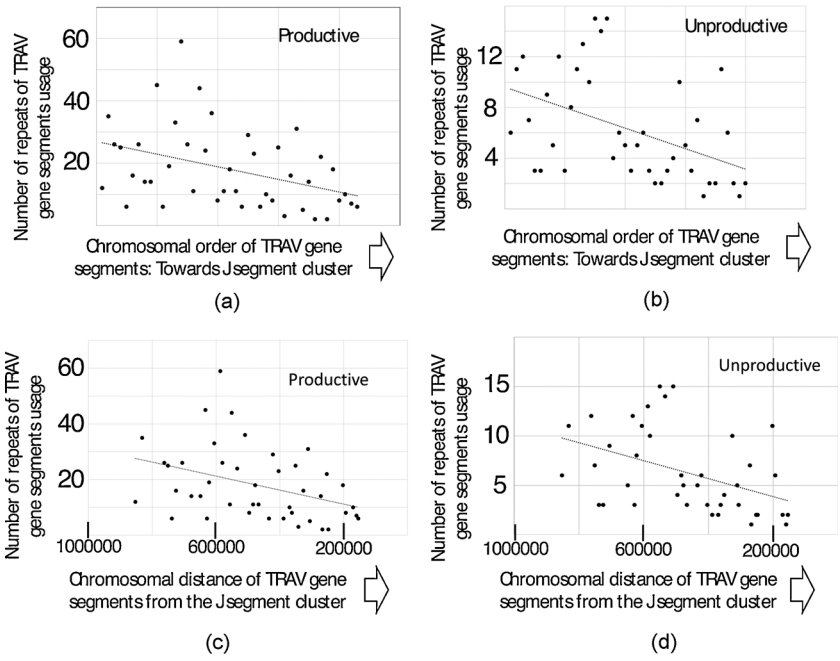

Fig. 2. TRAV usage, with respect to the TRAJ segment region, for the SKCM dataset. (A) Plot of TRAV gene segment usage (y-axis), according to TRAV-J recombination read recoveries, using algorithm-1 as indicated in **Results**, to recover 776 productive TRAV-J recombination reads from the metastatic SKCM WXS file set (Pearson's correlation coefficient = -0.393; p = 0.0093). **(B)** Plot of TRAV gene segment usage (y-axis), according to TRAV-J recombination read recoveries, using algorithm-1 as indicated in **Results**, to recover 252 unproductive TRAV-J recombination reads from the metastatic SKCM WXS file set (Pearson's correlation coefficient = -0.451; p = 0.0035). **(C)** Same as (A), except TRAV gene segment usage is plotted against actual genome (nucleotide) distance from the beginning of the TRAJ gene segment cluster (J61; reference genome version hg38, chr14: 22,475,316; genome.ucsc.edu) (Pearson's correlation coefficient = -0.381; p = 0.0116). **(D)** Same as (B), except TRAV gene segment usage is plotted against actual genome distance from the beginning of the TRAJ gene segment cluster (Pearson's correlation coefficient = -0.429; p = 0.0056).

segment gene cluster. And again, this separate TRA recombination read dataset indicated a statistically significant, greater usage of the V gene segments from the distal half of the TRAV gene cluster (Fig. 1B, p = 0.0136; Table S1).

