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Center of Experimental Rheumatology

DUDLI LAB Low Back Pain Chronic Regional Pain Syndrome

Extracellular Matrix Degradation-Driven Accumulation of Cytotoxic and Inflammatory Vδ1 T-cells in Modic Type 1 Changes

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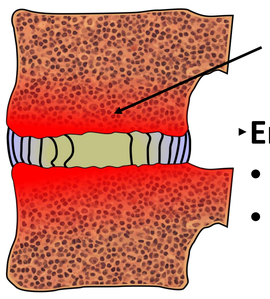
Background



Modic Type 1 changes (MC1)

- associate with pain and inflammation

Endplate damage

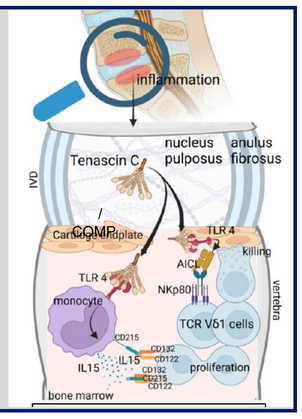
- always occurs with MC1
- Exposes the immune-privileged intervertebral disc (IVD) to immune cells of the bone marrow

Aim

Reveal immune cell subsets accumulating in MC1, discover why they accumulate there and what functional consequences it might have for the surrounding tissue.

Proposed disease model

- ECM degradation leads to the activation of monocytes and the induction of stress-induced molecules in bone marrow resident cells
- Production of IL15 by activated monocytes allows the proliferation of TCR Vδ1⁺ cells. These T cells kill stressed cells and contribute to tissue damage



