MYOFIBROBLAST TO T-ZONE RETICULAR CELL RATIO IDENTIFIES UNICENTRIC CASTLEMAN DISEASE LYMPH NODES

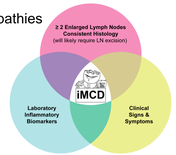
Joshua D Brandstadter[1,2], Mechthild Lütge[3], Megan S Lim[4], Adam Bagg[5], Ira D. Miller[1], Michael V. Gonzalez[1], Bridget Austin[1], Salvatore F. Priore[5], Burkhard Ludewig[3], David C. Fajgenbaum[1] and Ivan Maillard[2,6]

1. Center for Cytokine Storm Treatment & Laboratory, University of Pennsylvania, Philadelphia, USA. 2. Division of Hematology and Oncology, University of Pennsylvania, Philadelphia, PA, USA 3. Institute of Immunobiology, Kantonsspital St. Gallen, St Gallen, Switzerland. 4. Department of Pathology and Laboratory Medicine, Memorial Sloan Kettering Cancer Center, New York, New York, USA, 5. Department of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia, PA, USA. 6. Division of Hematologic Malignancies, Memorial Sloan Kettering Cancer Center, New York, New York, USA

INTRODUCTION

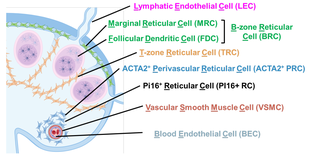
Castleman Disease is a rare, poorly understood polyclonal lymphoproliferative disorder

- Castleman Disease is a collection of lymphadenopathies defined by the histologic appearance
  - Idiopathic multicentric Castleman Disease (iMCD)
  - HHV8-associated multicentric Castleman Disease
  - POEMS-associated multicentric Castleman Disease
  - Unicentric Castleman Disease (UCD)
- **Discordant interpretations of LN biopsies**
  - Non-specific histologic features
  - Intranodal heterogeneity
  - Close histo-mimetics
  - Rare disease
- Siltuximab is the only FDA approved treatment for iMCD
  - Only ~1/3 of patients respond
  - No consensus second line for refractory patients



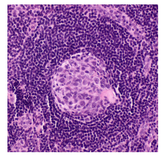
Lymph node stromal cells are heterogenous, specialized fibroblasts and endothelial cells with important immunoregulatory roles

- Host defense
- Autoimmunity
- **Alloimmunity**
- Lymphoma microenvironment
- Checkpoint inhibition
- CAR T cell activation



Recent data implicates lymph node stromal cells in Castleman pathogenesis with possible clonal growth in the unicentric Castleman Disease

- Lymph node stromal cells are histologically aberrant
- CXCL13 is up in disease flare in multicentric disease
- Genetic evidence suggests clonality in lymph node stromal cells in unicentric disease
  - HUMARA assay shows monoclonal pattern of X-chromosome inactivation in unicentric Castleman lymph nodes
  - No heavy chain or T-cell receptor gene rearrangements



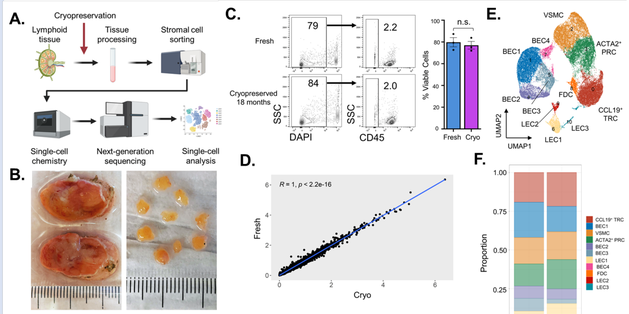
METHODS

Amassing the largest collection of single cell RNA sequencing data for human lymph node stromal cells

- **Resting/normal: 2 lymph nodes**
- **Reactive: 5 lymph nodes**
- **Unicentric Castleman: 5 lymph nodes**
- **Other: 11 lymph nodes**
  - Discordant pathologist interpretations, "reactive with Castleman-like changes," multifocal lymphadenopathy, systemic symptoms

METHODS (CONT.)

Figure 1. Whole-tissue cryopreservation enables biobanking to study lymph node stromal cells.

