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海外特別研究員としての派遣期間を終了しましたので、下記のとおり報告いたします。
なお、下記及び別紙記載の内容については相違ありません。

記

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免疫担当細胞のサブタイプスイッチのメカニズムに関する検討

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5. 所期の目的の遂行状況及び成果…書式任意 **書式任意（A4 判相当 3 ページ以上、英語で記入も可）**
（研究・調査実施状況及びその成果の発表・関係学会への参加状況等）
（注）「6. 研究発表」以降については様式 10－別紙 1～4 に記入の上、併せて提出するこ

Background and Aim of this study

Atherosclerosis is a chronic inflammatory disease of large and medium-sized arteries. Its autoimmune component is manifest in autoantibodies to apolipoprotein B (APOB), the main lipoprotein in low density lipoprotein (LDL), and to oxidized LDL, as well as robust CD4 T cell responses (Saigusa et al., 2020). Individuals without cardiovascular disease (CVD) have a detectable number of FoxP3+ CD4 T regulatory cells that specifically recognize epitopes in APOB (Kimura et al., 2018). Although FoxP3 is the canonical, lineage-defining transcription factor for regulatory T cells (Tregs), it is not known whether these APOB-specific T cells indeed have regulatory function. This cannot be addressed directly, because the number of ApoB-specific CD4 T cells recovered is too low for in vitro suppression assays.

Mouse studies have shown that ApoB-specific Tregs lose expression of many Treg genes with both age and high fat diet (Wolf et al., 2020). Consistent with this, individuals with subclinical cardiovascular disease (sCVD) harbor APOB-specific CD4 T cells that express FoxP3 along with T-helper transcription factors like ROR-γt and T-bet (Kimura et al., 2018), suggesting that these cells are no longer regulatory but instead assume a changed, pro-inflammatory phenotype. This earlier study (Kimura et al., 2018) was conducted by flow cytometry, resolving only 5 transcription factors and no other cellular details. Here, we use current single cell RNA-sequencing (scRNA-Seq) technology to obtain single cell transcriptomes and T cell receptor (TCR) sequences. Comparing the transcriptomes of ApoB-specific CD4 T cells with transcriptomes of bona fide Tregs can address transcriptomic similarities and differences. Studying the TCR repertoire addresses the clonality of the ApoB-specific CD4 T cell response.

CD4 T cells express TCRs that are heterodimers of one α and one β chain and the subunits of CD3 needed for surface expression and signaling. The CD3 subunit sequences are invariant, but the α and β TCR chains undergo somatic recombination, using different alleles of the V and J subunits and a template-free section that defines the most variable CDR3 region. With 5' single cell RNA-sequencing, TCR CDR3 sequences can be assembled (Stubbington et al., 2016), thus reflecting the full repertoire of TCRs found in the sample. Cells expressing the same TCRα and β are called clonotypes (Soto et al., 2020). Tregs express the transcription factor FoxP3 and the high affinity IL-2 receptor CD25. Other CD4 T cells serve as helpers: T-helper-1 (Th1) express T-bet and secrete interferon-γ. In human blood, Th1 cells express the chemokine receptor CXCR3. Th2 cells express GATA3 and secrete IL-4, IL-5 and IL-13, Th17 express ROR-γt and secrete IL-17A and IL-17F, and follicular helper CD4 T cells (TFH) express Bcl6 and secrete IL-21.

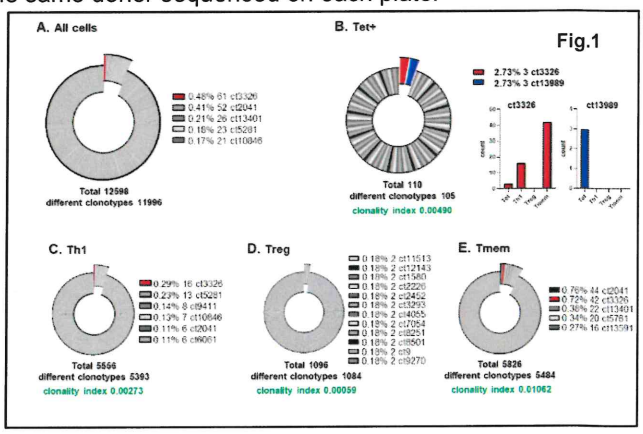
APOB-specific CD4 T cells can be detected by MHC-II peptide tetramers in humans and mice. Tetramers are recombinant MHC-II molecules loaded with the peptide epitope. In the case of human APOB,