To further verify and understand the above results, we assessed V-gene segment usage using additional approaches and datasets, using algorithm-1 in all remaining cases for this report. Thus, we next evaluated 776 productive TRA recombination reads representing metastatic melanoma (SKCM) WXS files. Results indicated that within this dataset, there was a greater usage of TRAV segments distal to the TRAJ gene segment cluster (Fig. 1C, p = 0.0042; Table S1). We then assessed the TRAV gene segment usage for 252 unproductive TRA recombination reads (*i.e.*, reads with a frameshift or stop codon) from the same, metastatic SKCM dataset, which indicated the same skewed V gene segment usage (Fig. 1D, p = 0.0002; Table S1). The analyses of the SKCM productive and unproductive recombination reads were repeated using a Pearson's correlation test, *i.e.*, by plotting the TRAV usage against the genomic order of V-occurrence with respect to the TRAJ-segment cluster (Fig. 2; Table S2). In the cases of both the productive and unproductive recombination reads, the Pearson's correlation coefficients were statistically significant (Fig. 2A; productive TRA recombinations, p = 0.0093; Fig. 2B, unproductive recombinations, p = 0.0035). The preceding approach was repeated using actual nucleotide distances of the TRAV gene segments from the end of the TRAJ gene segment cluster (J61; reference genome version hg38; 5′ nucleotide chr14: 22,475,316; genome.ucsc.edu), leading to the same results: a statistically significant Pearson's co-efficient correlating TRAV gene segment usage with distance from the J gene segment cluster (Fig. 2C, productive TRA recombinations, p = 0.0116; Fig. 2D, unproductive recombinations, p = 0.0056).

We repeated the above SKCM approach using a primary tumor, bladder cancer dataset (BLCA); and by using blood WXS files representing this BLCA dataset. Thus, 679 and 1167 productive TRA recombination reads from primary BLCA tumor and matching blood WXS files, respectively, were evaluated for TRAV usage with respect to the distance of the specific V gene segments from the J gene segment cluster. Both the assessments of the V usage representing the distal and proximal halves of the V gene segment cluster, for primary tumor and blood, respectively, and the Pearson's correlation assessment (Fig. 3; Table S3), were consistent with results observed for the SKCM dataset, although in the case of the Pearson's correlation coefficient for the BLCA primary tumor set, only a trend was observed. (See legend to Fig. 3 for all *p*-values.)

The above approach was repeated for the stomach adenocarcinoma dataset (STAD) and the kidney renal cell carcinoma data set (KIRC), using both primary tumor and blood WXS files in both cases as input files for the algorithm-1 (above), TRA recombination read searches. Both the assessments of the V usage representing the distal and proximal halves of the V gene segment cluster, for STAD (SOM Fig. S1A, p = 0.01, tumor; p = 0.005, blood; Table S4) and KIRC (Fig. S2A, p = .045, tumor; p = 0.17, blood; Table S5), for primary tumor and blood, respectively, and the Pearson's correlation assessments, were consistent with results observed for the SKCM and BLCA datasets. However, the *t*-test evaluating the V usage in the proximal and distal sections of the V gene segment region of the genome, with respect to the TRAJ region gene segment, for the KIRC dataset, only indicated a trend towards increased V-usage representing the distal portion of the TRAV gene segment region. (See legends to Figs. S1, S2 for the Pearson's correlation co-efficient p-values.)

Finally, we evaluated the relationship of the proximal and distal TRAJ gene segments with respect to the TRAV gene segments. We assessed the TRAV usage among the 10 most proximal, functional TRAJ gene segments, *i.e.*, the TRAJ segments closest to the TRAV gene segment cluster, with functionality determinations as catalogued by imgt.org (Table S7). And, we assessed the TRAV usage among the 10 most distal, functional TRAJ gene segments. For the pan-cancer algorithm-1 set, there was a trend towards distal TRAV's associated with distal TRAJ's (Table 1), and there was a statistically significant association of proximal TRAV's with proxima TRAJ's (p = 0.001, Table S7). We made the same assessments for the BLCA tumor and blood TRA recombination reads, and in both cases there was a statistically significant association of distal TRAV's with distal TRAJ's (Table 1, Tables S8, S9). For these latter two sets, there was no association of proximal TRAV's and proximal TRAJ's, not surprising given the relatively low numbers of proximal TRAV's in general (Figs. 1–3), particularly for cancer specific sets.

