The Effect of Conditional Macrophage Depletion on the Development of Injury-Induced Knee OA in Obese Transgenic MAFIA Mice

Chia-Lung Wu, Jenna McNeill, Kelsey Goon, Dianne Little, Kelly A. Kimmingler, Janet L. Huebner, Virginia B. Kraus, and Farshid Gulak
Duke University Medical Center, Durham, NC

All authors have no disclosures.

INTRODUCTION Macrophages (MΦs) have been recognized for their key role in obesity-associated inflammation as well as post-traumatic osteoarthritis (OA). While obesity has been shown to increase the severity of OA, the role of MΦs in regulating this effect is unknown. We hypothesized that MΦ depletion would mitigate OA severity following knee injury in obese mice.

METHODS Male Macrophage Fas-Induced Apoptosis (MAFIA) transgenic mice (The Jackson Lab) were placed on a high-fat (60% kcal fat) diet at 4 weeks of age (Fig 1A). MΦs in MAFIA mice are GFP+ and can be driven into apoptosis through the administration of a non-toxic reagent (AP20187). At 13 weeks of age, all mice underwent surgery to destabilize the medial meniscus (DMM) to induce knee OA in the left hind limb. Immediately after surgery, mice were treated with either vehicle solution or AP20187 to systemically deplete MΦs. At one week post-surgery, mice received a second MΦ depletion. To determine the effect of MΦ depletion on acute joint inflammation, mice (n = 5/group) were sacrificed the day after the second depletion (i.e. sacrificed at 0 wk post-depletion). To evaluate the effects of MΦ depletion on OA development, mice (n = 12/group) were sacrificed at 23 weeks of age (i.e. sacrificed at 9 wks post-depletion). Spleen and joint capsules were harvested to determine the extent of MΦ depletion by detecting GFP+ cells using flow cytometry. OA severity, osteophyte formation, and synovitis were determined by microCT and histological analyses. Immunohistochemistry (IHC) was used to identify various immune cells in the joint. Serum and synovial fluid cytokines were measured by ELISA. A p value less than 0.05 was considered significant.

RESULTS Obese mice lost a significant amount of weight after MΦ depletion (control 37.2 ± 0.9 g vs. depleted 25.2 ± 0.8 g), but gradually recovered to a similar weight as controls (control 50.1 ± 0.9 g vs. depleted 49.3 ± 4.6 g) at 23 weeks of age. Flow cytometry analyses showed that depleted mice had significantly lower MΦ numbers in both spleen (control 6.6% vs. depleted 5.1%, data not shown) and the DMM joints relative to non-depleted mice (Fig 1B). Decreased MΦs in the DMM joint was further confirmed by staining for F4/80, a general MΦ marker (Fig 1C). Staining for inducible nitric oxide synthase and CD206 demonstrated that both classically activated pro-inflammatory MΦs (M1 MΦs) and alternatively activated anti-inflammatory MΦs (M2 MΦs) were decreased in the DMM joint of the depleted mice (data not shown). MΦ-depleted obese mice exhibited improved metabolic profiles with reduced insulin and leptin levels (Fig 1D). Depleted mice also had decreased TGF-β concentration, but substantially increased granulocyte colony-stimulating factor (G-CSF) levels at the time of depletion (Fig 1D). MΦ depletion decreased osteophyte severity and joint bone mineral density (data not shown) in obese mice immediately after DMM (Fig 2A). However, MΦ depletion did not mitigate OA severity at any time points studied; rather, it increased joint synovitis at 9 weeks post-depletion (Fig 2B-C). IHC indicated that mice with MΦ depletion had significantly more neutrophils and CD3+ T cells, but not B cells, in the DMM joint compared to non-depleted mice (Fig 3A; data of T and B cells not shown). MΦ depleted mice had significantly up-regulated IL-1β, IL-6 and TNF-α levels in the synovial fluid of the DMM joint at the time of the treatment (Fig 3B).

DISCUSSION Our findings indicate that transient, systemic depletion of MΦs in obese MAFIA mice did not prevent post-traumatic OA. Although osteophytosis was less severe in the depleter mice at time of MΦ ablation, the depleted mice still developed a comparable osteophyte score to the control mice at 9 weeks post-depletion. Low osteophyte formation in the depleted mice at the time of treatment may be associated to their low serum TGF-β1 levels, as it has been shown that MΦs are important sources of TGF-β1 production. To our surprise, MΦ depletion did not decrease OA; it rather exacerbated synovitis. Currently, at least two phenotypes of MΦs have been identified: M1 and M2. The finding that both M1 and M2 MΦs were reduced in the synovium of the MΦ depleted joint implies that cells other than MΦs invaded into the joints of the depleted mice. The large number of infiltrated cells were later identified as T cells and neutrophils. Indeed, recent studies using distinct transgenic models that are capable of MΦ depletion also observed the development of neutrophilia following systemic removal of MΦs. Thus, it is likely that accumulated neutrophils, but not MΦs, are responsible for intensified inflammatory responses in our depleted mice. Furthermore, relative to the control mice, MΦ-depleted mice also exhibited significantly increased serum levels of G-CSF, a hormone essential for neutrophil differentiation and production. Our results suggest that despite their potential pro-inflammatory role, MΦs may be vital in maintaining the homeostasis of immune cells, partially through regulating G-CSF levels in obese mice.

SIGNIFICANCE Contrary to our hypothesis, we found that transient, systemic MΦ depletion in obese MAFIA mice did not mitigate cartilage degradation after joint injury, instead, it promoted joint synovitis by increasing serum G-CSF levels and infiltration of neutrophils into the DMM joint. Our study is significant for elucidating the immunomodulatory activity of MΦs in inflammation and joint injury in obesity.

ORS 2016 Annual Meeting Paper No. 0131