20240905 10X Symposium talk outline

Title: Foreign and autoreactive CD4 conventional and regulatory T cells in T1D and healthy subjects

Humans: Thomas H Edwards2\*, Janice Chen1\*, Vivian Gersuk2, David J Rawlings3, Jane Buckner1, Karen Cerosaletti1

Centers for 1Translational and 2Systems Immunology, and 3Seattle Children’s Research Center, Seattle WA; \*Co-1st authors

Abstract:

Regulatory T cell (Treg) dysfunction is implicated in type 1 diabetes (T1D), contributing to beta cell destruction by islet autoreactive conventional T cells (IAR Tconv). However, little is known about IAR Tregs. Previously, we observed limited TCR sharing between IAR Tconv and Treg. To investigate if this is unique to IAR T cells, we compared foreign antigen (ag) vs IAR CD4 Tconv and Treg in 3 HC and 3 T1D subjects using multimodal 10X sequencing. PBMC were stimulated with islet peptides, CEFX, or CD3/CD28 antibodies (ab) for 20 h. After, each donor and stimulation were stained with a unique hashtag ab, combined by stimulus, and stained for CD154 and CD137. Activated cells were enriched, stained with CITE-seq and flow abs and sorted for CD154+ and/or CD137+ cells. Polyclonal activated cells were added to the ag activated cells to enable clustering of ag reactive cells against the total CD4 landscape and run using 10X GEM-X XLEAP chemistry. The IAR and polyclonal activated Tconv and Treg cells showed similar transcriptomic signatures and cell recovery, while CEFX cells displayed a distinct transcriptomic profile and were recovered in greater numbers. Naïve and memory Treg and Tconv cells were distinguished by RNA-seq and CITE-seq. Ag specific cells were stringently gated using protein tags for ongoing investigation of transcript profiles and TCR repertoires of Treg vs Tconv cells between stimulations. This represents a technical advance to analyze both IAR Tconv and Tregs in relation to other specificities in T1D.

Talk notes/goals:

-This study is very much a WIP, and the audience will be of mixed background (not very much immunology expertise), so we want to focus on the technical details of the 10X run + data with enough background/context for it all to make sense.

-Provide sufficient context for a mixed audience to understand the motivation of the study and this particular experimental design.

-Provide a story of one of our earlier GEM-X XLEAP sequencing runs.

-Highlight technical/experimental details that may be of interest to a multidisciplinary audience.

-Motivate the overall conclusion that the experiment was executed successfully to allow us to explore the question that we set out to investigate.

Explanation of 10X/Illumina/BRI pipeline mix

-NextSeq200 (Marie), P4 XLEAP flowcell, 10X GEM-X for libraries (5' GEX/TCR/ FB)

Metrics…

-cell recovery percentages

-UMI counts per cell

-genes per cell

-multiplet rate

-percent cells with productive V-J spanning pair

-UMIs per cell boxplots of TRA/TRB

Plot pool ID on the UMAPs!

Identify other non GEM-X dataset to compare against.

Integrate by donorId

--and maybe also pool!

Optimize clustering resolution with unsupervised metrics: median silhouette coefficient, Davies-Bouldin index, Calinksi-Harabasz index

Then optimize embeddings with scDEED

Celltype inference with Seurat’s stuff?

Celltype calling from CITEseq (change name) data

-figs for Janice

--different log scalings

--some kind of shiny thing where we can interact with the figure??

----------------------------------------------Vivian------------------------------------------------------------

“GEM-X is new 10x chemistry that changes the capture and processing of the cells.  It has many improvements in capture efficiency, better detection of genes, better TCR detection and clonotype identification.  XLEAP is unrelated and is new chemistry from Illumina that changes the sequencing chemistry, improving the quality of sequencing data output.”

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------------------------------------------Matt D----------------------------------------------------------------

 If I were going to add anything, it would be in slides 9-11, or a comparison of this experiment to previous ones at/after slide 13 or 17.

A couple thoughts:

* You’re going to need to explain a little bit about what CEFX is, or at least why we’re using it.  Basically that it allows us to capture cells that recognize a bunch of different infectious agents.
* For slide 8, it would be great to have a simple cartoon.  Could be Tconv on the left, Treg on the right, IAR on top, CEFX-stimulated on the bottom.  Show a thin line between IAR Tconv and Treg indicating little sharing between them.  Then a question mark between CEFX Tconv and Treg.
* It would be really really nice if you had enough of a hint of results to come back to that cartoon at the end and say “here’s what we think is going on”.  I realize we may not be ready for that, or Karen may not want to announce that level of result yet.  If not, could show the cartoon again just to bring everyone back to the main question we’re trying to answer.
* A lot of the intro and goal of the project relates to Tconv vs. Treg.  Are you planning to show anything regarding identifying them?  I know you’re just working on that now so it may not be ready.

