Trem2 deficiency in Mice Causes Osteopenia

Alireza Nasoori[1], Jillian L. McCool[2], Benjamin Osipov[1], Deepa K. Murugesh[2], Nicholas R. Hum[2], Aimy Sebastian[2], Blaine A. Christiansen[1] and Gabriela G. Loots[1,2]

[1]*University of California Davis Health, Department of Orthopaedic Surgery, Sacramento, CA.*
[2]*Lawrence Livermore National Laboratory, Physical and Life Science Directorate, Livermore, CA.*

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INTRODUCTION: Mutations in the triggering receptor expressed on myeloid cells 2 (*TREM2*) have been shown to cause polycystic lipomembranous osteodysplasia with sclerosing leukoencephalopathy (PLOSL) or Nasu-Hakola disease (NHD), a recessively inherited progressive disorder that affects the bones and brain. NHD patients are highly susceptible to bone fractures, dementia, and premature death due to malfunction in cells from myeloid linages such as microglia and osteoclasts. While the pathogenic mechanisms of PLOSL in neurodegeneration have been extensively studied, the mechanisms contributing to bone loss have been minimally explored [1]. Recent studies have indicated that some subpopulations of macrophages that express high levels of *Trem2* mediate wound healing, phagocytosis, and cell clearance suggesting that *Trem2*^{high} macrophages participate in tissue remodeling and repair. Since macrophages are crucial in the healing process of damaged tissues, we sought to determine the immune features and responses of the MRL/MpJ (MRL) 'super healer' [2] mice that provide them with resistance to post traumatic osteoarthritis (PTOA). Conversely, we asked whether *Trem2* knockout mice (*Trem2*$^{-/-}$) are susceptible to joint degeneration. Accordingly, we found that *Trem2* deficiency led to osteopenia in the axial and appendicular skeleton and that these mice were also predisposed to osteoarthritis. In contrast, MRLs were resistant to PTOA and showed an increased number of *Trem2*^{high} macrophages post injury, relative to wildtype control mice, suggesting that *Trem2*^{high} macrophages may confer a pro-healing advantage in MRLs.

METHODS: Male and female B6, MRL, and *Trem2*$^{-/-}$ mice (10-12 weeks-old at the time of injury, 16-weeks old at the time of joint/bone analysis) were used in this study. At Day 0, mice were subjected to non-invasive anterior cruciate ligament (ACL) injury by using a single tibial compression overload (10-15M) with a loading rate of 1 mm/s (ElectroForce 3200, TA Instruments). At days 3, 7, 15 and 30 post injury, B6 and MRL injured and uninjured joints were harvested, digested to single cell suspension, and sequenced using the 10X Genomics and Illumina platforms; computational analysis was conducted as previously described by our group (Sebastian et al.2022). At 4- and 6- weeks post-injury mice were euthanized (16 weeks of age) whole knees, femurs and lumbar vertebrae were analyzed with micro-computed tomography to measure epiphyseal trabecular bone microstructure, osteophyte volume, trabecular and cortical bone volume and mass (μCT 35, SCANCO Medical AG). Whole joint histology to grade OA progression and synovitis was conducted on injured and uninjured mice. Knee joints, femurs and vertebrae were also examined histologically and by immunohistochemistry.

