Title: Differentiating pro and anti-inflammatory response using THP-1 monocytes and magnetic resonance imaging

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Structured Abstract:
Introduction: Individuals who survive acute myocardial infarction (AMI) typically experience progressive heart failure. After AMI, inflammatory response(s) stabilize the infarction by strengthening the heart muscle; however, unrestricted inflammatory response leads to excessive left ventricular remodeling. Differentiating pro- and anti-inflammatory signalling may establish when interventions should be introduced to curb unwanted tissue remodeling.

Monocytes are precursors of M1 (pro-inflammatory) and M2 (anti-inflammatory) macrophages. In general, M1 displays an iron storage phenotype while M2 exhibits an iron recycling phenotype. Hepcidin is a hormone expressed post-AMI (unpublished results from a canine model of AMI); is induced by pro-inflammatory signaling; and downregulates ferroportin (FPN), an iron export protein found on monocytes and macrophages. These features of iron metabolism may allow us to differentiate between pro- and anti-inflammatory responses post-AMI using magnetic resonance imaging (MRI).

Hypothesis: Changes in monocyte iron regulation are mediated by hepcidin and influence both cellular iron content and MR relaxation rates.

Methods: Human THP-1 monocytes are cultured for 7 days in the absence (-Fe) and presence (+Fe) of iron-supplemented medium, containing 25µM ferric nitrate. Upon withdrawal of iron supplement, cells are cultured a further 1 (1h-Fe), 2 (2h-Fe), 4 (4h-Fe) and 24 (24h-Fe) hours. Samples are also treated +/- 200 ng/ml hepcidin for up to 24 hours. At harvest, cells are either mounted on a gelatin phantom or lysed in RIPA/protease inhibitors (Roche) and sonicated. Protein is quantified using the BCA assay. Expression of iron export protein is assessed by Western blot using rabbit α-FPN as the primary antibody (Invitrogen). Relaxation rates are measured at 3T in gelatin phantoms as previously described. Longitudinal relaxation rate (R1), total transverse relaxation rate (R2*) and its irreversible component (R2) are assessed using a custom MATLAB program. The reversible component (R2') is calculated (R2* - R2). Using GraphPad Prism, one-way analysis of variance (ANOVA) and Tukey’s test provide comparisons between sample treatments.

Expected Results and Discussion: Our initial results indicate that THP-1 monocytes express FPN and are therefore poised to respond to hepcidin-mediated inflammation. Their relatively high transverse relaxation rates fluctuate in response to hepcidin and changes in extracellular iron. Similar to iron-exporting P19 cells we expect significant differences in the MR relaxation rates of monocytes and macrophages in response to hepcidin. This data may help us better understand progressive heart failure, a complex disease that may be partially alleviated by distinguishing between pro- and anti-inflammatory responses to cardiac injury.