The ablation of T cells exacerbates intervertebral disc degeneration following injury

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INTRODUCTION: CD3+ T lymphocytes, like CD4+ and γδ+ T cells, are critical mediators of tissue repair and rapidly infiltrate musculoskeletal tissues (e.g., bone and muscle) to stimulate repair post injury. However, the types of T cells that infiltrate during the acute IVD injury response and how they direct tissue repair remain understudied. Our recently published work shows an oscillatory, temporal regulation of CD3+ T cells at 3, 19, and 21 days post injury (dpi) during the acute IVD injury response in 12 week old C57BL/6J mice with less severe degeneration associated with γδ T cells infiltration in females (Clayton et al 2024). The acute injury response is a critical window (up to 3-4 weeks post injury) where infiltrating immune cells mediate tissue homeostasis and repair. In patients, γδ T cells accelerate tissue repair after infection-induced injury in the lungs, and preliminary data show the presence of γδ T cells in tissue from patient discectomies. The overarching hypothesis that CD3+ T cells are essential for IVD repair and the inhibition of the infiltration of γδ T cells specifically into the IVD acutely after injury exacerbates degeneration. Here, we solidify the protective role of CD3+ T cells in IVD tissue homeostasis post injury.

METHODS: IVD Injury: 5 Control (non-injured) and 5 injured (bilateral needle puncture) caudal IVDs were extracted from the same animal at 3, 19, or 21 dpi. Mice were either from wildtype "WT" 3 - 4 month old C57BL/6J female mice or T cell ablation mouse models. **T cell ablation mouse models:** 3.5-4 month old female T cell deficient mice, B6.129P2-Tcrbtm1Mom;Tcrdtm1Mom/J, were subjected to caudal IVD injuries. WT female mice were given intraperitoneal injections of a monoclonal, neutralizing antibody for γδ T cells twice before IVD injuries and every 6 days after to ablate γδ T cells IVD infiltration, n=3. IVDs were isolated at the aforementioned dpi's. **Flow cytometry**: Mice: For each group, 15 IVDs from 3 mice were pooled to render into a single cell suspension stained with the following antibodies: Fixable Viability Dye eFluor™ 780 (live/dead), CD45, CD19, CD3, CD4, CD8, Nk1.1/CD53, and TCRγδ, n=1-2. Human: Deidentified IVD tissue was collected from a female, 70 year old, White, female from L1-L5 and rendered into a single cell suspension before flow cytometry, n=2. **Histology**: Sagittal, midline sections from OCT embedded samples were stained with Safranin O/Fast Green and histopathological scoring performed n=2-3. **Statistical Analysis**: Plots were constructed in GraphPad Prism. Degeneration scores were analyzed using a paired t-tested or a mixed design 2-way ANOVA with Holm-Sidak post hoc analyses. The main effects of injury and genotype and the interaction: $p < 0.05$.

RESULTS: Comparative analysis of CD3+ T cells within the IVD was measured using flow cytometry at a previously determined key timepoint of T cell infiltration, 3 dpi, in injured young (12 weeks old) and aged (22 month old) C57BL6 female mice IVDs and from a female, 70 year old discectomy patient IVD sample. **(A)** We previously showed the presence of pro-repair γδ T cells in young mice (Clayton et al 2024), and a reduction in infiltration of γδ T cells with aging (unpublished). Here, we show that γδ T cells are present in human IVDs in proportions that resemble aged mice IVDs. (**B**) To determine the role of T cells in IVD degeneration progression, we conducted a needle puncture injury on the tail IVDs in T cell KO mice and determined that there is a clear effect of the needle puncture in inducing moderate IVD degeneration based on histopathological scoring at 21 dpi as shown in panel C. (**C**) Though under powered, there is a trending increase in degeneration score when comparing WT injured IVDs to injured IVDs from T cell KO mice. (**D**) We then performed intraperitoneal injections of a monoclonal, neutralizing antibody for γδ T cells and **(E)** observed a marked reduction in the number of γδ T cells that infiltrate the IVD at 3 dpi. **(F)** We then compared the effect of injury on WT control and injured IVDs, IgG injured IVDs, and TCR γδ T cells ablated mice at 19 dpi via histology. We discovered that there is a clear increase in degeneration scores of control, noninjured IVDs when compared to those with a needle puncture injury. **(G)** Most notably we observed a significant increase in degeneration score when comparing WT injured IVDs and IgG injected mice with injured IVDs (both moderate degeneration) to TCR γδ T cell ablated mice (severe degeneration).

