TNF is required for the induction but not the maintenance of compression-induced BME signals in murine tail vertebrae: limitations of anti-TNF therapy for degenerative disc disease

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INTRODUCTION: So called Modic Type 1 (MT1) changes found on MRI, are defined as areas of increased intensity seen on T2 weighted images of vertebrae adjacent to herniated discs. While this test is commonly used as a diagnostic for patients with low back pain (LBP) associated with degenerative disk disease (DDD), its natural history, cellular and molecular nature remain poorly understood. In contrast, similar MRI signals appear in ankylosing spondylitis (Asp), in which tumor necrosis factor (TNF) is known to be critically involved based on the remarkable success of anti-TNF therapy. To better understand MT1 and the role of TNF in this process, we developed a model of chronically loaded tail vertebrae in WT and TNF-Tg mice that closely resembles the radiology and histopathology of early DDD and Asp respectively. Common to both models was the conversion from a fatty “white” marrow to a hematopoietic “red” marrow. To the end of establishing a causal relationship between TNF and chronic compression induced MT1, and providing support for anti-TNF therapy for DDD as has been suggested by case reports, here we investigated: 1) the healing response of WT vertebrae when this load is removed, 2) compression-induced MT1 in TNF deficient mice, and 3) the efficacy of anti-TNF therapy to ameliorate BME and marrow changes in this model.

METHODS: Animals: Wild type C57bl/6 (WT), heterozygous TNF transgenic C57bl/6 (TNF-Tg), and TNF receptor 1 and 2 double knockout C57bl/6 (TNFR DKO) mice were used in this study. Surgery: Two 0.028” diameter titanium pins were implanted transcutaneously in the center of the 7th and 10th caudal vertebrae. Aluminum rings were attached to the implanted pins, which allow for chronic loading via manual tightening of four screws around which calibrated springs were placed. A load of 6X body weight was applied over an 8 week trial along with age matched unloaded controls. The load was then released and pins removed. Mice were euthanized at 8 or 14 weeks and the vertebrae were harvested, fixed, and processed for histological investigation. Imaging: Mice were anesthetized prior to MRI scans with a mixture of ketamine 60mg/kg and xylazine 4mg/kg injected I.P. to ensure minimal motion during scans. All instrumentation was removed prior to CE-MRI. We used a custom built small animal RF surface coil placed about the tail, which interfaced with a clinical 3 Tesla Siemens Trio MRI. A fat-suppressed, T1-weighted high-resolution scan was then performed. To assess contrast-enhanced (CE) MRI changes in the marrow space due to chronic loading, a high-resolution scan was acquired for CE-MRI. Normalized marrow contrast enhancement (NMCE), was calculated by subtracting the signal intensity of the region of interest (ROI) pre-contrast from the ROI Post-contrast, and dividing by the same measurement made in muscle at the base of the tail. Histology: The vertebrae were fixed in 10% buffered formalin for 72 hrs and then decalcified in 14% EDTA at room temperature (pH adjusted to 7.2) for 21 days. The vertebrae were then carefully embedded in paraffin for sectioning into 3µm sections. Sections were then stained with orangeG/alcian blue for histological examination.

RESULTS & DISCUSSION: TNFR DKO mice were resistant to compression-induced BME, as no significant changes in NMCE were observed (data not shown). The results from the recovery experiment revealed that the NMCE of WT vertebrae significantly decreased 3-fold (p<0.01) within 2 weeks, while the NMCE values in TNF-Tg vertebrae remained elevated, but had a significant decrease (p<0.05) by 6 weeks after the release of load (Figure 1A). Anti-TNF therapy failed to show a significant difference from placebo treated-continuous loaded WT mice (Figure 1B). Histological examination revealed that the changes seen in NMCE values corresponded to a significant decrease in the amount of vascular sinus area (30± 3% vs. 22± 5%; p<0.05), but not in cellularity (3,265 ± 235 vs. 2,972± 741 cells/mm²), for the loaded vs. release of load groups respectively. The absence of this response in TNFR DKO mice demonstrates that TNF signaling is required for the onset of load-induced BME. Interestingly, release from chronic loading reversed both NMCE and marrow vasculature, without a reversion to yellow marrow, suggesting that NMCE values have a strong dependence on vascularity and marrow perfusion, rather than simple cellular marrow conversion. Moreover, these outcomes are similar to MT2 changes observed in DDD patients, which are less painful than MT1, and occur secondary to stabilization and recovery from disc herniation. Finally, our results do not support the use of anti-TNF therapy for LBP, as has been suggested by some anecdotal case reports and small pilot studies of sciatica patients. Rather, our results suggest that biomechanical stability and return of normal loading of the joint is of greater importance in the treatment of DDD.

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Figure 1: Longitudinal NMCE values for unloading and Anti-TNF therapy. (A) WT controls and TNF-Tg mice are shown under loading and after the release of load at week 8. (B) WT mice are loaded through week 14, placebo and Anti-TNF treatments are started at week 9. * and # indicate a significant difference from baseline NMCE values for WT and TNF-Tg respectively (p<.05).