the best-defined epitope is APOB p18, sequence SLFFSAQPFEITAST. We previously constructed and validated a tetramer from the MHC-II allele DRB1*07:01 loaded with p18 (APOB-p18 DRB1*07:01 tetramer) (Kimura et al., 2018). In women without CVD, most APOB-p18 DRB1*07:01 tetramer positive CD4 T cells (tet+ cells) expressed FoxP3. In women with CVD documented by carotid B mode ultrasound, many of these cells expressed both ROR-γt and FoxP3, suggesting a transitional phenotype between Treg and Th17. The transcriptomes of tet+ cells in CVD cases and controls have not been studied before. No TCR sequences of tet+ cells have been reported.

Recent advances in scRNA-Seq enable the interrogation of the transcriptomes of thousands of individual cells. Here, we used an approach (10x Genomics 5') that allowed us to reconstruct the TCR α and β chains in addition to interrogating their transcriptomes. The experimental design was balanced, using sorted cells from 8 donors from the WIHS study. WIHS is an ongoing multi-center, prospective, observational cohort study of over 4,000 women with or at risk of HIV infection that was initiated in 1994. Cardiovascular risk in people living with HIV is elevated more than 2-fold compared to uninfected controls (Shah et al., 2018). In the present study, we sorted all tet+ cells from frozen peripheral blood mononuclear cells (PBMCs) from participants of the WIHS study. In addition, we sorted Tregs (CD25hi CD127-), Th1 cells (CXCR3+) and other (CXCR3-negative) memory T cells (Tmem). All four cell types were hash-tagged, as were donors, such that four cell types (tet+, Treg, Th1, Tmem) from 8 donors were run on each 10x Genomics Chromium microfluidic device. A small number of CD4 T cells from a healthy control sample were added to each plate to control for batch effects.

Results

We harvested PBMCs from 11 WIHS participants who expressed the DRB1*07:01 allele. Three samples had unsatisfactory cell numbers, resulting in 8 samples being included in the study, 2 each with no HIV and no CVD (HIV-CVD-), with HIV only (HIV+CVD-), with CVD only (HIV-CVD+) or with both HIV and subclinical CVD (HIV+CVD+). All PBMCs were gated for dump (CD8, CD14, CD16, CD19, CD56) negative and live. From CD3+TCRab+CD4+ T cells, APC and PE APOB-p18 DRB1*07:01 tetramer positive cells were sorted as tet+ cells. Treg, Th1 and Tmem cells were sorted from tetramer negative cells. The number of cells sorted and the number of transcriptomes and TCRα, TCRβ and TCRαβ pairs successfully assembled are shown in Figure 2. In all cases, the most limiting number of cells were the p18-DRB1*07:01 tetramer positive cells, of which between 99 and 281 were obtained per participant. The washing and loading procedure was optimized to preserve as many of these cells as possible. Doublets, low quality and proliferating cells were removed and batch effects were controlled by Harmony,(Korsunsky, 2019) taking advantage of spiked-in PBMCs from the same donor sequenced on each plate.



First, we analyzed the TCRα and β sequences in all sequenced cells. In total, we found 12,598 clonotypes, of which 11,996 were unique. Alpha and β chains were successfully assembled in approximately the same number of cells (15,774 and 16,647, respectively). 262 clonotypes were found in multiple cells, with the most common one found in 61 cells (Figure 1A). 11,734 clonotypes were found in only one cell each.

