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研究課題名（和文）Identification of potentially self-reactive T cell receptors and their candidate epitopes in a mouse model of rheumatoid arthritis

研究課題名（英文）Identification of potentially self-reactive T cell receptors and their candidate epitopes in a mouse model of rheumatoid arthritis

研究代表者

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交付決定額（研究期間全体）：（直接経費）　　3,200,000円

研究成果の概要（和文）：自己免疫疾患の原因となるT細胞受容体（TCR）分子は、集中的な研究にもかかわらず、ほとんど知られていません。本研究では関節リウマチモデルマウスの組織において、いくつかの自己反応性T細胞グループとそのTCRを同定した。我々は3つの自己反応性細胞群を発見した。そのうちの2つは非常に類似したTCRを持つ。もう一つは、遺伝子プログラムもTCRもユニークで、関節にのみ発生する。自己反応性Tconv TCRと正常なTregおよびTconv TCRの結果の類似性の比較は、自己免疫におけるTregからTconvへのレパートリーシフトという仮説を支持する。

研究成果の学術的意義や社会的意義
We provided a novel description of the gene programs and TCRs involved in autoimmunity and showed evidence of a Treg/Tconv repertoire shift. These results, increase our current understanding of the pathogenesis of autoimmune diseases and generate new hypothesis that can be tested in future research.

研究成果の概要（英文）：Autoimmune diseases such as rheumatoid arthritis (RA) are an important cause of morbidity and disability worldwide. In spite of intensive study, the T cell receptor (TCR) molecules responsible for autoimmune disease are largely unknown. By studying mice with impaired TCR signaling, it has been hypothesized that a shift in the TCRs from regulatory (Treg) to conventional (Tconv) cells plays a role in self-reactivity. Here, by using single cell sequencing, we identified several groups of autoreactive T cells and their TCRs in joints, lymph nodes and spleen of a mouse model of RA. We found three groups of self-reactive cells according to their gene programs, where two of them are also highly similar in their TCRs. The other group is unique in terms of both gene program and TCRs, and only occurs in joints. Moreover, we compared the similitude between self-reactive Tconv TCRs and normal Treg and Tconv TCRs and our results provide support for the hypothesis of the repertoire shift.

研究分野：Autoimmune disease

キーワード：Autoimmunity　T cell receptor　single cell sequencing

様　式　C－１９、F－１９－１、Z－１９（共通）

１．研究開始当初の背景

Antigen recognition by T cell receptors (TCRs) unleashes a signaling cascade in T cells, leading to the activation of antigen-specific T cells. ZAP-70 is a kinase that plays an important role in this process. During development, T cells must undergo a selection and maturation process which consists of the recognition of self-peptide-MHC complexes by TCRs in the thymus. In the generally accepted model of this process, developing T cells with strongly self-reactive TCRs are deleted, while moderate to slightly self-reactive cells will mature into conventional T cells (Tconvs). This selection process should prevent autoimmunity. Some T cells with a high self-reactive TCR may evade deletion, but will mature into regulatory T cells (Tregs), which instead of causing autoimmune responses, prevent them (Klein et al., 2019). SKG and ZAC mouse models consist of two independent mutations in the ZAP-70 gene (W163C and H165A, respectively) that result in different degrees of weakening of TCR signaling upon antigen recognition. Both mutations predispose mice to an autoimmune disease similar to rheumatoid arthritis, but while SKG mice require secondary stimulations (mannan), ZAC mice develop the disease spontaneously. The reason that attenuation of TCR signaling leads to autoimmunity remains unclear, but it has been proposed that defective TCR signaling may induce a shift in the T cell selection process allowing some moderate to high self-reactive cells to become Tconv cells instead of Treg cells; and a consequence of such a defect is that the host becomes susceptible to autoimmunity (Sakaguchi et al., 2003; Tanaka et al., 2010) (Figure 1). In line with this general model, it has previously been shown that there is an overlap in the normal thymic Treg repertoire and the repertoire of activated and potentially pathogenic T cells in Foxp3-deficient mice (Hsieh et al., 2006). Moreover, predominant T cells infiltrating target lesions in the Aire-/- mouse model of autoimmune prostatitis bear antigen receptors preferentially encountered in Treg cells of Aire+/+ mice (Malchow et al., 2016). However, these studies were performed by sequencing TCR-alpha chains in TCR-beta transgenic mice, and they do not represent a truly polyclonal setting.