4. Discussion

In this study we considered the possibility that the greater the

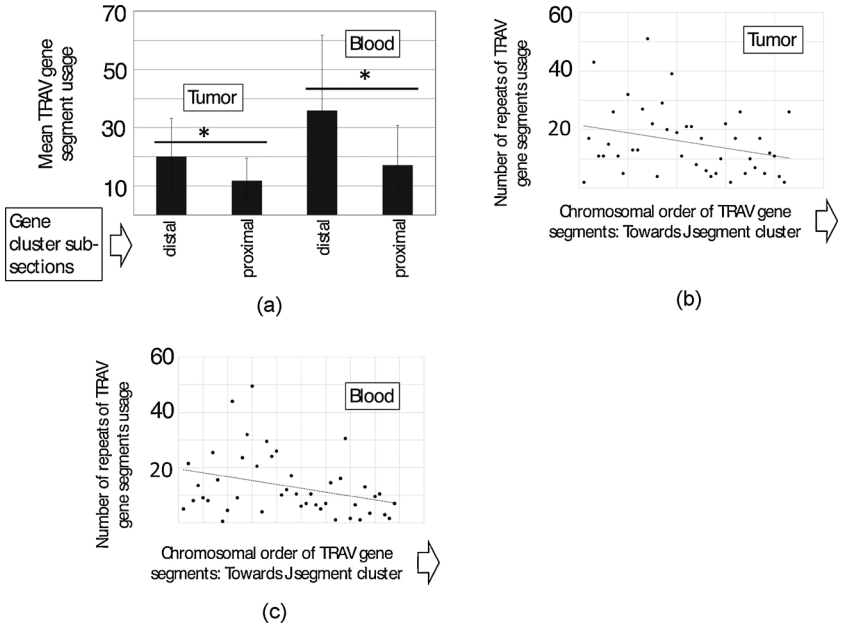

Fig. 3. TRAV gene segment usage, with respect to the TRAJ segment region, represented by the BLCA tumor and blood datasets. (A) Use of TRAV-J recombination read recovery algorithm-1, as described in **Results**, with a total of 679 TRAV-J recombination reads recovered from the BLCA primary tumor WXS file set; and use of TRAV-J recombination read recovery algorithm-1, with a total of 1167 TRAV-J recombination reads recovered from the BLCA blood WXS file set. The bar graphs indicate the average use of the specific TRAV gene segments in either the distal or proximal half of the TRAV segment region, with respect to the J segment region. The asterisks indicate statistical significance in the differences in the TRAV gene segment average usage, between the distal and proximal halves of the TRAV segment region (tumor, $p = 0.017$; blood, $p = 0.005$). **(B)** Plot of TRAV gene segment usage (y-axis), according to TRAV-J recombination read recoveries, using algorithm-1 as indicated in **Results**, to recover 679 productive TRAV-J recombination reads from the BLCA primary tumor WXS file set (Pearson's correlation coefficient = -0.289; $p = 0.0601$). **(C)** Plot of TRAV gene segment usage (y-axis), according to TRAV-J recombination read recoveries, using algorithm-1, to recover 1167 productive TRAV-J recombination reads from the BLCA blood WXS file set (Pearson's correlation coefficient = -0.318; $p = 0.0354$).

distance between a TRAV gene segment and the TRAJ gene segment cluster, the more likely the appearance of the TRAV gene segment in the set of TRAV-TRAJ recombinations obtained from the indicated human tumor and blood specimens. While distance did not simply or universally correlate with the frequency of TRAV usage, most of the results of the above analyses are consistent with the basic conclusion that, for the set of TRA recombination reads available, TRAV usage is greater as the distance from the J gene segment cluster increases. In addition, there was replicative data (blood and tumor, Table 1) indicating that distal TRAJ gene segments have a greater association with distal TRAV gene segments.

There have been previous reports of V gene segment positioning and V usage, in specific cases, for example the positioning of the TRGV segments, and TRGV gene segment usage, in mouse fetal thymocytes (Xiong et al., 2004). In this latter case, the V gene segment closer to the J segment predominated. However, as noted by others (Krangel, 2009), the TRG locus is highly compact, and may reflect biases in V usages due to functions distinct from the functions that impact V usages for other immune receptor loci.