Next, we analyzed the clonality of tet+, Th1, Tmem and Treg cells from all donors (Figure 1B-E). Among the tet+ TCR clonotypes, two were represented in 3 cells each and the remaining 108 were uniquely expressed in one cell each. The most common clonotype in Th1 cells was found in 16 cells, in Tmems in 44 cells, and in Tregs in 2 cells (Figure 1B-E). Since the number of clonotypes found multiple times is dependent on the total number of TCRs analyzed, we calculated the clonality index, which is defined as $1-H/\log_e(n)$ [H, Shannon index, the negative value of the sum of $p_i \log_e(p_i)$; i, from 1 to n] (Zhang et al., 2017). The highest clonality index (0.01) was found in Tmems, followed by tet+ cells (0.005) and Th1 (0.003). Tregs had the lowest clonality index (0.0006), meaning that they had the most diverse TCR repertoire of all CD4 T cells studied.

Because previous studies were based on TCRβ sequences obtained by DNA sequencing,(Lin et al., 2017) we re-analyzed the data for TCRβ only. We looked for Vβ and Jβ combinations in our samples. Even at this level of comparison, no VβJβ clonotypes were shared between tet+ and Treg only or between tet+, Treg and Tmem. Most VβJβ clonotypes were private for each cell type. More VβJβ clonotypes (10.8%) were shared between Th1, Tmem and Treg (7.3%). Seventeen VβJβ clonotypes were private to tet+ cells, whereas 51 were shared with Tmen, Treg and Th1. Fourteen to 22% of VβJβ clonotypes were private to each of the four patient groups, and there was no discernable systematic difference. Comparing TCRb only or TCRa only, the Tmem and Th1 cells are most oligoclonal, followed by Tregs, and Tet+ are most polyclonal, while comparing the combination of both TCRα and β, Tmem still has the highest clonality index, but now tet+ cells come next, followed by Th1, and Tregs are most polyclonal.

The most common combination was TCRβV20-1 combined with TCRβJ1-1, 1-2, 2-1 or 2-7 (Figure 2B). Constructing heatmaps for tet+, Th1, Tmem and Treg cells separately also shows TRβV20-1 as the most common V segment. Tet+ cells were enriched for TRβV20-1 combined with TRβJ2-1, whereas TRβV20-1 was more broadly associated with TRβJ1-1, 1-2, 2-1, 2-3 and 2-7 in Th1, Tmem and Treg cells. Also tet+ cells were enriched for TRBV4-2, 6-1 paired with TRBJ2-3. TRBV6-5 is paired with TRBJ1-5. Formal differential expression (DE) analysis for the VJ combinations for the 4 cell types showed no significant differences of TRβV and J usage among women with and without subclinical CVD.

Next, we clustered all cells based on their transcriptomes. UMAP with Louvain clustering identified 7 clusters. Treg transcriptomes clustered together (Figure 2A). Tmem and Th1 were intermingled, suggesting that their transcriptomes were not very different. Indeed, transcriptomic analysis showed that CXCR3- Tmem still contained Th1 cells, suggesting that not all human Th1 cells express CXCR3.

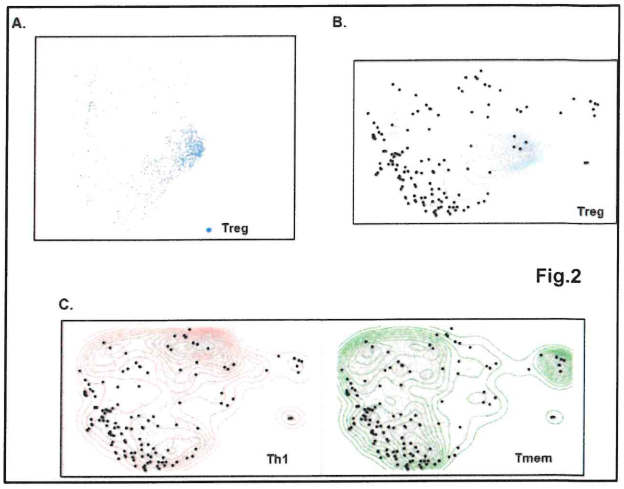


Fig.2

Projecting the transcriptomes of tet+ cells onto the Th1, Tmem and Treg UMAP showed that very few tet+ cells from HIV- women without CVD fell within the Treg cluster (Figure 2B). Instead, their transcriptomes placed them in the Tmem and Th1 clusters (Figure 2C).

