Controlling Monocyte Differentiation to Treat Ectopic Calcification

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Introduction

Ectopic calcification refers to abnormal deposition of calcium salts in soft or hard tissues, often taking the form of bone. A myriad of patients are affected by ectopic calcification including those with aortic stenosis, bioprosthetic valves, cardiac assist devices, diabetes, end-stage renal disease, coronary artery disease, arthritis, tumoral calcinosis and those who have sustained orthopedic fractures, hip arthroplasty, and brain and spinal injuries. The lack of therapeutics aimed at treating ectopic calcification has resulted in only temporal treatments including surgical removal, valve replacement (which also calcifies) and bisphosphonates for life threatening events. Previously, ectopic calcification was perceived as an unregulated and passive physicochemical process. However, recent observations have pointed out key similarities between bone and ectopic calcification. Ectopic calcification is now viewed as a highly cell-regulated process involving both inductive and inhibitory processes[1]. We hypothesize that ectopic calcification occurring in response to disease, trauma or biomaterial implantation is due to the absence and/or deficiency of factors and cells that facilitate active mineral regression. Osteoclasts are the main bone resorbing cell type and share a common precursor with macrophages, which may also aid in resorbing mineral. In this study, we investigated whether cell therapy with macrophages, or macrophages treated with the osteoclast inducing factor, RANKL, could induce mineral resorption at sites of ectopic calcification.

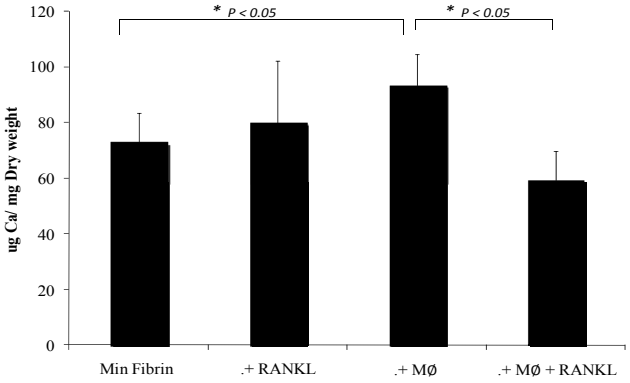
Methods

Murine bone marrow derived macrophages (BMDM) were derived from bone marrow monocytes by treatment with M-CSF as previously described[2]. The ability of BMDM to differentiate into osteoclasts was investigated *in vitro* by treatment with RANKL (40ng/ml), an osteoclast differentiating factor, and assessment of bone resorption using an osteologic disc assay. To examine the ability of BMDM to resorb mineral *in vivo*, microporous nanofibrous mineralized fibrin scaffolds (Min Fibrin) were fabricated as previously described[2]. Mineralized fibrin scaffolds were either untreated or loaded with RANKL (0.1 mg/ml), RANKL + BMDM, or BMDM alone. These scaffolds were then implanted subcutaneously into mice and examined at 14 days post implantation for calcium content.

Results

BMDM treated with RANKL were multinucleated, TRAP positive and showed the ability to resorb the mineralized surface in the osteologic disc assay, all characteristics of mature osteoclasts (data not shown). Thus, BMDM can serve as a precursor cell for osteoclastic differentiation.

The ability of exogenous BMDM and RANKL to promote mineral regression in mineralized fibrin scaffolds was then examined *in vivo*. Our preliminary data shows that RANKL treated scaffolds had similar calcium content as untreated scaffolds. In contrast, scaffolds seeded with macrophages alone had higher calcium content than either untreated or RANKL treated scaffolds. On the other hand, addition of macrophages and RANKL to the scaffolds significantly decreased calcium content compared to BMDM or RANKL alone (Figure).



Conclusions

In vitro, RANKL was sufficient to induce osteoclastic differentiation of BMDM. *In vivo*, BMDM alone had a pro-calcific effect on ectopic calcification. In contrast, exogenous BMDM treated with RANKL caused significant mineral regression. Treatment of scaffolds with RANKL alone did not stimulate mineral regression, suggesting a deficit in appropriate osteoclast precursors in the inflammatory response to the implanted mineralized materials. Our data suggest that BMDM treated with RANKL may provide a source for bone resorbing cells for treatment of ectopic calcification. Future studies to develop cell therapies for ectopic calcification that tightly control osteoclast differentation are in progress.

References

1. Giachelli, CM. Kidney International. 2009; May;75(9):890-7
2. Osthanon, T, et al. Biomaterials. 2008; 29:4091-4099