Also, some language specifics that would be good to keep in mind, both for what you put in slides and when you’re talking.  Specifics:

* XLEAP is new Illumina sequencing chemistry, not 10x
* GEM-X is 10x’s name for their new reagents and chip which they claim works better
* Surface marker quantification in 10x is not CITE-seq, even though people often call it that (it’s like Kleenex for tissues).  CITE-seq is a different protocol, 10x refers to their antibody barcode quantification as “Feature Barcode” technology
* 10x calls their TCR sequencing method “Single Cell Immune Profiling” or “V(D)J sequencing”
* It might also be important to know or point out that we hashtagged samples using DNA-barcoded antibodies and Feature Barcode technology. 10x has their own hashtagging kit that they call CellPlex, but that’s not what we use.

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--------------------------------------------Vivian--------------------------------------------------------------

 Cells were prepared on two days from 3 donors each.  For each batch, approximately 50,000 antigen-specific cells (I think they were Ag-specific?) were combined with 3000 filler cells (polyclonal???) and loaded on one channel of a 10x chip.  Viability was 91-92%.  Capture was ~18,300 and 19,100 respectively.

 GEX, TCR, and FB libraries were pooled and run on a NextSeq 2000 sequencer using the new P4 XLEAP chemistry, which offers enhanced data quality.

 Suggest you avoid BRI identification numbers like P390 or Cersaletti ID numbers.  Switch to labeling as “Donors 1,2,3…”  or “HC1,2,3 and T1D 1,2,3” perhaps

 Specify that previous work was plate sorted single cell analysis using SMARTseq to profile xxx (number of cells) when you show the circos plots.  And suggest you add an explanation of how those plots work and what they show.  Then Karen had nice verbiage to explain why we wanted to move to 10x to profile tens of thousands of cells.

 We chose to use GEM-X to maximize the chance for higher cell capture and gene and TCR detection since that was a critical component to this study.

 We did get 83-88% paired TCRs in the captured cells, a rate higher than we’ve observed with NextGEM. (check this with Matt D and Matt L)

 We talked about an alternative name for staining with cell surface markers and thought you could use “TotalSeq” as a one word label, if you like, instead of ADT.  And instead of RNAseq, TCRseq, and CiteSeq, you could use GEX, TCR, and FB.

 Suggest you be clear about data plots/figure that come from this 10x work vs from the sc plate sorts.  If you go back to the circos plots again at the end, use that to emphasize what this new analysis adds to the conclusions.

 On the Cell Ranger output plots, think about adding the metrics of genes detected, reads/cell, UMI/cell, % saturation, TCR paired detection

 Explain how the cell surface markers were used in the analysis and what the CD69 and CD154 tell us.

 Were differences observed between HC and T1D?  How did this new study add to our knowledge?

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------------------------------------------Talk outline skeleton----------------------------------------------

Slide 1: title

Slide 2: acknowledgements

Slide 3: outline

Slide 4: T cell intro

-Overall role in immunity/autoimmunity

-specifically, Treg and Tconv subsets

--Ask Alex Hu for Kenneth Lai’s cartoon slides

Slide 5: TCR intro

-what’s a T-cell receptor

-\*very\* brief VDJ recombination overview to explain diversity?

--"through a special process called recombination T-cells develop a wide repertoire of TCRs that play a key role in helping the immune system recognize things it has seen before”

-concepts of expansion/clones and how this might reveal relationships between Tcell subsets in some particular immune/autoimmune context

Slide 5: T cells in T1D

-autoimmune damage to pancreatic islet beta cells, how T effectors perhaps involved

-how Treg dysfunction could contribute

-Antigen-specific Tregs less well studied in T1D

Slide 6: prior work

-previous scRNAseq (plate-sorted) showed limited TCR sharing between IAR Tregs and Tconvs

Slide 7: outline again for signposting---------------------------------------------------------------

Slide 8: Present study motivation

-We want to compare the TCR relationships of (IAR-Treg:IAR-Tconv) vs (CEFX-stim-Treg:CEFX-stim-Tconv) to see if there’s anything distinct about Tregs in T1D vs adaptive immunity more broadly.

Slide 9: experimental scheme

-samples/T1D vs HC/CITEseq markers/stimulations

Slide 10: sample prep slide(s) from Janice

-samples were fresh blood, which lead to 2 pools rather than 1

-tested surface Ab markers/FB/oligos to check for saturated binding sites

Slide 11: Genomics core GEM-X / XLEAP experience / advice / feedback

-Any particular adjustments that had to be made?

-Anything other labs should watch out for?

-Sounds like it was pretty straightforward?

Slide 12: outline again for signposting-------------------------------------------------------------

Slide 13: QC/metrics

-cell loadings vs recovery

-UMI counts

-feature counts/mito

-cells passing QC

Slide 14: demultiplexing

Slide 15: cell counts by donor/stim

🡪 downsampling CEFX cells because there were so many

Slide 16: RNA UMAPs

Slide 17: TCR recovery figures

Slide 18: TCR airline on UMAP

🡪 conclusion is that we have the data in hand to investigate the question that we sought out to answer: compare the TCR relationships of (IAR-Treg:IAR-Tconv) vs (CEFX-stim-Treg:CEFX-stim-Tconv)

Slide 19: conclusions

Slide 20: acknowledgements again / fin