Accumulation of activated TCRαβ$^+$CD8$^+$ and TCRVδ1$^+$T cells in vertebral bone marrow of Modic change 1 patients

Jan Devan[1,2], Nick Herger[1,2], Irina Heggli[1,2], Melina Perez-Vertti Valdes[1,2], Tamara Mengis[1,2], Mazda Farshad[3], Oliver Distler[1], Stefan Dudli[1,2]
[1]*Center of Experimental Rheumatology, Department of Rheumatology, University Hospital Zurich, University of Zurich, CH,* [2]*Department of Physical Medicine and Rheumatology, Balgrist University Hospital, Balgrist Campus, University of Zurich, CH,* [3]*Department of Orthopedics, Balgrist University Hospital, University of Zurich, CH.* email:Jan.Devan@balgrist.ch **Disclosures: JD(N), NH(N), IH(N), MPV(N), TM(N), MF(N), OD(N), SD(N)**

INTRODUCTION: Modic type 1 changes (MC1) are painful vertebral bone marrow lesions defined by signal intensity changes on magnetic resonance imaging (MRI). Endplate damage is strongly associated with MC1, and endplate damage causes commingling of the immune-privileged disc with bone marrow leukocytes. Hence, an autoimmune response against disc tissue seems plausible in MC1. Evidence for the role of the adaptive immune system stems from both animal and human studies. For example, implanting nucleus pulposus cell surrogates into rat tail bone marrow leads to an infiltration of T cells around the surrogates and MC1-like signal intensity changes on MRI. Moreover, increased plasma cell infiltrates in human MC1 biopsies suggest B cell involvement in MC1. However, a comprehensive investigation of immune system remodeling in MC1 lesions is missing, and that could identify the underlying autoimmune mechanisms. This study therefore aimed to discover changes in the bone marrow immune cell composition in MC1.

METHODS: Vertebral bone marrow aspirates from patients undergoing lumbar spinal fusion surgery (n=17) were taken before screw insertion through the pedicle screw trajectory. In patients with MC1 (n=8), one aspirate was taken from the MC lesion, and one other from visually unaffected bone marrow adjacent to MC1. From patients with no MC1 (n=9), one vertebral bone marrow aspirate was processed. Mononuclear cells were isolated by density gradient centrifugation and single-cell suspensions were cryopreserved until the final analysis. For screening of dysregulated hematopoietic-origin cell populations in the MC1 bone marrow, we used a broad spectral flow cytometry-based immunophenotyping panel. To avoid batch effects, we optimized the multiplexing technique which allows the analysis of all samples together in one tube. We utilized the highly expressed markers CD45 on immune cells to create a unique fluorescent barcode on each sample. One to three out of five antibodies against CD45 but conjugated with different fluorochromes were used to create all 25 possible combinations. Barcoded samples were mixed and co-stained with 36 antibodies against surface markers, allowing us to distinguish main immune cell lineages such as B cells, T cells, NK cells, dendritic cells, monocytes/macrophages, basophils, and ILCs. Simultaneously, it allows us to follow the activation and functional maturation of these cell subsets. Samples were analyzed on Cytek Aurora spectral flow cytometer using SpectroFlo software. Demultiplexing and gating were performed in FlowJo and Prism software was used for statistical analysis.

RESULTS: Our analysis revealed that T cells were the only major immune cell subset with significantly increased frequency in MC1 patients (**Figure 1A**). Importantly, the accumulation of T cells in MC1 patient bone marrow was associated with changes in T cell functional maturation. The decreased proportion of naïve T cells and an increase in effector subsets suggest an ongoing stimulation of T cells in the tissue (**Figure 1B, C**). As a next step, we investigated which of the T cell subsets are responsible for the observed changes. This analysis revealed that the increase in T cell frequency is mainly driven by the significant increase in TCR αβ$^+$CD8$^+$ T cells and TCR Vδ1$^+$ T cells subsets (**Figure 1D**). Interestingly, no differences in the frequency of T cells as well as any of the T cell subsets were observed between cells aspirated from the MC1 lesion and the adjacent bone marrow unaffected by MC1 (**Figure 1A, D**). Finally, we investigated changes in the activation status of TCR αβ$^+$CD8$^+$ T cells and TCR Vδ1$^+$ T cells based on the expression of the CD69 molecule. Significantly increased proportion of CD69$^+$ TCR Vδ1$^+$ T cells and a trend toward increased activation in TCR αβ$^+$CD8$^+$ T cells in MC1 patient's bone marrow points to the ongoing immune response in MC1 mediated by these two T cell subsets (**Figure 1E**).

