Selective inhibition of the MCP-1-CCR2 ligand-receptor axis decreases systemic trafficking of macrophages in the presence of UHMWPE particles

INTRODUCTION:
Macrophages are key cells that regulate the inflammatory response to wear particles. Production of wear particles leads to a non-specific macrophage-mediated foreign body reaction that can lead to high local levels of pro-inflammatory cytokines and chemokines. Moreover, polyethylene particles induce systemic macrophage recruitment and pro-inflammatory cytokine release resulting in osteolysis. Monocyte chemoattractant protein-1 (MCP-1) is one of the most abundantly released chemokines and acts through its cell receptor CCR2. We hypothesized that wear particle-induced macrophage recruitment and bone loss can be mitigated by interruption of the MCP-1-CCR2 ligand-receptor axis. In this study, we show that disruption of the MCP-1 ligand-receptor axis is a viable strategy to mitigate systemic trafficking of macrophages and osteolysis in the presence of clinically relevant UHMWPE particles.

METHODS:
Fifty-six, 8-9 weeks old nude mice were divided into six groups. The experiment was approved by our institution’s Administrative Panel for Laboratory Animal Care (APLAC number 9037). Group 1 animals had UHMWPE particles infused into the distal femur via a minipump; the animals were also injected intraperitoneally with 0.1 mL of RS102895 (a soluble competitive CCR2B receptor inhibitor). Group 2 had infused UHMWPE particles; the animals were also injected intraperitoneally twice a week with 0.1 mL of the carrier solution only. Group 3 and 4 had infused UHMWPE particles and were injected via the tail vein with either CCR2/-/- or wild type reporter macrophages. Group 5 had a single injection of sterile saline solution into the femoral marrow space. Group 6 had a single injection of MCP-1 into the femoral marrow space to confirm the localization of migrated reporter macrophages induced by MCP-1. For Groups 1, 2, 5, and 6, tail vein injections were performed with the reporter macrophage cell line RAW264.7 that was transfected with the lentiviral vector to express the bioluminescent optical reporter gene firefly luciferase (luc), and a fluorescence reporter gene, green fluorescent protein (gfp). Animals underwent bioluminescent (BLI) imaging and microCT scanning at periodic intervals and immunohistochemical staining of retrieved specimens at sacrifice. Bioluminescence data, microCT data and quantitation of positive cells were analyzed by the nonparametric Mann-Whitney U tests (two-tailed).

RESULTS SECTION:
Local injection of MCP-1 into the distal femoral canal induced systemic recruitment of intravenously injected reporter macrophages; the ratio of BLI was 0.98 ± 0.10 (no MCP-1) vs 1.58 ± 0.31 (+MCP-1, p = 0.016) at day 4. When RS102895, the CCR2B antagonist was injected, we observed a significant decrease of systemic migration of macrophages using bioluminescence[the ratio of BLI (see Figure 1A) and immunohistochemistry (see Figure 2)]. The same trends were observed using primary cells (wild type macrophages and macrophages deficient in the CCR2 receptor) where the ratio of BLI was 0.99 ± 0.52 (CCR2-/- cells) vs 1.26 ± 0.54 (WT cells, p = 0.005) at day 10. MicroCT analysis confirmed the protective effect of the MCP-1 receptor antagonist on particle-induced bone loss. Total bone mineral density was significantly decreased for Group 2 (receiving particles but no antagonist) compared to Group 1 (receiving particles plus antagonist): 191.93 ± 41.34 vs 352.28 ± 36.65 (p = 0.015).

DISCUSSION:
Previously, it was assumed that the reaction to wear particles from joint replacements was a localized event. The current study provides strong experimental evidence of a direct relationship between the chemokine MCP-1 and systemic macrophage recruitment in the presence of UHMWPE particles. Limitations of our study include the use of a murine model that simulates biological processes in humans, and the use of reporter RAW 264.7 cells that are an immortal macrophage cell line. These cells proliferate after migration and could increase the bioluminescence signal within the region of interest. In consideration of this fact, we used primary wild type reporter macrophages which are not immortal cells, to confirm the results obtained with RAW cells. When the MCP-1 ligand-CCR2 receptor axis was interrupted by two interventions, macrophage trafficking was mitigated. Furthermore, disruption of the chemokine-receptor axis was associated with a decrease in the particle-associated adverse effects on bone mineral density. Using a clinically relevant murine model of continuous local UHMWPE wear particle infusion, our hypothesis has been confirmed; we demonstrated that disruption of the MCP-1 ligand-CCR2 receptor axis can mitigate systemic macrophage recruitment and particle-associated bone loss.

SIGNIFICANCE:
The inflammatory and foreign body reaction associated with wear debris interferes with initial prosthetic osseointegration and may lead to periprosthetic osteolysis, jeopardizing long-term implant stability. Thus, modulation of the MCP-1 ligand-CCR2 chemokine-receptor axis may provide a therapeutic strategy to diminish both the early and late adverse effects of wear particles.

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Figures:

Immunohistochemistry for Group 1 (A) and Group 2 (B). Reporter macrophages containing GFP (green), total macrophages stained with MOMA-2 (red), overlay of GFP and MOMA-2 images showing migrated reporter macrophages (yellow). Few reporter macrophages (arrows) were observed when MCP-1 receptor antagonist was injected. Cb = cortical bone. (original magnification x20).