Results 1 Cytotoxic proinflammatory TCRVδ1 cells accumulate in MC1

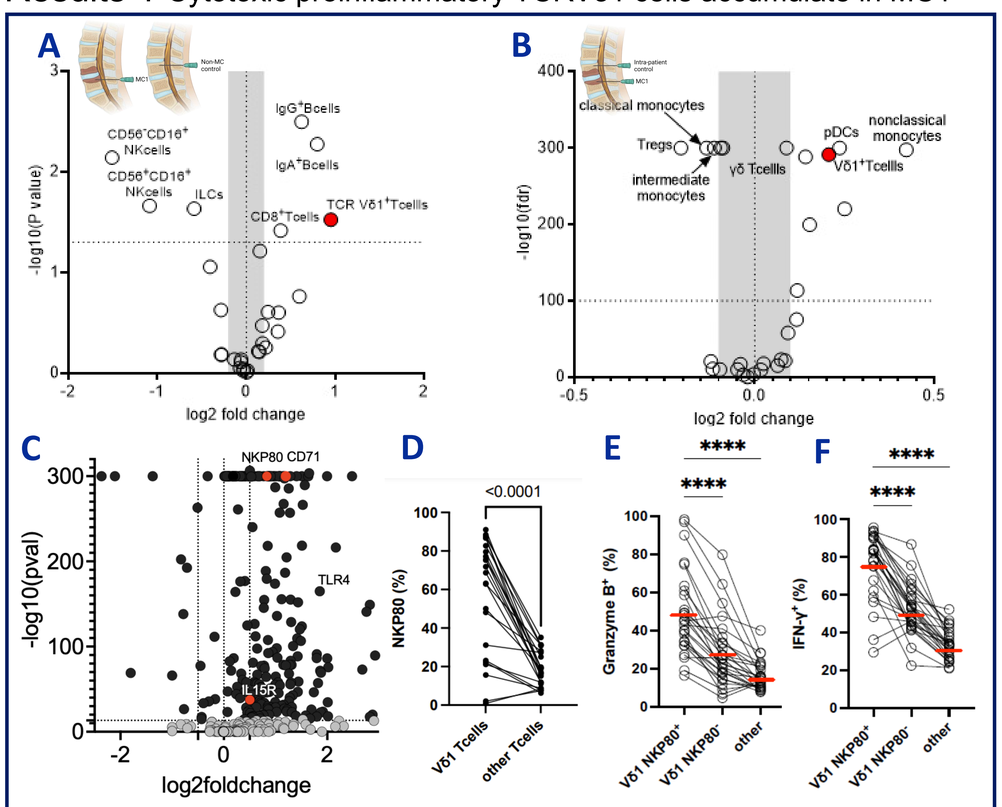


*Figure 1: **A**)Comparison of immune cell subset frequencies between MC1 patients and patients without MC1 or **B**) between MC1 patients and intrapatient controls.**C**) Differences in the surface expression of proteins on TCRVδ1 cells between MC1 lesions and intrapatient controls.**D**) Comparison of NKp80 on TCRVδ1 cells and other T cells. **E**) Comparison of Granzyme B on selected T cell subsets. **F**) Comparison of Interferon γ on selected T cell subsets. **G**) Comparison of IFN-γ in bone marrow plasma MC1 lesions and intrapatient controls*

Results 2 TCRVδ1 cells in MC1 can proliferate in response to IL15, which is produced by subset of myeloid cells enriched within the MC1 lesions

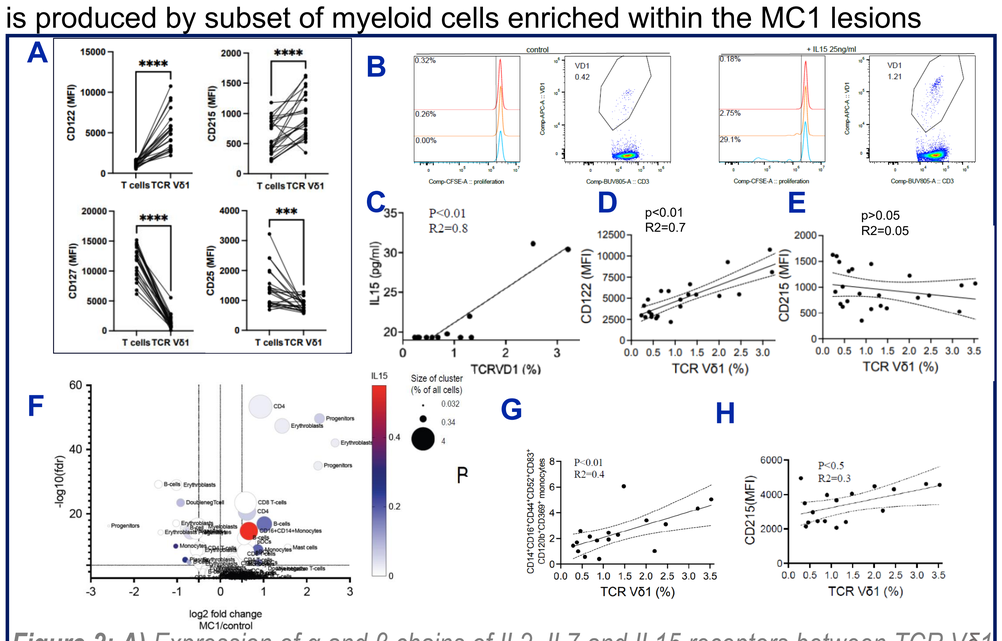


*Figure 2: **A**) Expression of α and β chains of IL2, IL7 and IL15 receptors between TCR Vδ1 cells and other T cells. **B**) Proliferation of T cell subsets in response to IL15 **C**) Correlation between bone marrow plasma levels of IL15 and the proportion of TCR Vδ1 cells **D**.) Correlation between IL2/IL15 β and the proportion of TCR Vδ1 cells. **E**) Correlation between IL15α and the proportion of TCR Vδ1 cells. **F**) Subset of CD14+CD16+ cells enriched in MC1 lesions is the main producer of IL15 in the vertebral bone marrow of MC1 patients. **G**) Correlation of IL15 producing myeloid cells and the proportion of TCR Vδ1 cells. **H**) Correlation of IL15Rα on IL-15 producing myeloid cells and the proportion of TCR Vδ1 cells.*

