Is matrix metalloproteinase-9 involved in the release of interleukin-1β by macrophages exposed to metal implant wear and corrosion products?

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INTRODUCTION: Wear and corrosion products from metal implants have been associated with adverse local tissue reactions that can lead to implant failure. However, the underlying mechanisms remain largely unknown. Previous studies have shown that exposure to Cr_2O_3 particles, CoCrMo particles, and Co^{2+}can induce the release of interleukin (IL)-1β by macrophages [1]. In addition, it has been reported that matrix metalloproteinase (MMP)-2, -3, and -9 can process human pro-IL-1β into mature biologically active forms [2]. Interestingly, we recently showed that exposing bone marrow-derived macrophages (BMDM) to Cr_2O_3 particles (unpublished results), CoCrMo particles [1], or Co^{2+} [3] can induce MMP-dependent IL-1β release. More specifically, MMP-2 and/or -9 appeared to be involved in IL-1β release induced by these particles and ions, as determined using an inhibitor specific for MMP-2 and -9. The objective of the present study was to determine if IL-1β release by BMDM exposed to Cr_2O_3 particles, CoCrMo particles, or Co^{2+} is MMP-9 dependent.

METHODS: This research has been approved by the local animal care committee. BMDM from 5- to 16-week-old wild-type and MMP-$9^{-/-}$ [4] female C57BL/6J mice (The Jackson Laboratory) were prepared as previously described [5], incubated post-harvest with β-mercaptoethanol (55 µM) and macrophage colony-stimulating factor (5 ng/mL) for 16 hours, then primed with lipopolysaccharide (500 ng/mL) for 3 hours. The primed BMDM, from both wild-type and MMP-$9^{-/-}$ mice, were exposed to commercially available spherical Cr_2O_3 particles (60-nm or 700-nm diameter; 0–3.5 million or 0–1500 particles per macrophage [p/mφ], respectively), spherical CoCrMo particles (3.4-µm diameter; 0–200 p/mφ), or Co^{2+} (0–24 ppm) for 18 hours, under cell culture conditions. At the end of the incubation period, culture supernatants were collected and processed as previously described [5]. IL-1β release was quantified using an enzyme-linked immunosorbent assay. Statistical analysis was performed using Prism v9.4.1 (GraphPad) software for macOS. Data sets were assumed to meet the assumptions of normality and homogeneity of variance. Statistical analysis was performed using a two-way analysis of variance (ANOVA) and the Tukey post hoc test. $p<0.05$ was considered significant. Data are presented as means ± SEM of 3 independent experiments, each performed with 3 replicate samples per condition.

RESULTS: Exposing BMDM from wild-type mice to Cr_2O_3 particles (60 nm or 700 nm) induced a concentration-dependent increase in IL-1β release of up to ca. 285% ($p=0.001$) and ca. 350% ($p<0.001$) with 3.5 million and 1500 p/mφ, respectively, relative to the negative control (0 p/mφ) (Figure 1A). Similarly, exposing the BMDM to CoCrMo particles (3.4 µm) or Co^{2+} induced a significant increase in IL-1β release of up to ca. 250% with 200 p/mφ ($p=0.001$) and ca. 760% with 24 ppm ($p=0.013$), respectively, relative to the negative control (Figure 1B, C). Interestingly, knocking out MMP-9 did not significantly decrease the release of IL-1β by BMDM exposed to Cr_2O_3 particles, CoCrMo particles, or Co^{2+} (Figure 1).

DISCUSSION: The exposure of BMDM from wild-type mice to 60-nm or 700-nm Cr_2O_3 particles, 3.4-µm CoCrMo particles, or Co^{2+} induced an increase in IL-1β release, as previously reported [1]. Furthermore, when Cr_2O_3 particle size and concentration were converted to volume, IL-1β release appeared to be dependent on the overall particle volume rather than particle size and concentration (data not shown). Interestingly, the BMDM from wild-type and MMP-$9^{-/-}$ mice exhibited similar levels of IL-1β release, strongly suggesting that this response to the particles and ions is MMP-9 independent. Since previous studies showed that inhibiting both MMP-2 and -9 (using MMP-2/MMP-9 inhibitor I) impaired IL-1β release by murine BMDM exposed to Cr_2O_3 particles (unpublished results), CoCrMo particles [1], or Co^{2+} [3], the present results suggest that MMP-2 is involved in this response.

SIGNIFICANCE: These results further elucidate molecular mechanisms involved in the response of macrophages to wear and corrosion products from metal implants. More broadly, the findings expand our understanding of the immune response mechanisms that can lead to implant failure and may ultimately help identify therapeutic targets to increase implant longevity by mitigating adverse local tissue reactions to wear particles and ions from metal implants.

REFERENCES: 1. Archibald J., M.A.Sc. thesis, University of Ottawa, 2020 and references therein; 2. Schönbeck et al., J. Immunol., 161, 3340–3346, 1998; 3. Lignereux et al., World Orthopaedic Research Congress of the ICORS, 2022; 4. Vu et al., Cell, 93, 411–422, 1998; 5. Ferko and Catelas, PLOS One, 13: e0199936, 2018.

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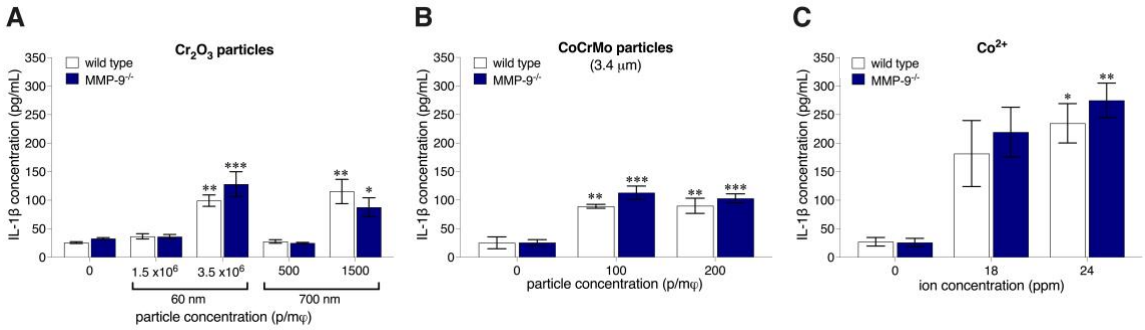

Figure 1. Release of interleukin (IL)-1β by bone marrow-derived macrophages (BMDM) prepared from wild-type and matrix metalloproteinase (MMP)-$9^{-/-}$ mice and exposed to Cr_2O_3 particles (A), CoCrMo particles (B), or Co^{2+} (C). The BMDM were primed with lipopolysaccharide (500 ng/mL) for 3 hours, then exposed to the particles and ions for 18 hours, under cell culture conditions. IL-1β release was analyzed by enzyme-linked immunosorbent assay. Asterisks (*, **, ***) indicate a significant difference ($p<0.05$, $p<0.01$ and $p<0.001$, respectively) between a given condition and its corresponding negative control (BMDM unexposed to the particles or ions). There was no significant difference between the release of IL-1β by BMDM from wild-type and MMP-$9^{-/-}$ mice. Data are presented as means ± SEM of 3 experiments, each performed with 3 replicate samples per condition.