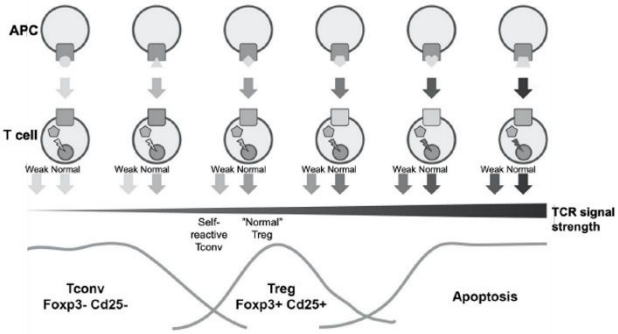

Figure 1. Fate of developing T cells according to their self-reactive potential under normal and TCR weakened signaling

２．研究の目的

The recent development of single cell barcoding, combined with high-throughput sequencing methods for the TCR has allowed the examination of paired alpha and beta chain TCR repertoires at unprecedented breadth and depth (De Simone et al., 2018). In this study, we aimed to investigate the underlying case of the autoimmune disease more deeply, by examining the effects of the H165A mutation on mature conventional T cell (Tconv) repertoire at the single cell level, we focused on the identification of candidate self-reactive TCRs obtained from joints of arthritic mice, and the comparison of their similitude to Treg and Tconv repertoires in WT mice. In addition we examined the transcriptomic profiles of self-reactive cells in joints and compared to the profiles of Tconvs from other tissues and from SKG and WT mice.

３．研究の方法

Tconv and Treg cells were sorted from joints, draining lymph nodes (dLN) and spleen of 4 arthritic ZAC mice, labeled with hashtag oligos, pooled and subjected to RNA+VDJ single cell sequencing by the 10x platform. Data analysis consisted of hashtag demultiplexing and quality control followed by dimensional reduction and clustering of each dataset independently. For transcriptomic analysis, differentially expressed genes were identified and gene set enrichment analysis was performed, together with trajectory analysis. For comparison of transcriptomic profiles, gene modules containing signature genes for the relevant gene expression clusters from ZAC joints were selected and module scores were calculated in the additional datasets. Repertoire analysis of individual datasets consisted of clonal expansion and overlap at the exact CDR3 level.

For similarity comparison of ZAC Tconv repertoires to our own WT and SKG data as well as to publicly available data we conducted clustering by CDR sequence as an approximation of paratope similarity. The approach used considers two TCRs to be similar if their identity of aligned CDR residues is >=90% for CDR1 and CDR2 and >=80% for CDR3, and if the minimum sequence alignment coverage is >=90%.

4. 研究成果

a) Phenotypical characterization of joint infiltrating Tconvs in ZAC mice

Analysis of joint Tconv single cell transcriptomes led to the identification of 6 gene expression clusters with similar distribution across replicates (Figure 2a-b). Cell identities were coarsely defined by exploring their expression of typical T cell markers (Figure 2c) and led to the identification of three Th17 clusters (J0, J1 and J2), one cluster identified as quiescent (J3), one NKT cluster (J4) and one cluster of proliferating cells (J5). In addition, we found that the Th17 clusters can be further distinguished by their expression of additional gene markers (i.e., Tnfsf11) (not shown), and by the enrichment of unique gene pathways: the response to interferon-gamma pathway in J0, the tumor necrosis factor-mediated signaling pathway in J2 and the cell chemotaxis pathway in J1 (Figure 2d). Trajectory analysis was performed setting the start in cluster J1 and revealed two possible trajectories: J1->J0->J2 and J1->J3 (Figure 2e). Interestingly, we detected gene expression patterns across pseudotime for some relevant genes such as Myc, Tnfsf11, Bhlhe40 and Igfbp7 (Figure 2f).

