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Center of Experimental Rheumatology

Single cell RNA sequencing of Modic type 1 change bone marrow cells reveals dendritic cells as central mediators of inflammatory processes

I. Heggli[1,2], D. Burri[3,4], J. Devan[1,2], N. Herger[1,2], T. Mengis[1,2], C.L. Laux[5], N.A. Farshad-Amacker[6], F. Wanivenhaus[5], M. Betz[5], J.M. Spirig[5], I. Unterfrauner[5], F. Brunner[2], M. Zavolan[3,4], O.Distler[1,2], M. Farshad[5], S. Dudli[1,2]

[1]Center of Experimental Rheumatology, Department of Rheumatology, University Hospital Zurich, University of Zurich, Switzerland; [2]Department of Physical Medicine and Rheumatology, Balgrist University Hospital, Balgrist Campus, University of Zurich, Switzerland; [3]Computational and Systems Biology, Biozentrum, University of Basel, Switzerland; [4]Swiss Institute of Bioinformatics, Switzerland; [5]Department of Orthopedics, Balgrist University Hospital, University of Zurich, Switzerland; [6]Department of Radiology, Balgrist University Hospital, University of Zurich, Switzerland

Background

What are Modic type 1 changes (MC1)?

- Vertebral bone marrow lesions visualized as signal intensity changes on T1-weighted (T1w) and T2w magnetic resonance images
- Prevalent (16%) in chronic low back pain (cLBP) patients.

What we know about the MC1 pathobiology:

- Key hallmark: Bone marrow inflammation
- Few studies suggest a role of neutrophils, monocytes, T-and B-cell cells in inflammatory MC1 processes[1,2,3]

What we do not have:

- a comprehensive understanding of the MC1 cellular composition

Relevance to identify key cellular players that drive inflammatory MC1 processes

- To provide grounds for future cell type specific pathomechanistic studies
- Laying the foundation for the development of disease-modifying MC1 treatments.

Objective

To resolve the MC1 bone marrow cell composition and to identify potential pathomechanistic relevant cell populations

Sample collection and single cell sequencing

From low back pain patients with MC1 undergoing lumbar spinal fusion:

- Per patient: One MC1 and one intra-patient control bone marrow biopsy (MC1: n=4; intra-patient controls: n=4) was collected

Creation of single cell suspension:

- Enzymatic digestion and flushing
- Depletion of CD45+CD66b+ neutrophils by cell sorting

Sequencing and bioinformatic preprocessing:

- 10'000 cells (10x Genomics) (**Figure 1**)

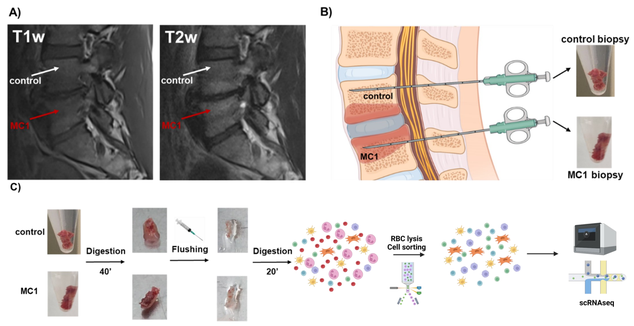

Figure 1: Study design and sample processing workflow. A) Representative MRI of a patient included in this study. B) Sample collection and representative image of collected biopsies. C) Workflow to isolate single cells

Bioinformatic analysis

- Read alignment, count matrix creation (Cell Ranger)
- Quality control, dimensionality reduction (principal component analysis) (*scater*)
- Highly variable genes (HVG) identification (*scran*)
- Clustering (*igraph* using Leiden algorithm)
- Data integration (*fastMNN*)
- Annotation (DISCO database, manually)

<u>MC1 vs. intra-patient-controls:</u>

- Cell fractions: Paired t-tests (*stats*)
- Pseudobulk differential expression analysis: *edgeR*, patient as secondary factor
  - Differentially expressed (DEGs): p-value < 0.05.
  - Hypergeometric overrepresentation analysis (ORA) of DEGs: WEB-based GEne SeT AnaLysis Toolkit with terms considered as significant for false discovery rate (FDR) < 0.2.
- Cell interactions: Cell chat

Patient demographics

Table 1: Patient characteristics. VAS=Visual analogue scale; ODI: Oswestry disability index; DD: degree of disc degeneration (Pfirrmann grade); TES: Total endplate score.