Overall, it is clear that access of the immune receptor gene locus, for transcription, facilitates the recombination process (Krangel, 2009). In addition, it is likely that specific V gene segment usage, or the frequency of usage of certain immune receptor V segments, is dictated by specific locations and activities of specific promoters (Krangel, 2009; Baker et al., 1998). In the case of mouse TRA locus, when thymocytes have reached the double positive stage of development (CD4+, CD8+), and when the TRA locus enhancer is activated, multiple rounds of V-J recombination are observed (Huang et al., 2005; Hawwari and Krangel, 2005; Carico et al., 2017), with an initial bias towards usage of TRAV gene segments close to the J gene segment cluster; and, as subsequent recombinations occur, there is a greater usage of distal V and J gene segments, consistent with data in this report (Table 1). The repetitive recombinations may be due to a selection, in the thymus, for TRAV and TRAJ usage that represents biochemical consequences of particular T-cell receptor polypeptides. For example, this repetitive re-establishment of the TRAV-TRAJ recombination may be required to have a TRAV-TRAJ combination that will lead to an α/β TCR heterodimer that will survive negative or positive selection (Huang et al., 2005). Thus, the presumption has been that the alteration in usage reflects selection and elimination of unusable TCR-α polypeptides. However, it has apparently not been confirmed that this sequential usage, or TRAV and TRAJ availability, is not simply linked to a progressive opening of the chromatin of the more and more distal TRAV and TRAJ gene segments, with increasing rounds of lymphocyte replication and the "positive feedback" impact of TRA locus recombination, which readjust the positions of open chromatin with respect to unrecombined, *i.e.*, distal TRAV gene segments. In short, increased usage of distal TRAV and TRAJ gene segments has been observed in a specific murine setting (Carico et al., 2017; Krangel et al., 2004), but the molecular basis, or facilitation for this trend has not be clearly established. Thus, the possibility remains such that an increase in distal TRAV gene segments simply reflects the expansion of the DNA break "target", or RAG protein target, similar to other DNA recombination events. That is, all other DNA recombination events are known to be more common

Table 1
Distribution of TRAV-gene segments among TRAJ-gene segments, with regard to proximity standards.

Recombination read collection, per **Results** text. Note: SOM tables with tabular data, in parentheses.	Functional TRAJ-gene segments, either proximal set of 10, or distal set of 10 gene segments. Note proximal J's are close to the V-gene segments; distal J's are far from the V-gene segments.	Average count of TRAV-gene segment usage for the distal half of the TRAV cluster. Note, distal V's are far from the J-gene segments.		p-values representing the significant differences of the indicate V-gene segment tallies
Complete set of recovered TRA recombination reads, algorithm-1 (Table S7)	Distal TRAJs	Distal TRAVs	49.50	p-value comparing distal TRAV counts, = 0.065
	Proximal TRAJs	Distal TRAVs	31.13	
TRA recombination reads, recovered from BLCA tumor Table S8)	Distal TRAJs	Distal TRAVs	3.92	p-value comparing distal TRAV counts = 0.028
	Proximal TRAJs	Distal TRAVs	1.79	
TRA recombination reads recovered from BLCA blood (Table S9)	Distal TRAJs	Distal TRAVs	7.92	p-value comparing distal TRAV counts = 0.017
	Proximal TRAJs	Distal TRAVs	3.67	

with more genomic space, as a stochastic process of effectively, DNA damage.