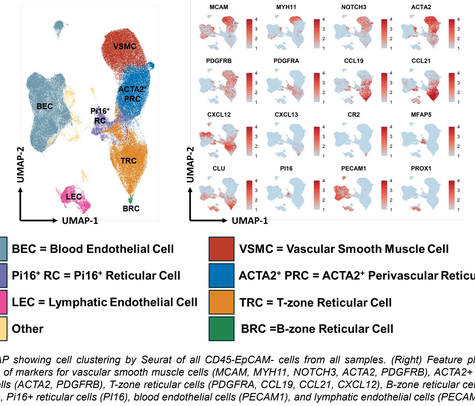


Modified from Brandstadter et al., EJI. 2023.

Whole tissue cryopreservation preserves cell viability to allow biobanking of lymphoid tissue. (A) Schematic overview of the procedure to process lymphoid tissue for single-cell RNA sequencing. (B) Fresh tonsils received as pathology excess specimen (top) and after gross dissection and cutting into 2–3mm pieces (bottom). As pictured, eight-to-ten pieces (ca. 150 mg total) were placed in each cryovial with 1 mL DMSO-containing cryopreservative. (C) Three LNs were processed fresh or after cryopreservation for 18 months prior to enzymatic digestion, CD45−EpCAM− cell sorting, and single-cell RNA sequencing. Flow cytometry analysis of freshly processed (top) and cryopreserved (bottom) LN cells. The first column represents live/dead discrimination by DAPI uptake among all singlets. The second column shows the percentage of CD45− cells among live singlets in the fresh and cryopreserved tissue. Viability on all samples is also shown as a bar graph (n = 3 LNs, p = 0.64 by Student's t-test). (D) Linear regression of gene expression between freshly processed and cryopreserved LNs. Pearson correlation with associated p-value listed in graph. (E) UMAP showing Seurat-based clustering with labeled cell types based on expression of known markers. (F) Colors in each bar define proportions of each cell-type within the entire sample.

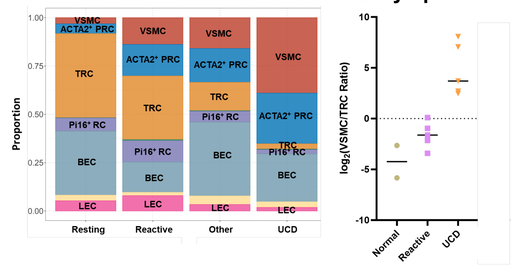
RESULTS

Figure 2. Heterogenous lymph node fibroblasts and endothelial cells can be identified by single-cell RNA sequencing.



(Left) UMAP showing cell clustering by Seurat of all CD45-EpCAM- cells from all samples. (Right) Feature plots showing expression of markers for vascular smooth muscle cells (MCAM, MYH11, NOTCH3, ACTA2, PDGFRB), ACTA2+ perivascular reticular cells (ACTA2, PDGFRB), T-zone reticular cells (PDGFRA, CCL19, CCL21, CXCL12), B-zone reticular cells (CXCL13, CR2, CLU), Pi16+ reticular cells (PI16), blood endothelial cells (PECAM1), and lymphatic endothelial cells (PECAM1, PROX1).

Figure 3. Vascular smooth muscles cells are differentially abundant in Unicentric Castleman Disease lymph nodes.

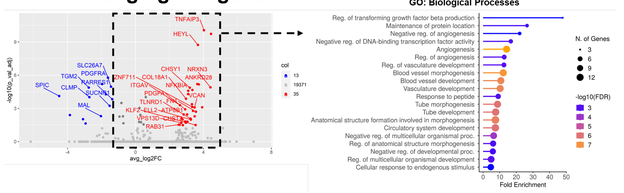


(Left) Average proportions of all cell-types for each group of samples (n=2 for resting; n=5 for reactive, n=5 for UCD, n=11 for other). These cell-types include vascular smooth muscle cells (VSMC), ACTA2+ perivascular reticular cells (ACTA2+ PRC), T-zone reticular cells (TRC), Pi16+ reticular cells (Pi16+ RC), blood endothelial cells (BEC), and lymphatic endothelial cells (LEC). (Right) Log-transformed ratio of VSMCs to TRCs for resting, reactive, and UCD lymph nodes.

Figure 4. VSMC/TRC ratio suggests UCD diagnosis compared to controls.

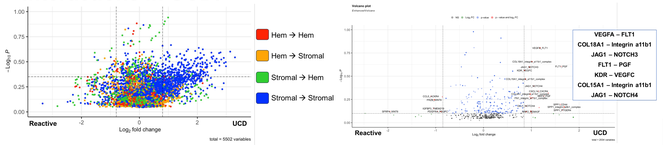
(Left) UMAPs for each individual sample of "other" histology lymph nodes that was not neither definitely Castleman or not-Castleman histology. UMAP clusters and colors correspond to those identified for all samples collectively in Figure 2A. Some of these lymph nodes were classified as "other" due to discordant interpretations between pathologists, described as "reactive with Castleman-like changes," or presented with multifocal lymphadenopathy and/or systemic symptoms making unicentric Castleman an unlikely diagnosis. (Right) Log-transformed ratio of VSMCs to TRCs for resting, reactive, and UCD lymph nodes in addition to "other" lymph nodes with red circle around all samples with VSMC/TRC ratio resembling UCD lymph nodes.