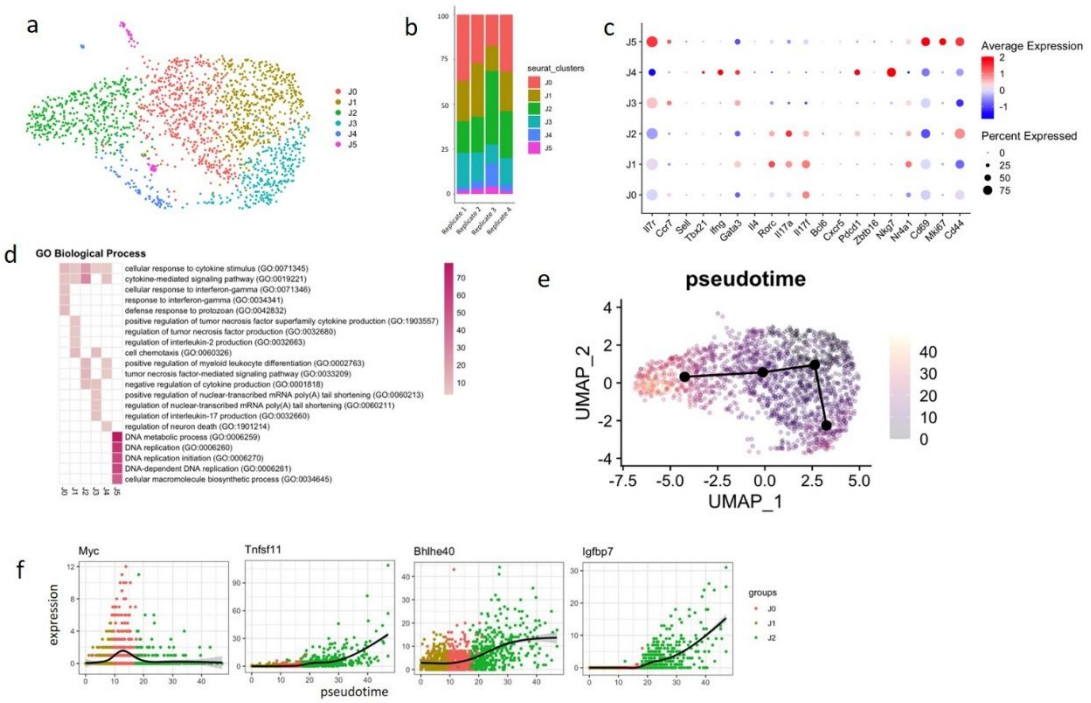

Figure 2. Phenotypical characterization of joint infiltrating Tconvs in ZAC mice. (description in the main text).

b) Repertoire analysis of joint infiltrating Tconvs in ZAC mice

Since cells from all replicates are extensively expanded we classified the clones by their size as: singletons (clone size=1), small (clone size from 2-5), medium (clone size from 6-15) and large (clone size>15). Wide expansion was evident in most clusters except J3 and J4 (Figure 3a-b), in addition, we observed an increased proportion of singletons in J2 as compared to J0 and J1 (Th17 clusters)(Figure 3b). Repertoire overlap calculated by morisita index at the exact paired CDR3 sequence level, uncovered a degree of overlap occurring among trajectory-related clusters (J0, J1, J2 and J3) (Figure 3c) supporting the notion that these clusters are part of a differentiation path. In addition, broad overlap is evident among clusters J0 and J1, but the overlap is much smaller among these clusters and J2, suggesting a unique repertoire for J2. Interestingly, six public clones at the paired chain (gene usage and CDR3 sequence) level were identified, five of which were expanded in at least one subject, including 3 clones in the medium clone size category and one in the large category (Figure 3d); this suggests that expanded clones are likely to be shared across subjects as a

consequence of antigen specificity.