While the murine studies have indeed indicated a likely favoring of the use of the distal TRAV and TRAJ gene segments for the initially produced repertoire, to our knowledge, this is the first confirmation of such a predominance of, particularly, the distal TRAV gene segment usage in the human setting; in the periphery in any species; and in particular, for TRA recombinations representing tumor infiltrating lymphocytes (TILs), as indicated by a plethora of studies that have correlated the recovery of immune receptor recombination reads form tumor exome files with features of TILs (Callahan et al., 2018a,b; Roca et al., 2019; Tong et al., 2018, 2017; Chobrutskiy et al., 2018a; Zaman et al., 2018a,b; Patel et al., 2019; Clark et al., 2019; Samy et al., 2017; Tu et al., 2017b; Gill et al., 2016; Chobrutskiy et al., 2018b; Brown et al., 2015; Levy et al., 2016; Li et al., 2017). The high likelihood that TILs demonstrate such skewing raises the question of whether this apparent restriction on the TRA repertoire has an impact on disease course. Thus, disease course may be impacted by the HLA allele-V-J combinations that are effective; CDR3s that are available and bind to antigen; and now, the relevance of the variations among V (and J) gene segments sequences as would be effectively dictated by genomic position. These three aspects of TRA gene segment usage restrictions may be inter-related, an issue that would have to be addressed with future studies. For example, are there certain HLA allele-V-J combinations that are particularly suited to binding certain epitopes, but as such, require the availability of a V very close to the J gene segment cluster? And, is there evolutionary pressure on HLA alleles, such that there are more and more HLA alleles in a given population that encode HLA proteins able to effectively interact with TCRs represented by distal TRA V and J-segments?

Ethical approval

This article does not contain any studies with human participants.

Declaration of Competing Interest

Authors have nothing to declare.

Acknowledgements

Authors wish to acknowledge the support of USF research computing and the taxpayers of the State of Florida. BIC is a recipient of a USF Morsani College of Medicine RISE fellowship.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.molimm.2019.10.010.

References

Attaf, M., Malik, A., Severinsen, M.C., et al., 2018. Major TCR repertoire perturbation by immunodominant HLA-B(*)44:03-Restricted CMV-Specific t cells. Front. Immunol. 9, 2539.

Callahan, B.M., Tong, W.L., Blanck, G., 2018a. T cell receptor-beta J usage, in combination with particular HLA class II alleles, correlates with better cancer survival rates. Immunol. Res. 66, 219–223.

Callahan, B.M., Yavorski, J.M., Tu, Y.N., et al., 2018b. T-cell receptor-beta V and J usage, in combination with particular HLA class I and class II alleles, correlates with cancer survival patterns. Cancer Immunol., Immunother.: CII. 67, 885–892.

Cui, J.H., Jin, Y.B., Lin, K.R., et al., 2018. Characterization of peripheral blood TCR repertoire in patients with ankylosing spondylitis by high-throughput sequencing. Hum. Immunol. 79, 485–490.

Di Sante, G., Tolusso, B., Fedele, A.L., et al., 2015. Collagen specific T-Cell repertoire and HLA-DR alleles: biomarkers of active refractory rheumatoid arthritis. EBioMedicine 2, 2037–2045.

Faham, M., Carlton, V., Moorhead, M., et al., 2017. Discovery of t cell receptor beta motifs specific to HLA-B27-Positive ankylosing spondylitis by deep repertoire sequence analysis. Arthritis Rheumatol. 69, 774–784.

Hosie, L., Pachnio, A., Zuo, J., Pearce, H., Riddell, S., Moss, P., 2017. Cytomegalovirus-specific t cells restricted by HLA-Cw*0702 increase markedly with age and dominate the CD8(+) T-Cell repertoire in older people. Front. Immunol. 8, 1776.

Roca, A.M., Chobrutskiy, B.I., Callahan, B.M., Blanck, G., 2019. T-cell receptor V and J usage paired with specific HLA alleles associates with distinct cervical cancer survival rates. Hum. Immunol.

Tong, W.L., Callahan, B.M., Tu, Y.N., Zaman, S., Chobrutskiy, B.I., Blanck, G., 2018. Immune receptor recombinations from breast cancer exome files, independently and in combination with specific HLA alleles, correlate with better survival rates. Breast Cancer Res. Treat.