To study the influence of CVD on the transcriptomes of Tet+ cells, we reclustered Th1, Tmem and Treg cells separately for CVD- and CVD+ in each subject and projected the corresponding tet+ cells on the contour-plots (Figure 3A, B). There was no visible difference. To formally and quantitatively analyze the distance between tet+ and other cell types, we calculated the distance of each tet+ cell transcriptome from the calculated bulk transcriptomes of Treg, Th1 and Tmem, separately for women with and without CVD, again focusing on the HIV- subjects. Tet+ cell transcriptomes were more similar to Th1 and Tmem than to Tregs (Figure 3C). For formal statistical analysis, we plotted the cumulative histograms of the distances of tet+ cells from Tmem (Figure 3D), Treg (Figure 3E) and Th1 (Figure 3F). Tet+ cells from women with CVD showed were significantly closer to Tmem transcriptomes (Figure 3D) and tended to be further removed from Tregs than those from women without CVD (Figure 3E). There was no difference in the distance from Th1 (Figure 3F). These findings are summarized in Figure 3G in a modified Ternary plot. Thus, we conclude that tet+ cells from women with CVD are closer to memory T cell transcriptomes and away from Treg transcriptomes.

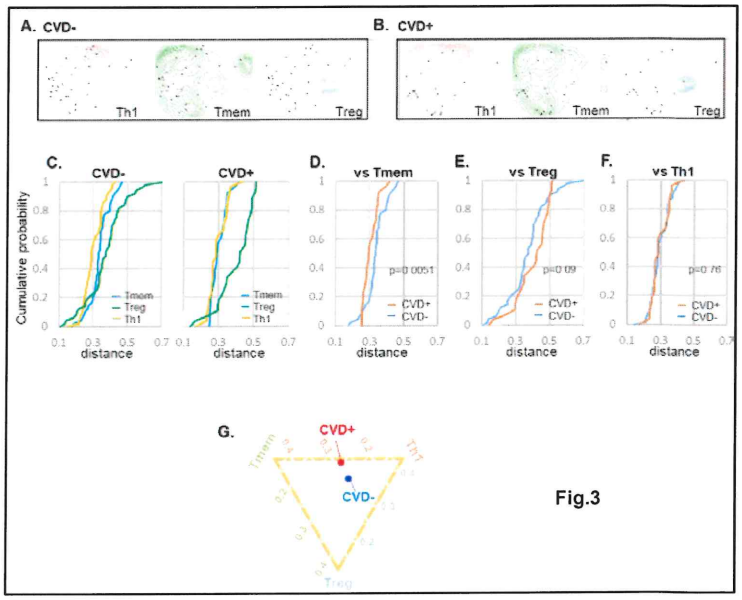


Fig.3

Next, we investigated the expression of which genes drove the repositioning of tet+ cells with cardiovascular disease in HIV- subjects. Using Seurat, we determined the significantly (adjusted $p<0.05$) differentially expressed genes between tet+ cells and Th1, Tregs and Tmem.

In CVD- women, tet+ cells expressed significantly more TYMS, CENPA, CBS, CALD1, CPS1, FOXP2, CATSPERD, CLEC7A, FAM86B2 and NR6A1 than Th1 and Tmem cells from the same participants. Thymidylate cyclase, the product of the TYMS gene, is involved in mitochondrial thymidylate biosynthesis. CENPA encodes a histone H3-like protein found in centromeric nucleosomes. CBS encodes cystathionine beta-synthase hydro-lyase, catalyzing the first step of the transsulfuration pathway. CALD1 encodes Caldesmon 1, actin- and myosin-binding protein implicated in the regulation of actomyosin interactions in smooth muscle and nonmuscle cells. CPS1 is involved in the urea cycle of ureotelic animals where the enzyme plays an important role in removing excess ammonia from the cell. FOXP2 may play a role in developing neural, gastrointestinal and cardiovascular tissues. CATSPERD is involved in sperm cell hyperactivation. CLEC7A is necessary for the TLR2-mediated inflammatory response and for TLR2-mediated activation of NF-kappa-B. NR6A1 may be involved in the regulation of lipid metabolism. FAM86B2 encodes a protein of unknown function.

