Plasma Fibronectin Modulates Foreign Body Response to Biomaterials

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Statement of Purpose: Inflammatory responses to implanted biomaterials severely limit the functional lifetime of implanted devices.[1] These responses are directed, in part, by proteins adsorbed onto the biomaterial, including extracellular adhesive proteins.[1] For example, Busuttil et al. found that in fibrinogen-knockout mice, leukocyte adhesion was diminished in the acute inflammatory response.[2] In light of this, we investigated the effects of an ubiquitous extracellular matrix adhesive protein, fibronectin (FN), on both acute and chronic inflammation.

Methods: Because deletion of the gene for FN is embryonically lethal, plasma conditional FN knockout mice (pFNko) have been generated (129Sv/C57BL6; gift from H. P. Erickson and R. O. Hynes) were generated via the Cre-loxP conditional gene knock out system, which allows the role of FN to be studied in adults.[3] Briefly, mice containing a floxed (fl; loxP-site containing) fibronectin allele were crossed with mice expressing Cre under the control of the interferon and polyinosinic-polycytidic acid (pI-pC) Mx promoter. This procedure generates mice carrying the null allele (pFN(fl/fl)/Mx-Cre+; pFN(fl/fl) mice carrying the Mx-Cre transgene). This system has been shown to completely delete the gene in the liver,[4] which is the main location of plasma fibronectin production. The deletion of the fibronectin gene was induced in 8-12 week-old female mice by 3 intraperitoneal (ip) injections of 250 µg pI-pC at 2 day intervals. Blood samples were taken to verify the absence of pFN for knock out-induced mice. Western blot analysis showed that plasma FN in the knockout mouse was reduced to less than 2% of the WT, 3 days post pI-pC injections. Polyethylene terapthalate (PET) discs were implanted either ip or subcutaneously (sc) to examine the acute and chronic inflammatory responses, respectively. To examine acute responses, at 16 hr, discs were explanted and adherent cells were either trypsinized and total leukocyte cell counts were performed, or fixed and stained with May-Grunwald-Geimsa stain for differential cell counts. To examine chronic responses, at 14 days, discs were explanted, histological sections were made and stained with Verhoeff-van Geisson stain for nuclei (dark blue) and collagen (pink).

Results/Discussion: Control experiments showed there was no effect of the pI-pC observed in the WT mice (WT +/- pI-pC). Experiments examining the acute inflammatory response showed no differences in leukocyte recruitment and adhesion for either total leukocyte or differential cell counts between the pFNko and the WT mice (**Figure 1**). In contrast, significant differences in the foreign body response were revealed between the pFNko and WT mice, at 14 days (**Figure 2**). In particular, the fibrous capsule surrounding the implanted discs was ~2x thicker for the pFNko compared to the WT mice.

Conclusion:

These results indicate an important role of plasma FN in chronic, but not acute, inflammatory responses to implanted synthetic materials. These data are consistent with suggestions that FN plays a role in wound healing.[5] Our findings have a wide impact, with applications toward the engineering of biomaterials that modulate the foreign body response.

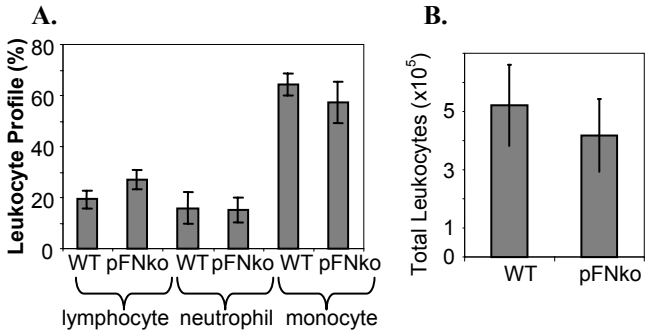

Figure 1. Acute inflammatory reaction to implanted PET discs, showing no differences between conditional plasma fibronectin knockout (pFNko) and control (WT) mice, for both differential (A.) and total (B.) leukocyte cell counts.

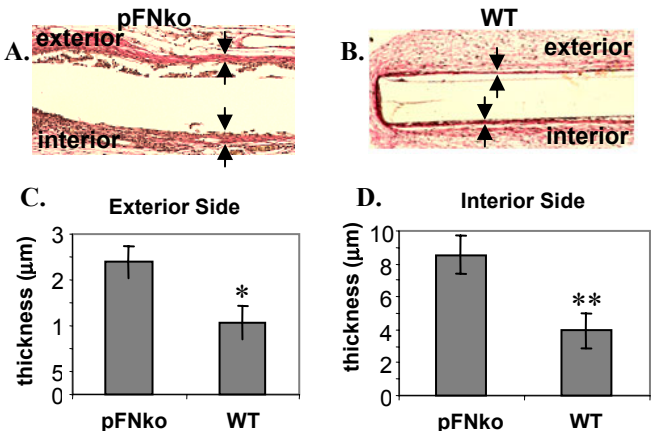

Figure 2. Foreign body reaction to implanted PET discs, demonstrating that plasma fibronectin conditional knockout mice (pFNko) produce thicker capsules than control mice (WT) at 14 days. Fibrous capsules (arrows, A. and B.) were found to be thicker on interior side of the implant (facing muscle) than the exterior side (facing skin), for all implants. Analyses between the pFNko and WT were therefore performed on the exterior and interior groups separately. Quantification of capsule thickness revealed capsules were ~2x thicker in the pFNko compared to the WT for both exterior (C.) and interior (D.) sides of the implant (ANOVA: * $p<0.04$, exterior side; ** $p<0.02$, interior side).

References: [1] Anderson, *Annu Rev Mat Res* (2001); [2] Busuttil et al., J Thromb Haemost (2004); [3] Sakai et al., *Nat Med* (2001); [4] Kuhn et al., *Science* (1995); [5] Singer et al. *N Engl J Med* (1999).

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