DISCUSSION: The results highlight a potential protective role of γδ T cells in intervertebral disc (IVD) injury and degeneration. Flow cytometry analysis confirmed that γδ T cells infiltrate the IVD following injury, with proportions in human IVDs resembling those of aged mice, supporting the idea that aging is associated with reduced γδ T cell presence. Functional studies using T cell knockout mice and γδ T cell depletion further demonstrated that loss of T cells, particularly γδ T cells, exacerbates disc degeneration. While needle puncture consistently induced moderate degeneration in wild-type animals, γδ T cell ablation resulted in significantly more severe degeneration, indicating that these cells may contribute to disc repair or protection following injury. Together, these findings suggest that γδ T cells play a key immunomodulatory role in maintaining IVD integrity after injury.

SIGNIFICANCE: This study will identify critical CD3+ T cell subtypes to target for therapeutic treatments of IVD repair to prevent degeneration.

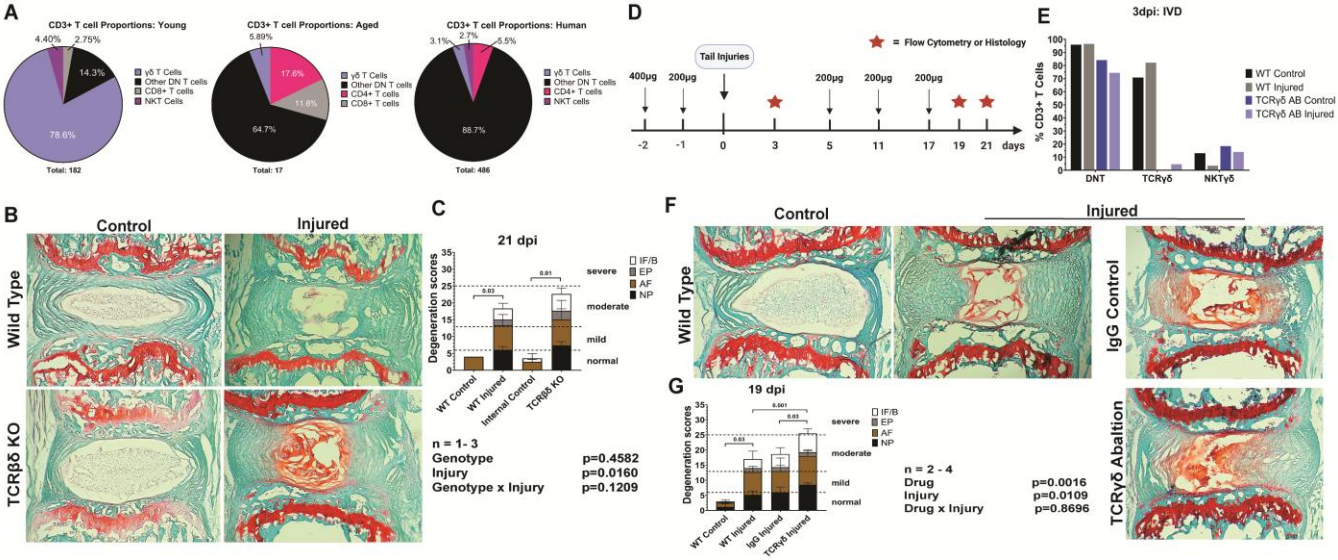

Figure: **(A)** The proportions of infiltrating CD3+ T cells in injured IVDs based on flow cytometry analysis. qPCR analysis showing the relative fold changes of immune cell gene expression of injured IVDs (bars) above control samples (black dotted line). **(B)** Histology of control and injured IVDs from 21 dpi C57BL6 WT and T cell KO female mice. **(C)** Histopathological scoring of IVDs from panel B. **(D)** Timeline of intraperitoneal injections of a γδ T cell neutralizing antibody or an IgG control antibody in conjunction with IVD tail injuries. **(E)** At 3dpi, the γδ T cell neutralizing antibody has selectively depleted the TCR γδ T cells within the IVD. **(F)** Histology of control and injured IVDs from 19 dpi C57BL6 WT, IgG Control, and T cell ablated (neutralizing antibody) female mice. **(G)** Histopathological scoring of IVDs from panel F.