ID	Age	sex	Height [cm]	weight [kg]	BMI [kg/m^2]	smoker	VAS.back	VAS.leg	ODI	DD control	DD MC1	TES control	TES MC1	MC extent of vertebral body height
1	54	m	172	66	22.3	no	3	2	22	2	5	2	6	> 50 %
2	44	f	180	74	22.8	yes	8	9	50	2	4	2	6	> 50 %
3	61	f	164	103	38.3	no	7	7	53	3	5	3	6	> 50 %
4	68	f	152	44.7	19.3	no	5	5	43	3	5	3	6	> 50 %
mean ± sd	56.8 ± 10.2		167 ± 11.9	71.9 ± 24.1	25.7 ± 8.6		5.8 ± 2.2	5.8 ± 3	42 ± 14	2.5 ± 0.6	4.8 ± 0.5	2.5 ± 0.6	6 ± 0	
percentage		75 % f				25 % smoker								100 % > 50 %

Clustering reveals 74 clusters

Cells retained after quality control:

- Total: 69'415 (MC1: 37'365; controls: 32'050)

74 main clusters (Figure 2A)

→Assignment to 18 main clusters (**Figure 2B**)

1. B-cell progenitors
2. B-cells
3. CD4 T-cells
4. CD8 T-cells
5. Conventional dendritic cells (cDCs)
6. Double negative T-cells
7. Erythroblasts
8. Innate lymphoid cells (ILCs)
9. Macrophages
10. Mast cells
11. Monocytes
12. Myeloblasts
13. Natural killer cells (NKCs)
14. Plasma B-cells
15. Plasmacytoid dendritic cells (pDCs)
16. Progenitors
17. Stromal cells
18. Contaminating neutrophils

→ Removal of contaminating neutrophils and erythroblasts from further analysis

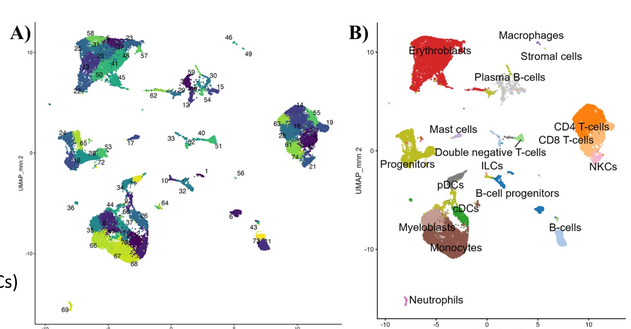

Figure 2: UMAP of Modic type 1 change (MC1) and intra-patient control bone marrow cells. A) 74 cell clusters. B) Clusters assigned to 18 main cell types.

Fractions of plasmacytoid dendritic cells (pDCs), T-cells, and conventional dendritic cells (cDCs) were higher in MC1 than control bone marrow

Higher cell fraction in MC1:

- Plasmacytoid dendritic cells (pDCs)*
- T-cells (CD4 and CD8 T-cells)
- Conventional dendritic cells (cDCs)

*consistently higher in MC1 among all patients

Lower cell fractions in MC1:

- Double negative T-cells
- B-cell progenitors
- Myeloblasts

Table 2: Cell type fractions in MC1 and intra-patient control bone marrow after depletion of CD66b+CD45+ cells by cell sorting.