Tet+ cells expressed significantly more TTC24, SLC17A9, ITGA7, GPC3, C5AR1, ASB14, GRTP1 and AKAP12 than CXCR3+ CD4 Th1 cells from the same HIV-CVD- participants. C5AR1, also known as CD88, is the main receptor for complement C5a and is a known target in autoimmune and inflammatory diseases. ITGA7 is an integrin alpha subunit that pairs with beta1 to form the main laminin receptor. SLC17A9 encodes a protein involved in vesicular storage and exocytosis of ATP. GPC3 encodes glypican, a cell surface proteoglycan that contains heparan sulfate side chains. The product of AKAP12 mediates the subcellular compartmentation of protein kinases A and C. TTC24, GRTP1 and ASB14 encode proteins of unknown function.

Tet+ cells expressed significantly more WDFY3, SPC25, SKA1, MZB1, MELK, HJURP, HIST2H3A, GZMK, DSCAML1, CDC45, and ANLN than CXCR3- CD4 Tmem cells from the same HIV-CVD- participants. WDFY3 encodes a phosphatidylinositol 3-phosphate-binding protein that functions as a master conductor for aggregate clearance by autophagy. SPC25 and SKA1 are part of the kinetochore complex and needed for proper chromosome separation. MZB1 promotes IgM assembly and secretion, but its role in T cells is unknown. MELK is a serine/threonine-protein kinase involved in various processes including cell cycle regulation. HJURP encodes a centromeric protein that plays a central role in the

incorporation and maintenance of histone H3-like variant CENPA at centromeres. HIST2H3A encodes a core component of the nucleosome. GZMK encodes granzyme K. CDC45 is required for initiation of chromosomal DNA replication. ANLN is an actin-binding protein that is required for cytokinesis. Interestingly, tet+ cells in CVD- subjects also overexpress an antisense gene to IFN-γ (IFNG.AS).

In women with CVD, protein-coding genes including IL17D, PTGIR, TMEM198, ADGRA2, LURAP1, and CLNK were significantly higher in tet+ cells than in Th1 and Tmem cells from the same HIV-CVD+ subjects. Most notably, IL17D encodes interleukin IL-17D, also known as IL-27. This pro-inflammatory cytokine induces expression of IL-8 and GM-CSF, known drivers of atherosclerosis. PTGIR encodes the prostacyclin receptor, a very important vasodilator and inhibitor of thrombosis. TMEM198 promotes LRP6 phosphorylation by casein kinases and thereby plays a role in Wnt signaling. ADGR2 plays a key role in Wnt7-specific responses, such as endothelial cell sprouting. LURAP1 acts as an activator of the canonical NF-kappa-B pathway and drive the production of proinflammatory cytokines. CLNK encodes a protein which enhances CD3-triggered activation of T-cells and subsequent IL2 production. SLC86A2 and SLC12A5 were also higher in tet+ cells, and encode sodium/nucleoside cotransporter.

In women with CVD, protein-coding genes including MMP28, COL18A1, and TIAF1 were significantly higher in tet+ cells than in Th1 cells from the same HIV-CVD+ subjects. The matrix metalloproteinase MMP28 can degrade casein and probably extracellular matrix proteins. MMP28 in tet+ cells was also significantly higher than in Tregs. COL18A1 encodes collagen XVIII, which also has anti-angiogenic properties. TIAF1 encodes TGFB1-Induced Anti-Apoptotic Factor 1, which inhibits the cytotoxic effects of TNFα. CGN encodes a protein of unknown function.

In CVD+ participants, VWDE, TIMP3, SCOC.AS, LEMD1, CCDC142, LDHD, ARAP3, and APOBEC3B were significantly more highly expressed in tet+ cells than in CXCR3-Tmem. VWDE encode von Willebrand factor D and EGF domain, a protein with unknown function. TIMP3 encodes an inhibitor of matrix metalloproteinases, a group of peptidases involved in degradation of the extracellular matrix. LEMD1 is a paralog of TMPO, which encodes Thymopoietin. CCDC142 and ARAP3 encode proteins of unknown function. SCOC.AS is an anti-sense RNA of SCOC, which encodes a short coiled-coiled domain-containing protein that localizes to the Golgi apparatus. LDHD encodes lactate dehydrogenase. CBX2 is an important epigenetic reader involved in cell proliferation and differentiation.