Figure 5. UCD vascular smooth muscles cells express more TNF and angiogenic genes.



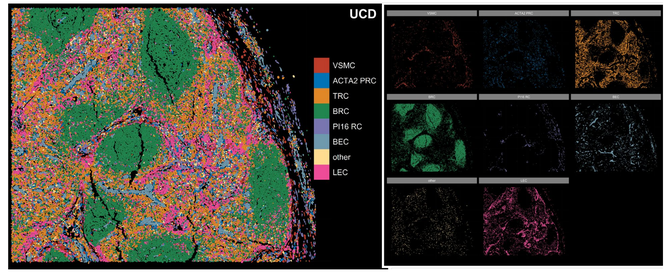
(Left) Volcano plot shows vascular smooth muscle cell differentially expressed genes. All genes with adjusted p values < 0.05 and log2 fold-change > 1 are labelled and colored for UCD (red) and reactive (blue). (Right) The 35 genes differentially expressed by vascular smooth muscle cells in UCD were then analyzed by gene ontology (biological process database) to identify patterns of gene expression.

Figure 6. Interaction analysis identifies stromal-stromal interactions in UCD.

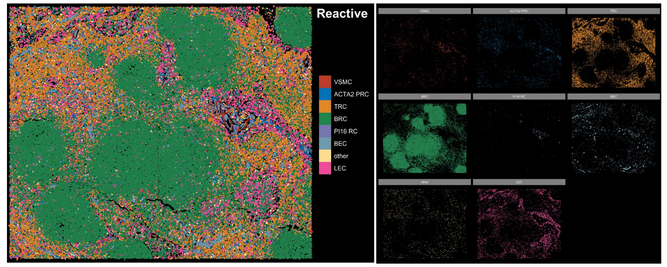


(Left) Volcano plot shows differentially expressed ligand/receptor pairs between UCD on the right and reactive on the left. Dots are colored based on the cell-type of the ligand and receptor. (Right) Volcano plot shows differentially expressed ligand/receptor pairs between UCD on the right and reactive on the left with pairs labeled when adjusted p values < 0.05 and log2 fold-change > 1. A selection of the ligand/receptor pairs differentially expressed by UCD is highlighted separately in a box.

Figure 7. Spatial transcriptomics localizes VSMCs to the subcortical, perivascular, and medullary regions



The 10X Xenium platform for spatial transcriptomics used a 550-probe panel (500 pre-defined probes targeting 'immuno-oncology' genes plus 50 custom probes) with single-cell resolution to define gene expression within a microscopic field of view on FFPE tissue. The transcriptomic signatures defined in the gene expression database were used as a reference to define the same stromal cell subsets within the lymph node architecture. The above representative image shows several follicles near the cortex of a unicentric Castleman Disease lymph node. Vascular smooth muscle cells (VSMC) in red are visible around vessels and below the cortex. As expected, B-zone reticular cells (BRC) are identified in green within the germinal centers while T-zone reticular cells (TRC) in orange are found within the interfollicular space. Blood and lymphatic endothelial cells (BEC, VEC) are also identified in vascular patterns and other reticular cell populations are identified.



The above representative image shows several follicles near the cortex of a reactive histology lymph node. Vascular smooth muscle cells (VSMC) are again observed in red around vessels and below the cortex, as they were seen in the unicentric Castleman Disease lymph node. Similarly, B-zone reticular cells (BRC) in green and T-zone reticular cells (TRC) in orange are observed in the germinal centers and interfollicular spaces, respectively. Blood and lymphatic endothelial cells (BEC, VEC) are identified in vascular patterns and other reticular cell populations are seen in similar distribution to as they were observed in the unicentric Castleman disease lymph node.

CONCLUSIONS

We demonstrate that specific changes in lymph node stromal cells can be leveraged to help diagnose Castleman Disease.

- New tools, including whole-tissue cryopreservation, enable the study of LNSCs in human lymphoid tissue
- VSMCs are differentially abundant in unicentric Castleman compared to otherwise inflammatory controls
- VSMC/TRC ratio may assist in the diagnosis of unicentric Castleman Disease

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