RESULTS: Following tibial compression induced ACL rupture, MRLs mice did not develop PTOA and showed a significant increase of multiple macrophage populations at early timepoints post injury when compared to the control B6 strain which showed severe PTOA. MRLs showed a sustained elevation in *Trem2*^{high} populations of macrophage throughout the injury time course, while B6 had a spike in *Trem2*^{high} populations at Day 3 and Day 7 post injury that trended back towards baseline beyond Day 15 (Figure 1A). This is consistent with previous studies indicating that macrophages expressing high levels of *Trem2* can enhance wound healing, phagocytosis, and cell clearance. Examination of *Trem2*$^{-/-}$ knockout mice determined that global deletion of *Trem2* caused severe osteopenia. MicroCT analysis of lumbar vertebrae and femora indicated that BV/TV, Conn Den, Tb.N, BMD and TMD of trabecular bone were significantly lower in *Trem2*$^{-/-}$ than B6 wildtype controls (Figure 1C,D), but femoral cortical parameters were unaffected. We also found that *Trem2*$^{-/-}$ uninjured joints displayed a significant loss of proteoglycan, hyaluronic acid, and chondroitin sulfate staining and an uneven articular cartilage surface with signs of fibrillation (Figure 1B), suggesting that these mice are susceptible to osteoarthritis. Semi-quantitative OARSI scoring indicated that uninjured *Trem2*$^{-/-}$ joints had a significantly higher OA score, on average, compared to B6, consistent with an OA phenotype.

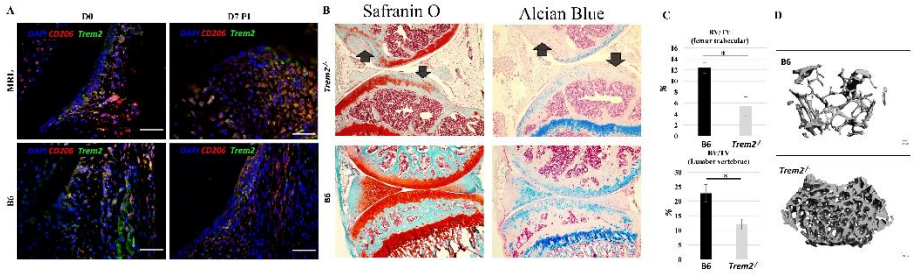

Figure 1. Trem2 is important for bone and cartilage homeostasis. ***(A)*** *Immunohistochemistry shows an increase of Trem2 signal (green) in the synovium at day 7 post injury compared to uninjured (left column), corresponding to M2 macrophages (CD206*$^{+}$*; red/DAPI; blue).* ***(B)****. Trem2*$^{-/-}$ *mice show osteoarthritis (arrows, diminished proteoglycan [Safranin O], hyaluronic acid, and chondroitin sulfate [Alcian Blue]) 10x magnification, and* ***(C)*** *trabecular bone loss;* ***(D)*** *microCT images.*

DISCUSSION: *Trem2* deficiency leads to severe osteopenia in mice axial and appendicular bones and predisposes them to osteoarthritis. This highlights the importance of Trem2 as macrophage receptors in the skeletal system. To determine which parts of the skeletal phenotype are driven by osteoclasts and which by macrophages, future studies should employ conditional knockout alleles. Gain of function studies are also warranted to determine if the elevated levels of Trem2^{+} cells in the MRLs are responsible for the post traumatic osteoarthritis protective phenotype.

SIGNIFICANCE/CLINICAL RELEVANCE: This study showed that MRLs possess significantly more *Trem2*^{high} macrophages in the injured joint than B6, correlating with the OA resistant phenotype documented for this strain. Conversely, *Trem2* deficient animals exhibit spontaneous osteoarthritis suggesting that *Trem2* is essential for maintaining a healthy joint. The results of this study can help inform new "cell-based" strategies for human subjects after ACL injuries before ACL reconstruction by increasing the number of Trem2+ macrophages in the joint, to help the healing process and potentially prevent the initiation of PTOA. The results highlight the effectiveness of *Trem2* in joint and skeleton homeostasis which can be potentially employed as medical modalities for trauma (e.g. ACL injuries) and genetic disorders (e.g. NHD).

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References:

1. Otero K, Shinohara M, Zhao H, Cella M, Gilfillan S, Colucci A, et al. TREM2 and beta-catenin regulate bone homeostasis by controlling the rate of osteoclastogenesis. J Immunol. Mar 15 2012;188(6):2612-21. Epub 20120206.
2. Heydemann A. The super super-healing MRL mouse strain. Front Biol (Beijing). Dec 1 2012;7(6):522-38.