

# Integrin-Directed Modulation of Macrophage Response to Biomaterials

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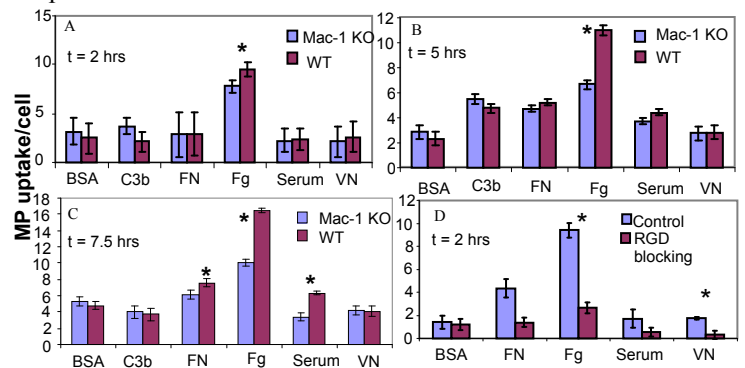
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**Background:** Macrophages recruited to the site of biomaterial implantation are the primary mediators of the chronic foreign body response to implanted materials.<sup>1</sup> Since foreign body response limits performance and functional life of numerous implanted biomaterials/medical devices, various approaches have been investigated to modulate macrophage interactions with biomaterial surfaces to mitigate this response.<sup>2</sup> The integrin family of cell surface receptors mediates cell adhesion to biomaterials through adhesive proteins spontaneously adsorbed on biomaterial surfaces.<sup>3</sup> 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.<sup>4</sup> 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.

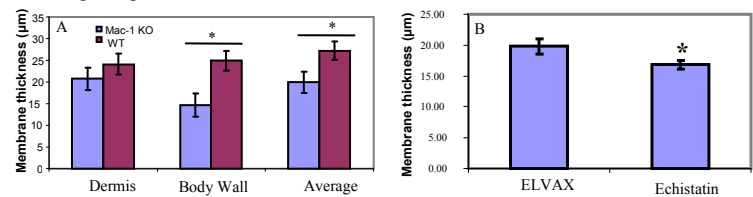
**Methods:** Macrophages matured from bone marrow harvested from C57BL/6J mice and Mac-1 KO mice were used to study macrophage phagocytosis and inflammatory response. Polystyrene microparticles (MPs) coated with proteins such as fibronectin (FN), fibrinogen (Fg), Vitronectin (VN), bovine serum albumin (BSA) and Serum were incubated with the macrophages at cell:MP ratio of 1:40 for different time points. The number of PS particles phagocytosed, were quantified at 2, 5 and 7 h using a fluorescence plate reader. In order to study the role of Mac-1 in foreign body response to implanted non-particulate biomaterials, Polyethylene terephthalate (PET) discs were implanted subcutaneously in Mac-1 KO and WT mice. To quantify capsule thickness on blocking RGD-binding, PET discs coated with ELVAX polymer loaded with Echistatin (protein isolated from snake venom containing RGD binding sequence) were implanted subcutaneously in WT mice. After 2 weeks the mice were sacrificed and the discs were fixed and stained in order to determine the thickness of the fibrous capsule formed around the disk.

**Results and Discussion:** We quantified macrophage phagocytosis of protein coated PS MPs. Mac-1 KO macrophages phagocytosed 40% fewer MPs compared to WT (Fig 1 A, B & C) when coated with proteins Fg and FN, known ligands for Mac-1. Due to the differences in the number of phagocytosed MPs, we infer that Mac-1 is able to

mediate macrophage phagocytosis of PS MPs. The thickness of fibrous capsule formed around the implanted discs was lower in Mac-1 KO mice as compared to WT indicating a role of Mac-1 in fibrous capsule formation and the foreign body response to bulk biomaterials. (Figure 2A) The thickness of fibrous capsule formed around the discs coated with Echistatin loaded ELVAX was lower as compared to ELVAX controls indicating a role of RGD-binding integrins such as  $\alpha_v\beta_3$  in fibrous capsule formation. (Figure 2B) These results indicate that integrins Mac-1 and RGD-binding integrins such as  $\alpha_v\beta_3$  can play a role in macrophage adhesion, phagocytosis and inflammatory response to biomaterials and MPs. 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: Integrins Mac-1 and RGD-binding integrins such as  $\alpha_v\beta_3$  modulates phagocytic uptake of protein opsonized PS MPs by murine macrophage.** Macrophages from Mac-1 KO and WT mice were incubated with 40 MPs/cell for A) 2 h B) 5 h C) 7.5 h. The number of Fg, FN and Serum coated MPs phagocytosed by Mac-1 KO macrophages is significantly lower than WT.D) WT macrophages were incubated with 20 MPs/cell for 2 h with 0.25 $\mu$ M RGD for blocking adhesion and phagocytosis. The number of Fg, and VN coated MPs phagocytosed is significantly lower with RGD blocking compared to controls.



**Figure 2: Integrin Mac-1 and RGD-binding integrins such as  $\alpha_v\beta_3$  modulates the foreign body reaction to PET discs implanted subcutaneously for 14 days.** A) Measurement of thickness capsule revealed thinner capsules around PET implants in Mac-1 KO mice compared to wild type control for both body wall and dermis faces of the implant. B) Measurement of thickness capsule revealed thinner capsules around PET implants coated with Echistatin loaded ELVAX polymer compared to only ELVAX controls.

**References:** [1] Anderson, J.M., Biological responses to materials. Ann Review of Materials Research, 2001. 31: p. 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. Journal of Biomedical Materials Research Part A 2008 Jun 15;85A(4):1043-51.[3] Aderem, A. and D.M. Underhill, Mechanisms of phagocytosis in macrophages. Ann Review of Immunology, 1999. 17: p. 593-623.[4] Altieri, D.C., P.M. Mannucci, and A.M. Capitanio, Binding of Fibrinogen to Human-Monocytes. J. Clinical Investigation, 1986. 78(4): p. 968-976.