

NEUTRALIZING OSTEOCLAST DIFFERENTIATION FACTOR AND MACROPHAGE COLONY STIMULATING FACTOR DECREASES PARTICLE-INDUCED OSTEOCLAST DIFFERENTIATION IN VITRO.

R MacQuarrie, G Richardson, YF Chen, GI Anderson

Depts of Surgery and Biomedical Engineering, Dalhousie University, Halifax, Canada.

INTRODUCTION: Arthroplasty wear particles initiate aseptic loosening partly by increasing the release of inflammatory mediators and increasing osteoclast numbers¹. Increased osteolysis depends not only on the activity per osteoclast but also on the total number of osteoclasts acting locally. In normal osteoclast differentiation both macrophage colony stimulating factor (M-CSF) and osteoclast differentiation factor (ODF) are key factors. We investigated the effects of blocking macrophage colony stimulating factor and osteoclast differentiation factor in the presence of various arthroplasty wear particles on the induction of osteoclast differentiation from mouse bone marrow populations.

METHODS: Mouse bone marrow cells (8×10^6) obtained from CD1 (6 week old female) were cultured in 35 mm dishes for 7 days in MEM with 15% foetal calf serum supplemented with VitD₃, Vit C and antibiotics. Particles were added at the following concentrations: polyethylene (PE) 100 ug/well, titanium (Ti) 100 ug/well and cobalt chrome (CoCr) 10 ug/well. High molecular weight polyethylene (PE) particulates were generated by a hip simulator², the polymethylmethacrylate (PMMA) was Simplex powder, Titanium particulates (Ti) were purchased and sieved to yield particulates with a mean diameter less than 5 microns, and the Cobalt chrome (CoCr) particulates were from metal on metal tribology experiments and were a gift from Dr John Medley, University of Waterloo, Ontario, Canada. ODF neutralizing antibodies (anti-ODF Ab) were applied on days 1, 4 and 6 at 0.1 and 0.2 ug/ml. M-CSF neutralizing antibodies (anti-M-CSF Ab) were applied on days 2, 4, and 6 at 1.25 ug/ml. After fixing the cells in 10% formaldehyde, the cells were stained for tartrate resistant acid phosphatase (TRAP), an osteoclast marker. TRAP positive colonies were counted using a grid overlay system to calculate the area of TRAP+ colonies / 35 mm dish¹.

RESULTS: Ti and PE particulates tended to increase the TRAP+ colony area / dish. Anti-ODF

Ab (at both concentrations) significantly decreased TRAP+ colony area in the presence of

Ti and PE ($p < 0.05$) and in control groups. In 2 of 3 experiments, there was no significant effect of adding anti-M-CSF Ab to control groups. However, there was a reduction in TRAP+ colony area ($p < 0.05$) in the dishes that had received anti-M-CSF-Ab with either PE or Ti particles. These neutralizing antibodies eliminated the particle-induced increase in osteoclast differentiation. CoCr particulates did not induce an increase in TRAP+ colony area at 10ug/ml but there was a trend to decrease TRAP+ colony area similar to controls with anti-M-CSF Ab and anti-ODF Ab.

DISCUSSION & CONCLUSIONS: M-CSF and ODF are both known to stimulate normal osteoclast differentiation but their role in particle-induced osteoclast differentiation is unknown. Our results suggest that both ODF and, to a lesser extent, M-CSF, play a role in particle-induced osteoclast differentiation. Given that ODF has a naturally occurring inhibitor, namely osteoprotegerin, this mechanism may prove to be a novel way to modify the response to particulates by osteoclasts and their precursors in vivo^{3,4}.

REFERENCES: ¹ G Anderson, R MacQuarrie, C Osinga, YF Chen, M Langman, and R Gilbert (2001) *J Biomed Materials Research – Applied Biomaterials* – in press. ² Essner A, Wang A, Stark C, Dumbleton JH. (1996) *Biomaterials* 17: 865-871. ³ J Lories, FP Luyten (2001) *Clinical Rheumatology* 20: 1; 3-9. ⁴ P Collin-Osdoby, L Rothe, M Nelson, F Anderson, W Maloney, P Osdoby. (2001) *JBC* March 23, MS M010153200 on line.

ACKNOWLEDGEMENTS: This work was supported by an operating grant from The Arthritis Society of Canada and a Clinical Research Fellowship from the Dept of Surgery, Dalhousie University, Canada.