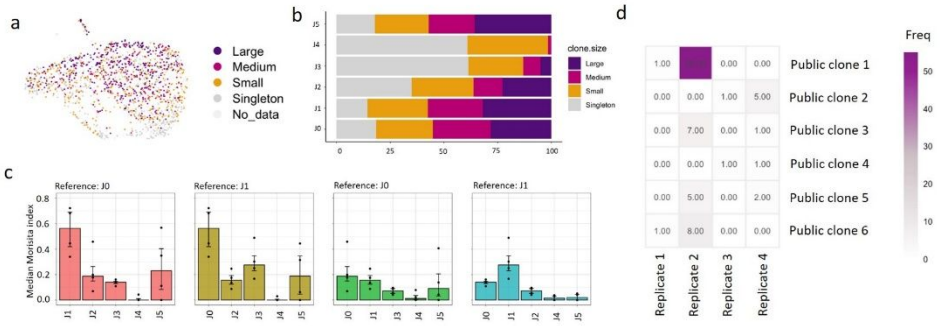

Figure 3. Repertoire analysis of joint infiltrating Tconvs in ZAC mice. (description in the main text).

c) Identification of ZAC joint gene expression signatures in other tissues and mouse models

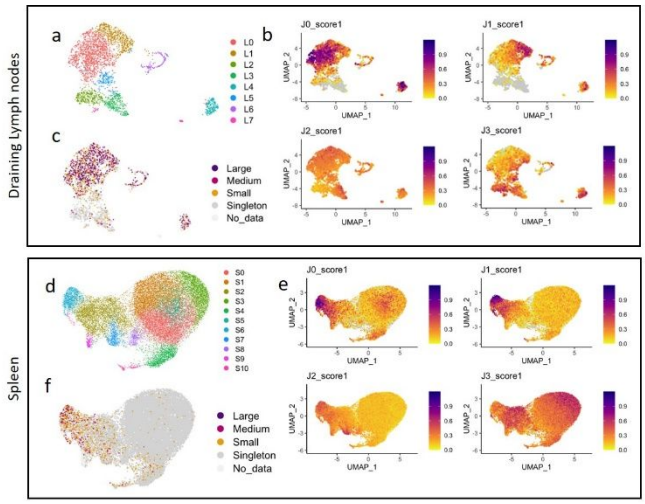

Figure 4. Gene expression analysis of dLN and Spleen Tconvs and comparison with relevant gene programs observed in Joints (description in the main text).

In dLN, Tconvs were grouped in 8 gene expression clusters (Figure 4a). Gene expression clusters were annotated in the same way as in joint cells and the following groups were defined: Intermediate (L0), Th17 (L1), Naïve (L2), Folicular T cells (Tfh) (L3), Memory Th17 (L4), Intermediate (L5) and Proliferating (L6).

Our comparison of gene programs with joint tissues by module score calculation revealed that the gene program of J0 was the most strongly identified in dLN and was higher in L0 and L4, whereas J1 gene program is concentrated in L1 and L4. J3 program was mildly detected and mostly observed in L2 and L4. Interestingly, the gene program represented by J2 was weakly observed in dLN, suggesting that J2 represents a program specific of joints (Figure 4b). In addition, broad clonal expansion was observed in clusters L0, L1, L4 and L6. (Figure 4c).

Regarding spleen, we analyzed Tconvs from 2 ZAC mice, 2 WT and 2 unarthritic SKG mice. Eleven gene expression clusters were identified (Figure 4d) and annotated as: Naïve (S0, S1, S3), Intermediate (S2), Unidentified (S4), IFN gamma responsive (S5), Th17 (S6), Tfh (S7), Early activated T cells (S8), NKT (S9) and Proliferating (S10). Module scores calculation showed that the gene programs of J0 and J1 were most strongly identified in S6 (Th17) (Figure 4e), specially in ZAP-70 mutant mice (not shown). J3 module scores were lower and mostly detected in S3 (Naïve), and J2 gene program expression was in general very low and detected in S7 (Tfh) and S6 (Th17). Together with our findings in dLN, our results support the notion that J2 corresponds to a gene program exclusively activated in joints.