	Overall	Control	MC1	P-value MC1 vs. control	Fractional change MC1 relative to control	Direction
Plasmacytoid dendritic cells (pDCs)	3.06 ± 1.1	2.4 ± 0.73	3.73 ± 1.05	0.12	55.42	↑
CD4 T-cells	11.76 ± 6.78	9.97 ± 3.99	13.55 ± 9.1	0.29	35.91	↑
CD8 T-cells	9.17 ± 5.14	7.83 ± 4.03	10.5 ± 6.38	0.24	34.10	↑
Concentiona dendritic cells (cDCs)	2.49 ± 0.6	2.13 ± 0.38	2.85 ± 0.58	0.20	33.80	↑
B-cells	4.65 ± 2.45	4.4 ± 1.77	4.9 ± 3.28	0.58	11.36	
Mast cells	1.23 ± 0.4	1.17 ± 0.58	1.29 ± 0.15	0.63	10.26	
Stromal cells	0.21 ± 0.1	0.2 ± 0.11	0.22 ± 0.1	0.79	10.00	
ILCs	0.11 ± 0.08	0.11 ± 0.09	0.12 ± 0.07	0.83	9.09	
Plasma B-cells	5.69 ± 1.69	5.64 ± 1.37	5.75 ± 2.19	0.90	1.95	
Natural killer cells (NKCs)	1.59 ± 0.77	1.58 ± 0.57	1.6 ± 1.03	0.95	1.27	
Monocytes	16.8 ± 5.13	17.51 ± 5.52	16.08 ± 5.44	0.31	-8.17	↓
Macrophages	0.33 ± 0.42	0.34 ± 0.49	0.31 ± 0.42	0.60	-8.82	↓
Progenitors	11.85 ± 4.8	12.61 ± 3.26	11.09 ± 6.46	0.50	-12.05	↓
Myeloblasts	5.72 ± 1.81	6.48 ± 1.83	4.96 ± 1.67	0.12	-23.46	↓
B-cell progenitors	3.18 ± 3.08	3.63 ± 4.27	2.72 ± 1.82	0.51	-25.07	↓
Double negative T-cells	1.88 ± 2.48	2.35 ± 3.31	1.41 ± 1.68	0.33	-40.00	↓
Contaminating neutrophils	1.28 ± 0.82	1.43 ± 0.41	1.13 ± 1.20	NA	NA	
Erythroblasts	19.34 ± 4.83	20.59 ± 2.52	18.09 ± 6.63	NA	NA	

Most transcriptomic changes occur in MC1 plasma B-cells and cDCs

Plasma B-cells (1118 DEGs) and cDCs (913 DEGs) had the highest number of DEGs between MC1 and intra-patient controls

pDCs **Strongest (and only consistent) fractional increase in MC1**

cDCs **Among the top two cell types with most transcriptomic changes**

➢ **Further exploration of dendritic cells in MC1**

Dendritic cells in MC1 bone marrow make more cellular interactions and have a pro-inflammatory transcriptome suggestive for T-cell activation

	Cell interactions MC1 vs. control	Transcriptomic changes MC1 vs. control
pDCs	**+ 46 %** Attributed to interaction increase with other pDCs, CD4 T-cells, and cDCs	Adaptive immune response; Immune response; Interleukin-2 production; T cell activation; Response to purine-containing compound; $-\log_{10}$ FDR
cDCs	**+ 26 %** Attributed to interaction increase with other cDCs, CD4 T-cells, and pDCs	Leukocyte differentiation; Regulation of GTPase activity; Regulation of cell-cell adhesion; Leukocyte migration; T cell activation; $-\log_{10}$ FDR

Discussion

Dendritic cells in MC1:

➢ Potential regulators orchestrating inflammatory processes

➢ Both dendritic cell types seem to be involved in T-cell activation

➢ Explanation for increased T-cell fractions

Potential initiation of an adaptive immune processes in MC1

Potential activation mechanism of pDCs in MC1:

- pDCs expand and become activated by neutrophil extracellular traps (NETs) (purine containing compounds)

→ Neutrophils infiltrate and are activated in MC1[1,2]

TAKE HOME MESSAGE

Dendritic cells expand in MC1 bone marrow and might be potential key players driving inflammatory processes

IMPLICATIONS

Dendritic cells might be potential treatment targets to alleviate inflammation in MC1

References: [1]Heggli I et al (2023). Ann Rheum Dis. [2]Dudli S et al. (2022). Osteoarthr Cartl Open. [3]Dudli S et al. (2017). Euro Spine J.

Dudli Lab: www.dudli-lab.ch

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