DISCUSSION: Using a fluorescent barcoding approach and multidimensional flow cytometry, we performed a comprehensive immunophenotyping analysis of bone marrow-derived cells of hematopoietic origin from MC1 and control patients.
We discovered an increased T cell proportion in MC1 patients' bone marrow driven by the accumulation of activated TCR αβ$^+$CD8$^+$ T cell and TCR Vδ1$^+$ T cell populations and associated with an increased proportion of effector T cells. We speculate that naïve CD8$^+$ T cells might get primed by high quantities of yet unknown antigens released from damaged IVD. Additionally, the presence of bacteria, which is discussed to play a role in MC1 could be the source of antigens. Damage of IVD and the stress response can also activate TCR Vδ1$^+$ T cells without the need for antigens. The exact mode of TCR Vδ1$^+$ T cells activation in MC1 thus remains to be elucidated.
The changes in proportions and activation of TCR αβ$^+$CD8$^+$ T cells and TCR Vδ1$^+$ T cell populations represent a novel aspect of MC1 immunopathology. Understanding the immunological role of these T cell subsets in bone marrow inflammation might reveal pathogenetic mechanisms driving MC1 development.

SIGNIFICANCE: This *ex vivo* analysis provides the first direct evidence of specific T cell subset accumulation in MC1. Discovery of TCR αβ$^+$CD8$^+$ T cell and TCR Vδ1$^+$ T cell accumulation in MC1 changes might represent the key step towards understanding the mechanisms driving changes of the immune system in MC1 and ultimately to targeted treatments for MC1.

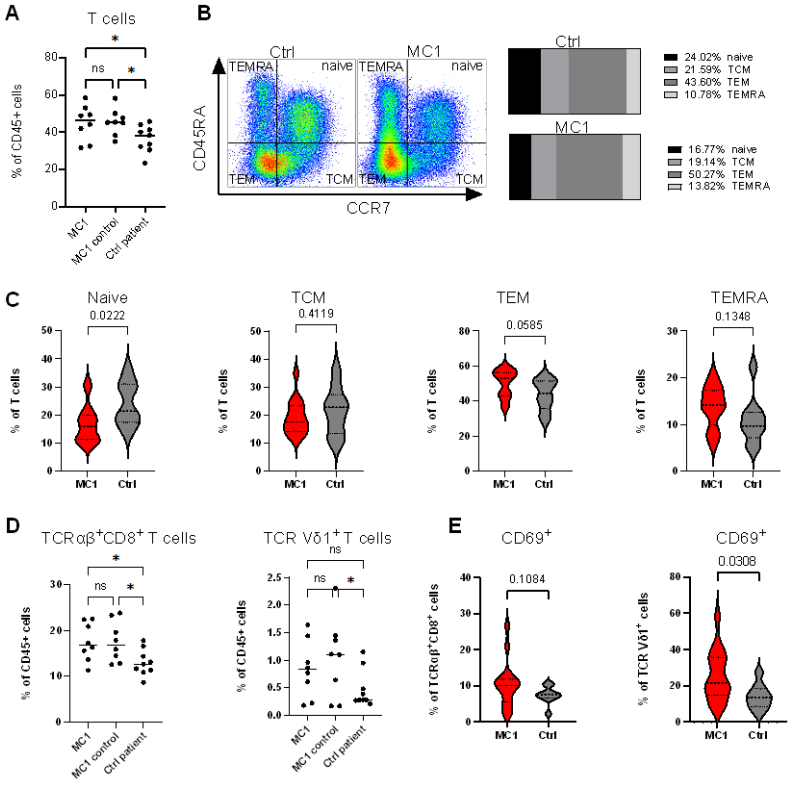

Figure 1. A.) Comparison of T cell frequencies in MC1, intrapatient control, and patients without MC1. **B**.) **Left**: Representative example of differences in T-cell functional maturation (naïve [CCR7$^+$CD45RA$^+$], T central memory – TCM-[CCR7$^+$CD45RA-], T effector memory – TEM- [CCR7$^-$CD45RA$^-$], T effector memory RA – [CCR7$^-$CD45RA$^+$]). **Right**: Average frequencies of T cell functional maturation stages in MC1 and control patient. **C**.) Comparison of T-cell functional maturation stages between MC1 and control patients. **D**.) Comparison of TCR αβ$^+$CD8$^+$ and TCR Vδ1$^+$ T cell frequencies in MC1, intrapatient control, and patients without MC1. **E**.) Comparison of CD69 expression on TCR αβ$^+$CD8$^+$ and TCR Vδ1$^+$ T cells from MC1 or control patients.