Finally, although at a lower degree as compared to other tissues, clonal expansion was evident in spleen, specially in ZAC mice, and largely concentrated in cluster S6 (Figure 4f).

d) Integrated repertoire analysis of all tissues

We next analyzed the repertoire overlap at the paired exact cdr3 level across tissues, and mouse models. In general, no overlap across different mice was detected, even in mice of the same condition. In terms of overlap across tissues from same mice, we found significant overlap among Th17 cells from different tissues, as evidenced by high values of Morisita index between S6, L1, L4, J0 and J1, also consistent with the gene program sharing and expansion observed in these clusters. Nevertheless, the degree of overlap with J2 which also corresponds to a Th17 phenotype much reduced (black squares)

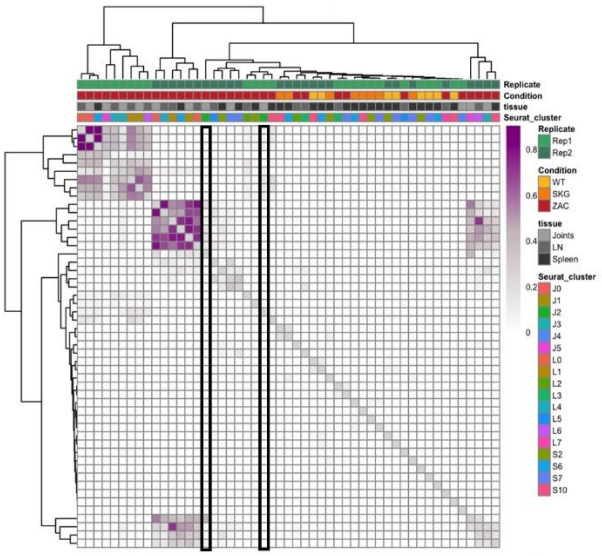

Figure 5. Exact paired cdr3 repertoire overlap as measured by Morisita index across tissues and mouse models

(Figure 5). So far, our results indicate that J2 cluster represents a unique group of joint infiltrating Tconv cells with an exclusive transcriptional program and a unique repertoire.

e) Machine learning data analysis of ZAC Tconv repertoire similarity

Finally, in order to test the hypothesis of a repertoire shift between Tconv and Treg repertoires in TCR weakened mice, we conducted clustering by CDR sequence as an approximation of paratope similarity. The approach used considers two TCRs to be similar if their identity of aligned CDR residues is >=90% for CDR1 and CDR2 and >=80% for CDR3, and if the minimum sequence alignment coverage is >=90%. With this strategy, we clustered the TCR beta chain sequences from Joint Th17 cells with Treg and Tconv sequences obtained from spleen of WT mice by our lab, and sequences obtained from publicly available data. Given the high exact overlap observed between clusters J0 and J1, we combined the two repertoires for these comparisons and J2 overlap was analyzed separately.

In the three datasets tested, we consistently found that the similarity between both groups of Joint Th17 TCRs and Treg TCRs from WT mice was higher than the similarity between Joint Th17 and Tconv TCRs from the same WT mice, statistical significance was calculated by the Fischer test and p value is shown in Table 1 and Table 2. Our result supports the hypothesis that a Treg/Tconv shift occurs in mice with weakened TCR signaling.

