Integrin-Directed Modulation of Macrophage Response to Biomaterials

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Background: Macrophages recruited to the site of biomaterial implantation are the primary mediators of the chronic foreign body response to implanted materials.[1] Since foreign body response limits performance and functional life of numerous implanted biomaterials/medical devices, various approaches have been investigated to modulate macrophage interactions with biomaterial surfaces to mitigate this response.[2] The integrin family of cell surface receptors mediates cell adhesion to biomaterials through adhesive proteins spontaneously adsorbed on biomaterial surfaces.[3] We have investigated the role of integrin Mac-1 in macrophage inflammatory processes such as phagocytosis and inflammatory cytokine secretion in response to particulate biomaterials. Mac-1 binding to adsorbed proteins has shown to mediate phagocyte recruitment and adhesion to implanted material.[4] We have also investigated the in vivo foreign body response to subcutaneously implanted biomaterials in Mac-1 KO mice compared to WT control. We are also investigating the role of other integrins such as $\alpha_V\beta_3$ in macrophage phagocytosis by blocking with RGD peptide which is the binding motif present in different proteins for integrin binding. By studying the phagocytosis, inflammatory and foreign body response of macrophages from integrin knockout mice, we aim to identify the role of various integrins such as Mac-1 in macrophage adhesion to and phagocytosis of biomaterials.

Methods: Macrophages matured from bone marrow harvested from C57BL/6J mice and Mac-1 KO mice were used to study macrophage phagocytosis and inflammatory cytokine response. Polystyrene microparticles (MPs) coated with LPS (100EU/ml) and proteins such as fibronectin (FN), fibrinogen (Fg), Vitronectin (VN), bovine serum albumin (BSA) and Serum were incubated with the macrophages at cell:MP ratio of 1:10 for 24h for cytokine secretion. A negative control of cells incubated with no MPs and positive control of cells incubated with 1µg/ml LPS was also set up to study baseline cytokine secretion and activation by a strong inflammatory signal. For the RGD blocking experiments macrophages were incubated with 2.5 µM RGD in macrophage media for 1 h prior to feeding MPs to the cells. After 24 h, the supernatant was collected and frozen at -20˚C for cytokine analysis using sandwich ELISA and MP phagocytosis was quantified. A detectable level of endotoxin on MPs has been shown to be a prerequisite for inflammatory cytokine secretion.[5] Hence we have compared phagocytosis and subsequent cytokine secretion of MPs coated with known level of endotoxin to MPs with undetectable endotoxin levels.

Results and Discussion: The absence of Mac-1 integrin on macrophage surface down regulates TNF-α and IL-6 secretion upon uptake of serum and VN opsonized PS MPs, (Figure 1) indicating a role of integrin Mac-1 in inflammatory cytokine secretion upon phagocytosis of particulate biomaterials. Interestingly, there was a significant decrease in MP uptake and the production of both TNF- α and IL-6 at 24 h for samples incubated with RGD peptide for all the protein coatings (Figure 2 & 3), suggesting a major role played by RGD binding integrins in inflammatory response mounted against particulate biomaterials by macrophages. These results indicate that integrins Mac-1 and RGD-binding integrins such as $\alpha_V\beta_3$ can play a role in macrophage phagocytosis and inflammatory response to particulate biomaterials. Once their role in inflammatory response to biomaterials is established, integrin blocking therapies can be developed to mitigate the macrophage inflammatory response and thus improve functional life of biomaterials.

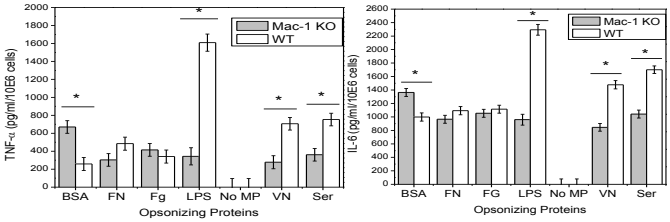

Figure 1. Integrin Mac-1 modulates inflammatory cytokine secretion from macrophages upon exposure to protein and LPS coated PS MPs. Quantification of (A) TNF-α and (B) IL-6 secretion from Mac-1 KO and WT macrophages upon exposure to protein and LPS coated PS MPs for 24 h

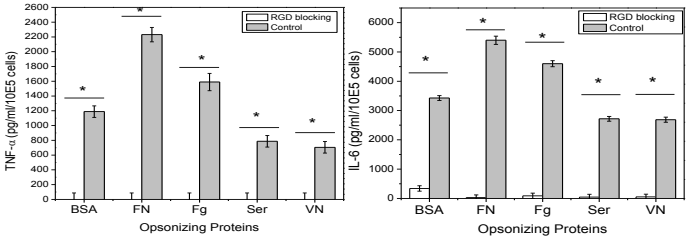

Figure 2. **Macrophage cytokine secretion upon exposure to protein coated PS MPs is modulated by blocking RGD-binding integrins**. Quantification of (A) TNF-α and (B) IL-6 secretion from WT macrophages upon blocking RGD receptors by soluble RGD (2.5 µM) and exposure to protein and LPS coated PS MPs for 24 h.

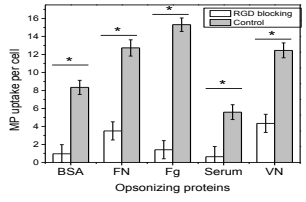

Figure 3. Phagocytosis of protein opsonized PS MPs by macrophage is modulated by blocking RGD-binding integrins with soluble RGD peptide. MP uptake of all protein coated MPs is reduced as compared to controls at 24 h after blocking with 2.5 µM RGD peptide.

References: [1] Anderson, J.M., Biological responses to materials. Ann Rev Mater Res, 2001;31:81-110. [2] Ren WP, Markel DC, Schwendener R, Ding YH, Wu B, Wooley PH. Macrophage depletion diminishes implant-wear-induced inflammatory osteolysis in a mouse model. J Biomed Mater Res Part A 2008;85A(4):1043-51.[3] Aderem, A. and D.M. Underhill, Mechanisms of phagocytosis in macrophages. Ann Rev Immuno 1999;17:593-623.[4] Altieri, D.C., P.M. Mannucci, and A.M. Capitanio, Binding of Fibrinogen to Human-Monocytes. J. Clin Invest, 1986;78(4):968-976. [5] Y. Bi, J. M. Seabold, S. G. Kaar, A. A. Ragab, V. M. Goldberg, J. M. Anderson, E. M. Greenfield, Adherent endotoxin on orthopedic wear particles stimulates cytokine production and osteoclast differentiation, J Bone Miner Res, 2001;16:2082-2091.