Results 3 Extracellular matrix degradation product COMP enriched in MC1 induces surface IL15 expression on myeloid cells

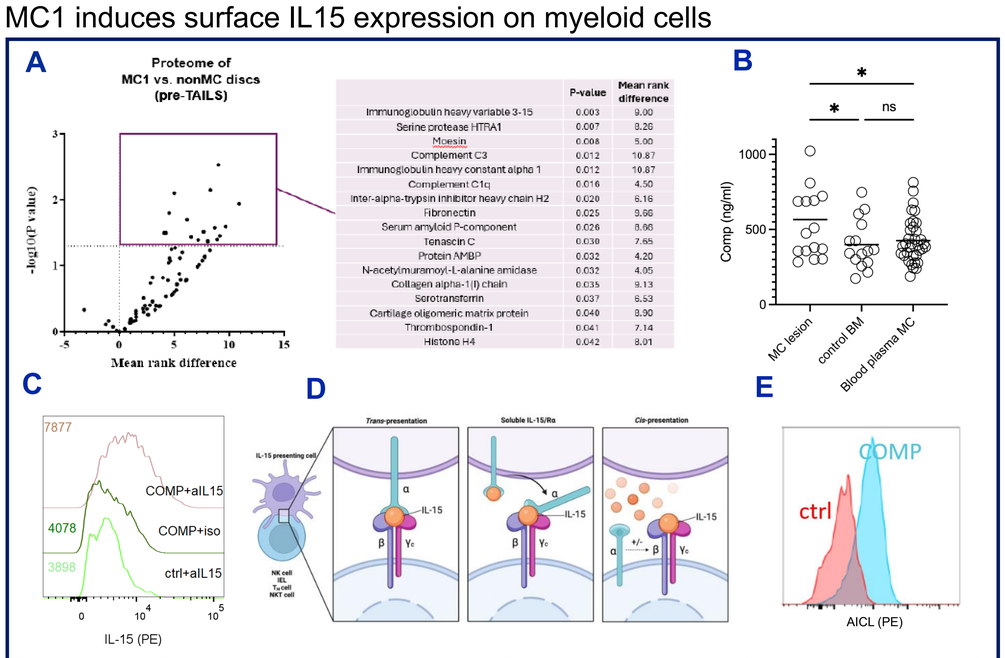


*Figure 3: **A**) Difference in the proteome of IVDs associated with MC1 and control discs. **B**) Comparison of COMP levels in bone marrow and blood plasma **C**)Upregulation of surface IL-15 on monocytes in response to COMP **D**) Modes of IL15 presentation (From PMID: 38405398) **E**) Upregulation of surface AICL on THP1 cells in response to COMP*

METHODS: Vertebral bone marrow aspirates were collected from cLBP patients with MC1 (n=22, MC1+intra-patient control=8+8, control patients=6) undergoing lumbar spinal fusion, mononuclear cells were isolated and investigated by massively parallel flow cytometry. T-cell functional properties were analysed with flow cytometry: 1.) *ex vivo* analysis of Granzyme B, 2.) cytokine release after *in vitro* stimulation with PMA/ionomycin, and 3.) proliferation in response to IL-15 followed with CFSE labelling. Single-cell RNA-sequencing (scRNA-seq) was performed on enzymatically digested, neutrophils-depleted MC1 and intra-patient control biopsies (n=4+4). Extracellular matrix proteins of intervertebral discs were analysed by LC-MS/MS. Cartilage oligomeric matrix protein (COMP) was quantified in bone marrow plasma with ELISA. Finally, bone marrow-derived mononuclear cells were treated with COMP, and cytokine production was analysed using flow cytometry.

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