In CVD- participants, IL7R was significantly higher expressed in tet+ cells, and DUSP4 and HLA.DPB1 were significantly lower expressed, than in Tregs from the same CVD- subjects. DUSP4 encodes the dual specificity protein phosphatase, and regulates mitogenic signal transduction by dephosphorylating both Thr and Tyr residues on MAP kinases ERK1 and ERK2.

In CVD+ participants, NELL2, THEMIS, CTSL and MMP28 were significantly higher expressed in tet+ cells than in Tregs. NELL2 encodes a protein required for neuron survival through the modulation of MAPK pathways. THEMIS plays a central role in late thymocyte development by controlling both positive and negative T-cell selection. CTSL encodes Cathepsin L, which is associated with the risk of cardiovascular mortality.

Summary of this study

This is the first study of tet+ T cells in subjects with and without CVD by scRNA-sequencing. The position of tet+ cells places them closer to Th1 and Tmem than Treg. The previously reported expression of FoxP3 in these same cells (Kimura et al., 2018) suggested that they were Tregs, but the present data suggest that they are not true Tregs, although they share many genes with the Treg transcriptome. Only one gene, IL7R, was significantly higher expressed in tet+ cells than in Tregs in CVD- subjects. However, tet+ cells transcriptomes from subjects with CVD were placed even further away from Tregs and closer to Tmem. Based on these data, we propose to call tet+ cells in healthy subjects Treg-like (rather than Tregs).

The finding that the same clonotypes appeared in some tet+, Tmem and Th1 cells, combined with the finding that tet+ cells are more similar to Tmem and Th1 than Tregs supports the idea that tet+ cells are not derived from true Tregs. If they were, one would expect shared clonotypes between tet+ and Tregs. It is known that induced Tregs (iTregs) derive from conventional CD4 T cells and are unstable. Our findings support the concept that tet+ cells may share some features with iTregs in women without cardiovascular disease, and lose these features in women with cardiovascular disease

In mouse studies of bulk Tregs, some showed plasticity and acquired traits of Th17, Th1 (Wolf et al., 2020), or TFH (Gaddis et al., 2018). The present data are consistent with these findings and now sharpen the focus on tet+ cells in humans. In the only study of tet+ T cells in mice (Wolf et al., 2020) identified by mouse ApoB-p6 tetramer, the tet+ cells showed more effector and central memory markers compared to non-tet+ cells, which was further exacerbated by atherosclerosis (in *Apoe*$^{-/-}$ mice). Consistent with this, we show here that the transcriptomes of tet+ cells in women with CVD are significantly closer to Tmem than to Treg transcriptomes. Mouse tet+ cells also showed more proliferation than tet- cells. Although we did not directly measure proliferation in the present study, we find centromere and kinetochore genes enriched in tet+ cells, suggesting potential proliferation. Lineage tracker mice showed more exTregs among mouse tet+ cells than in ApoB-non-reactive cells. Lineage tracking is not possible in humans. Based on our data, we propose that tet+ CD4 cells in humans start out Treg-like and move significantly closer to Tmem cells in humans with CVD.

Our clonotype analysis showed that Tmems have the highest clonality index, probably reflecting the fact that this population contains clones of memory cells that had responded to previous infections (Picarda and Benedict, 2018). Some chronic viral infections like cytomegaly virus (CMV) induce a large and stable memory CD8 and CD4 T cell response. Surprisingly, the tet+ cells have a lower clonality index, suggesting a diverse population.

In conclusion, we report the first single cell transcriptomes of tet+ cells in humans. Although the transcriptomes show some similarities with Tregs in women without cardiovascular disease, their transcriptomes are markedly different from Tregs sorted from PBMCs as CD25hi and CD127-. Also, there are no shared clonotypes between tet+ cells and Tregs, calling into question the concept that these cells derive from Tregs.

References

Gaddis, D.E., Padgett, L.E., Wu, R., McSkimming, C., Romines, V., Taylor, A.M., McNamara, C.A., Kronenberg, M., Crotty, S., Thomas, M.J., et al. (2018). Apolipoprotein AI prevents regulatory to follicular helper T cell switching during atherosclerosis. Nat Commun *9*, 1095.