Table 1. Repertoire overlap of ZAC J0+J1 repertoires with WT Treg and WT Tconv repertoires from different sources

Study	WT celltype	Pairs with J0+J1	Do not pair with J0+J1	Ratio (J0+J1/no J0+J1)	p value
Own	Tconv	6681	14343	0.31777968	8.98E-07
	Treg	7020	13601	0.340429659	
Logunova et al., 2020	Tconv	110090	265100	0.293424665	3.15E-72
	Treg	40731	86448	0.320265138	
Lu et al., 2020	Tconv	6899	9990	0.408490734	2.98E-09
	Treg	6065	7650	0.442216551	

Table 2. Repertoire overlap of ZAC J2 repertoire with WT Treg and WT Tconv repertoires from different sources

Study	WT celltype	Pairs with J2	Do not pair with J2	Ratio (J2/no J2)	p value
Own	Tconv	2413	18611	0.114773592	1.20E-05
	Treg	2656	17965	0.128800737	
Logunova et al., 2020	Tconv	40609	334581	0.108235827	4.08E-31
	Treg	15282	111897	0.120161347	
Lu et al., 2020	Tconv	2456	14433	0.145420096	2.65E-06
	Treg	2263	11452	0.165001823	

In conclusion, our study provided the identification of a transcriptionally unique group of Th17 cells infiltrating joints of arthritic ZAC mice which are also unique in terms of repertoire. In addition, by contrasting Joint Th17 repertoire similitude with Treg or Tconv repertoires in normal mice, we provided evidence supporting the hypothesis of the induction of a shift in the T cell selection process as a consequence of defective TCR signaling which further allows some moderate to high self-reactive cells to become Tconv cells instead of Treg cells resulting in an increased susceptibility to autoimmunity. This study expands our knowledge of the driver factors of autoimmune disease and the specific group of T cells responsible for clinical manifestations.

５．主な発表論文等

〔雑誌論文〕　計2件（うち査読付論文　2件／うち国際共著　2件／うちオープンアクセス　2件）

１．著者名 Teraguchi Shunsuke、Saputri Dianita S.、Llamas-Covarrubias Mara Anais、Davila Ana、Diez Diego、Nazlica Sedat Aybars、Rozewicki John、Ismanto Hendra S.、Wilamowski Jan、Xie Jiaqi、Xu Zichang、Loza-Lopez Martin de Jesus、van Eerden Floris J.、Li Songling、Standley Daron M.	４．巻 18
２．論文標題 Methods for sequence and structural analysis of B and T cell receptor repertoires	５．発行年 2020年
３．雑誌名 Computational and Structural Biotechnology Journal	６．最初と最後の頁 2000～2011
掲載論文のDOI（デジタルオブジェクト識別子） 10.1016/j.csbj.2020.07.008	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 該当する

１．著者名 Atsushi Tanaka, Shinji Maeda, Takashi Nomura, Mara Anais Llamas-Covarrubias, Satoshi Tanaka, Lin Jin, Ee Lyn Lim, Hiromasa Morikawa, Yohko Kitagawa, et al.	４．巻 220
２．論文標題 Construction of a T cell receptor signaling range for spontaneous development of autoimmune disease	５．発行年 2023年
３．雑誌名 Journal of Experimental Medicine (JEM)	６．最初と最後の頁 e20220386
掲載論文のDOI（デジタルオブジェクト識別子） 10.1084/jem.20220386	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 該当する

〔学会発表〕　計1件（うち招待講演　0件／うち国際学会　1件）

１．発表者名 Mara Llamas-Covarrubias, Atsushi Tanaka, Martin Loza-Lopez, Diego Diez, Shimon Sakaguchi, Daron Standley
２．発表標題 Single cell sequencing reveals a Tconv tissue adaptation process influenced by the TCR in a mouse model of rheumatoid arthritis
３．学会等名 18th International Congress of Immunology（国際学会）
４．発表年 2023年

〔図書〕　計0件

〔産業財産権〕

〔その他〕

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6．研究組織

	氏名 （ローマ字氏名） （研究者番号）	所属研究機関・部局・職 （機関番号）	備考
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研究協力者	田中　淳 (Tanaka Atsushi)		
研究協力者	ディエス　ディエゴ (Diez Diego)		

7．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

8．本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関