**Introduction:** Mutations in the triggering receptor expressed on myeloid cells 2 (TREM2) have been shown to cause polycystic lipomembranous osteodysplasia with sclerosis (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 lineages 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<sub>high</sub> 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<sup>-/-</sup>) 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<sub>high</sub> macrophages post injury, relative to wildtype control mice, suggesting that Trem2<sub>high</sub> macrophages may confer a pro-healing advantage in MRLs.

**Methods:** Male and female B6, MRL, and Trem2<sup>-/-</sup> 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 mass and (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<sub>high</sub> populations of macrophage throughout the injury time course, while B6 had a spike in Trem2<sub>high</sub> 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<sub>high</sub> knockout mice determined that global deletion of Trem2 caused severe osteopenia. MicroCT analysis of lumbar vertebrae and femora indicated that BV/TV, Conn Den, Th.N, BMD and TMD of trabecular bone were significantly lower in Trem2<sup>-/-</sup> than B6 wildtype controls (Figure 1C,D), but femoral cortical parameters were unaffected. We also found that Trem2<sup>-/-</sup> 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<sup>-/-</sup> joints had a significantly higher OA score, on average, compared to B6, consistent with an OA phenotype.

**Discussion:** Trem2 deficiency leads to severe osteopenia in mice axial and appendicular bones and predisposes them to osteoarthritis. This highlights the importance of Trem2<sup>-/-</sup> macrophages as regulators of bone and joint development and function. The loss of Trem2<sup>-/-</sup> macrophages post injury in the MRLs strain is of particular interest as these macrophages are crucial for the post traumatic osteoarthritis protective phenotype.

**Significance/clinical relevance:** This study showed that MRLs possess significantly more Trem2<sub>high</sub> 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<sub>high</sub> 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:**