Gregersen, I., Sandanger, Ø., Askevold, E.T., Sagen, E.L., Yang, K., Holm, S., Pedersen, T.M., Skjelland, M., Krohg-Sørensen, K., Hansen, T.V., et al. (2017). Interleukin 27 is increased in carotid atherosclerosis and promotes NLRP3 inflammasome activation. PLoS One *12*, e0188387.

Han, A., Glanville, J., Hansmann, L., and Davis, M.M. (2014). Linking T-cell receptor sequence to functional phenotype at the single-cell level. Nat Biotechnol *32*, 684–692.

Kimura, T., Kobiyama, K., Winkels, H., Tse, K., Miller, J., Vassallo, M., Wolf, D., Ryden, C., Orecchioni, M., Dileepan, T., et al. (2018). Regulatory $CD4^+$ T Cells Recognize Major Histocompatibility Complex Class II Molecule–Restricted Peptide Epitopes of Apolipoprotein B. Circulation *138*, 1130–1143.

Korsunsky, I. (2019). Fast, sensitive and accurate integration of single-cell data with Harmony. Nature Methods *16*, 16.

Lin, Z., Qian, S., Gong, Y., Ren, J., Zhao, L., Wang, D., Wang, X., Zhang, Y., Wang, Z., and Zhang, Q. (2017). Deep sequencing of the T cell receptor β repertoire reveals signature patterns and clonal drift in atherosclerotic plaques and patients. Oncotarget *8*, 99312–99322.

Picarda, G., and Benedict, C.A. (2018). Cytomegalovirus: Shape-Shifting the Immune System. J Immunol *200*, 3881–3889.

Saigusa, R., Winkels, H., and Ley, K. (2020). T cell subsets and functions in atherosclerosis. Nat Rev Cardiol *17*, 387–401.

Shah, A.S.V., Stelzle, D., Lee, K.K., Beck, E.J., Alam, S., Clifford, S., Longenecker, C.T., Strachan, F., Bagchi, S., Whiteley, W., et al. (2018). Global Burden of Atherosclerotic Cardiovascular Disease in People Living With HIV: Systematic Review and Meta-Analysis. Circulation *138*, 1100–1112.

Soto, C., Bombardi, R.G., Kozhevnikov, M., Sinkovits, R.S., Chen, E.C., Branchizio, A., Kose, N., Day, S.B., Pilkinton, M., Gujral, M., et al. (2020). High Frequency of Shared Clonotypes in Human T Cell Receptor Repertoires. Cell Reports *32*, 107882.

Stubbington, M.J.T., Lönnberg, T., Proserpio, V., Clare, S., Speak, A.O., Dougan, G., and Teichmann, S.A. (2016). T cell fate and clonality inference from single-cell transcriptomes. Nat Methods *13*, 329–332.

Venturi, V., Quigley, M.F., Greenaway, H.Y., Ng, P.C., Ende, Z.S., McIntosh, T., Asher, T.E., Almeida, J.R., Levy, S., Price, D.A., et al. (2011). A Mechanism for TCR Sharing between T Cell Subsets and Individuals Revealed by Pyrosequencing. J.I. *186*, 4285–4294.

Wolf, D., and Ley, K. (2019). Immunity and Inflammation in Atherosclerosis. Circulation Research *124*, 315–327.

Wolf, D., Gerhardt, T., Winkels, H., Michel, N.A., Pramod, A.B., Ghosheh, Y., Brunel, S., Buscher, K., Miller, J., McArdle, S., et al. (2020). Pathogenic Autoimmunity in Atherosclerosis Evolves From Initially Protective Apolipoprotein B100-Reactive CD4+ T-Regulatory Cells. Circulation *142*, 1279–1293.

Zhang, L., Cham, J., Paciorek, A., Trager, J., Sheikh, N., and Fong, L. (2017). 3D: diversity, dynamics, differential testing – a proposed pipeline for analysis of next-generation sequencing T cell repertoire data. BMC Bioinformatics *18*, 129.