Zeng, G., Huang, Y., Huang, Y., Lyu, Z., Lesniak, D., Randhawa, P., 2016. Antigen-specificity of t cell infiltrates in biopsies with t cell-mediated rejection and BK polyomavirus viremia: analysis by next generation sequencing. Am. J. Transplant. 16, 3131–3138.

Reiser, J.B., Gregoire, C., Darnault, C., et al., 2002. A T cell receptor CDR3beta loop undergoes conformational changes of unprecedented magnitude upon binding to a peptide/MHC class I complex. Immunity 16, 345–354.

Tsuchiya, Y., Namiuchi, Y., Wako, H., Tsurui, H., 2018. A study of CDR3 loop dynamics reveals distinct mechanisms of peptide recognition by T-cell receptors exhibiting different levels of cross-reactivity. Immunology 153, 466–478.

Hernandez-Munain, C., 2015. Recent insights into the transcriptional control of the Tcra/Tcrd locus by distant enhancers during the development of T-lymphocytes. Transcription 6, 65–73.

Carico, Z., Krangel, M.S., 2015. Chromatin dynamics and the development of the TCRalpha and TCRdelta repertoires. Adv. Immunol. 128, 307–361.

Choi, N.M., Feeney, A.J., 2014. CTCF and ncRNA regulate the three-dimensional structure of antigen receptor loci to facilitate V(D)J recombination. Front. Immunol. 5, 49.

Tong, W.L., Tu, Y.N., Samy, M.D., Sexton, W.J., Blanck, G., 2017. Identification of immunoglobulin V(D)J recombinations in solid tumor specimen exome files: evidence for high level B-cell infiltrates in breast cancer. Hum. Vaccin. Immunother. 13, 501–506.

Chobrutskiy, B.I., Zaman, S., Tong, W.L., Diviney, A., Blanck, G., 2018a. Recovery of T-cell receptor V(D)J recombination reads from lower grade glioma exome files correlates with reduced survival and advanced cancer grade. J. Neurooncol.

Zaman, S., Chobrutskiy, B.I., Patel, J.S., Callahan, B.M., Tong, W.L., Blanck, G., 2018a. Mutant cytoskeletal and ECM peptides sensitive to the ST14 protease are associated with a worse outcome for glioblastoma multiforme. Biochem. Biophys. Res. Commun.

Zaman, S., Chobrutskiy, B.I., Patel, J.S., et al., 2018b. MMP7 sensitivity of mutant ECM proteins: an indicator of melanoma survival rates and T-cell infiltration. Clin. Biochem.

Patel, J.S., Callahan, B.M., Chobrutskiy, B.I., Blanck, G., 2019. Matrix-metalloprotease resistant mucin-16 (MUC16) peptide mutants represent a worse lung adenocarcinoma outcome. Proteomics Clin. Appl. e1800155.

Clark, K.R., Tong, W.L., Callahan, B.M., Yavorski, J.M., Tu, Y.N., Blanck, G., 2019. TRB-J1 usage, in combination with the HLA-A*01:01 allele, represents an apparent survival advantage for uterine corpus endometrial carcinoma: comparisons with microscopic assessments of lymphocyte infiltrates. Int. J. Immunogenet. 46, 31–37.

Kinskey, J.C., Tu, Y.N., Tong, W.L., Yavorski, J.M., Blanck, G., 2018. Recovery of immunoglobulin VJ recombinations from pancreatic Cancer exome files strongly correlates with reduced survival. Cancer Microenviron. 11, 51–59.

Mai, A.T., Tong, W.L., Tu, Y.N., Blanck, G., 2018. TcR-alpha recombinations in renal cell carcinoma exome files correlate with an intermediate level of T-cell exhaustion biomarkers. Int. Immunol. 30, 35–40.

