Modulating Macrophage Response to Wear Particles to Mitigate Peri-Implant Osteolysis

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Background: Total joint replacements have been widely used to return mobility to patients suffering from arthritis; however a considerable number of these fail due to periprosthetic osteolysis generally believed to be caused by wear debris generated during mechanical loading of the joints[1]. Ultra high molecular weight polyethylene (UHMWPE) components are used in the majority of artificial joints in the US and hence UHMWPE wear particles (0.1-10 μm diameter) are the most abundant and reactive type of wear particles retrieved from the periprosthetic tissue during revision surgeries[2]. Upon phagocytosing wear particles, macrophages are activated and release cytokines such as IL-1β, IL-6 and TNF-α[3]. These cytokines have been shown to activate osteoclasts and cause bone resorption around implants[1]. Wear debris particles generated in the body are coated with adhesive proteins which facilitate macrophage adhesion and thus activation. Integrins, such as Mac-1 are known to mediate macrophage adhesion to adsorbed proteins. In this work we aim to delineate the role of Mac-1 receptor in macrophage adhesion to and phagocytosis of UHMWPE wear debris. Identification of the primary mediator in the osteolytic response will enable targeting of integrin receptors for anti-adhesion therapies for peri-implant osteolysis.

Methods: Macrophages matured from bone marrow harvested from C57BL/6J mice and Mac-1 KO mice were used to study macrophage phagocytosis and inflammatory response. The purity of the culture was determined by staining for F4/80 and CD11b, murine macrophage markers using flow cytometry. Macrophages from Mac-1 KO mice were stained for CD11b to verify knockout of Mac-1 expression. UHMWPE microparticles (MPs, 5μm) coated with fluorescently labeled extracellular matrix proteins such as fibronectin (FN) and bovine serum albumin (BSA) were fed to macrophages using an inverted cell culture system. Cell viability was determined when cell were incubated with an increasing dose of MPs. The number of UHMWPE particles phagocytosed, were quantified at 1 hr using a fluorescent plate reader. After incubating with 10 MPs per cell for 48 hrs, supernatant was collected for cytokine analysis of TNF-α and IL-6, measured by sandwich ELISA.

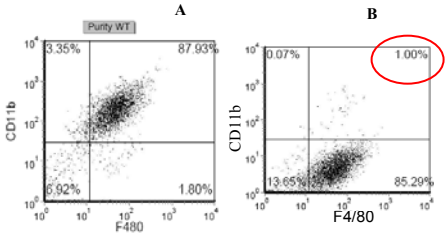

Figure 1. (A). Purity of the culture determined to be >87% as shown by staining for F4/80 and CD11b. **(B)** Macrophages obtained from Mac-1 KO mice are verified for knock down of Mac-1 expression as <1% cells stain for CD11b.

Results and Discussion: The purity of the macrophage culture was determined to be >87% **(Fig 1A)**. The knock down of Mac-1 receptor on macrophages from Mac-1 KO mice was verified by staining for CD11b which forms the α chain of Mac-1 integrin **(Fig 1B)**. We found that the >75% cells remained viable when they phagocytose 10 MPs, hence we selected cell : MPs ratio for subsequent experiments **(Fig 2)**. We quantified that Mac-1 KO macrophages phagocytosed 50% fewer MPs compared to WT **(Fig 3)**. Additionally, MP phagocytosis was ligand-dependant; for example the number of BSA coated MPs phagocytosed is ~2X greater than FN coated MPs. Due to the differences in the number of phagocytosed MPs we can conclude that Mac-1 is able to mediate macrophage phagocytosis of UHMWPE MPs. Mac-1 KO macrophages released significantly less (~50%) of both inflammatory cytokines TNF-α and IL-6 as compared to WT**(Fig 4)**. Thus we see that the inflammatory response of macrophages to UHMWPE particles can be mediated through Mac-1. These results indicate that Mac-1 can play a role in macrophage adhesion, phagocytosis and inflammatory response to UHMWPE MPs. Once the role of Mac-1 in this process is established integrin blocking therapies can be developed to prevent adhesion of macrophages to MPs and thus arrest the cascade of reactions leading to periprosthetic osteolysis.

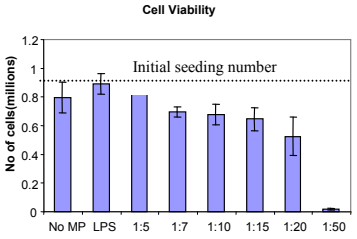

Figure 2: Quantification of viable WT macrophages when incubated with different cell: MPs ratios for 12 hours. MPs were precoated with FN. Initial seeding - 0.9 million cells

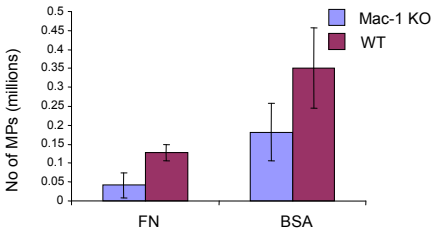

Figure 3: Quantification of the number of UHMWPE MPs phagocytosed by Mac-1 KO and WT macrophages when fed 10 MPs per cell for 1 hour

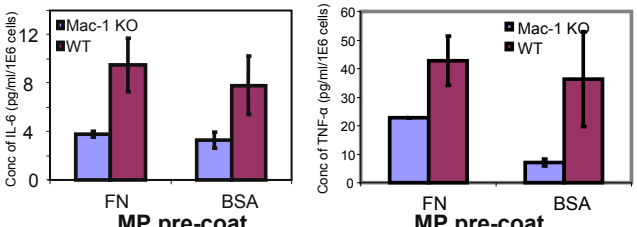

Figure 4: Quantification of TNF-α and IL-6 cytokine secretion from Mac-1 KO and WT macrophages when incubated with 10 MPs per cell for 48 hours.

References

[1] Bauer, T. W. & Schils, J. The pathology of total joint arthroplasty.II. Mechanisms of implant failure. *Skeletal Radiol.* **28**, 483-497 (1999).
[2] Mabrey, J. D. *et al.* Standardized analysis of UHMWPE wear particles from failed total joint arthroplasties. *J. Biomed. Mater. Res.* **63**, 475-483 (2002)
[3] Ingham, E. & Fisher, J. The role of macrophages in osteolysis of total joint replacement. *Biomaterials* **26**, 1271-1286 (2005)