Samy, M.D., Tong, W.L., Yavorski, J.M., Sexton, W.J., Blanck, G., 2017. T cell receptor gene recombinations in human tumor specimen exome files: detection of T cell receptor-beta VDJ recombinations associates with a favorable oncologic outcome for bladder cancer. Cancer Immunol., Immunother.: CII. 66, 403–410.

Tu, Y.N., Tong, W.L., Fawcett, T.J., Blanck, G., 2017a. Lung tumor exome files with T-cell receptor recombinations: a mouse model of T-cell infiltrates reflecting mutation burdens. Lab. Invest. 97, 1516–1520.

Tu, Y.N., Tong, W.L., Samy, M.D., Yavorski, J.M., Kim, M., Blanck, G., 2017b. Assessing microenvironment immunogenicity using tumor specimen exomes: co-detection of TcR-alpha/beta V(D)J recombinations correlates with PD-1 expression. International journal of cancer. Int. J. Cancer Suppl. 140, 2568–2576.

Tu, Y.N., Tong, W.L., Yavorski, J.M., Blanck, G., 2018. Immunogenomics: a negative prostate Cancer outcome associated with TcR-gamma/delta recombinations. Cancer Microenviron. 11, 41–49.

Xiong, N., Baker, J.E., Kang, C., Raulet, D.H., 2004. The genomic arrangement of T cell receptor variable genes is a determinant of the developmental rearrangement pattern. Proc. Natl. Acad. Sci. U.S.A. 101, 260–265.

Krangel, M.S., 2009.) Mechanics of T cell receptor gene rearrangement. Curr. Opin. Immunol. 21, 133–139.

Baker, J.E., Cado, D., Raulet, D.H., 1998. Developmentally programmed rearrangement of T cell receptor Vgamma genes is controlled by sequences immediately upstream of the Vgamma genes. Immunity 9, 159–168.

Huang, C.Y., Sleckman, B.P., Kanagawa, O., 2005. Revision of T cell receptor {alpha} chain genes is required for normal T lymphocyte development. Proc. Natl. Acad. Sci. U.S.A. 102, 14356–14361.

Hawwari, A., Krangel, M.S., 2005. Regulation of TCR delta and alpha repertoires by local and long-distance control of variable gene segment chromatin structure. J. Exp. Med. 202, 467–472.

Carico, Z.M., Roy Choudhury, K., Zhang, B., Zhuang, Y., Krangel, M.S., 2017. Tcrd rearrangement redirects a processive tcra recombination program to expand the tcra repertoire. Cell Rep. 19, 2157–2173.

Krangel, M.S., Carabana, J., Abbarategui, I., Schlimgen, R., Hawwari, A., 2004. Enforcing order within a complex locus: current perspectives on the control of V(D)J

recombination at the murine T-cell receptor alpha/delta locus. Immunol. Rev. 200, 224–232.
Gill, T.R., Samy, M.D., Butler, S.N., Mauro, J.A., Sexton, W.J., Blanck, G., 2016. Detection of productively rearranged TcR-alpha V-J sequences in TCGA exome files: implications for tumor immunoscoring and recovery of antitumor T-cells. Cancer Inform. 15, 23–28.
Chobrutskiy, B.I., Zaman, S., Diviney, A., Mihyu, M.M., Blanck, G., 2018b. T-cell receptor-alpha CDR3 domain chemical features correlate with survival rates in bladder cancer. J. Cancer Res. Clin. Oncol.
Brown, S.D., Raeburn, L.A., Holt, R.A., 2015. Profiling tissue-resident T cell repertoires by RNA sequencing. Genome Med. 7, 125.
Levy, E., Marty, R., Garate Calderon, V., et al., 2016. Immune DNA signature of T-cell infiltration in breast tumor exomes. Sci. Rep. 6, 30064.
Li, B., Li, T., Wang, B., et al., 2017. Ultrasensitive detection of TCR hypervariable-region sequences in solid-tissue RNA-seq data. Nat. Genet. 49, 482–483.