Biologic Scaffolds Composed of Mammalian Extracellular Matrix Promote a Constructive Macrophage Phenotype

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Statement of Purpose: Macrophages are a heterogeneous cell population capable of obtaining distinct phenotypes with diverse functions. Classically activated pro-inflammatory (M1) macrophages propagate a pro-inflammatory immune response while constructive (M2) macrophages are immunomodulatory and promote tissue repair. A heterogeneous macrophage cell population is essential for skeletal muscle regeneration after injury. Paracrine effects of polarized macrophages influence the expansion and differentiation of progenitor cells following skeletal muscle injury. Specifically, paracrine effector molecules derived from M1 and M2 macrophages promote the proliferation and myogenesis of skeletal muscle myoblasts, respectively. Moreover, specific depletion of M2 macrophages results in impaired inherent regeneration and the formation of scar tissue.

Biologic scaffolds composed of mammalian extracellular matrix (ECM) have been used to promote constructive tissue remodeling in a variety of tissues including skeletal muscle. The mechanisms responsible for this constructive response include modulation of the host innate immune response, among others. Specifically, ECM implantation sites have been shown to be associated with a predominant M2 macrophage phenotype. It remains unclear if bioactive ECM degradation products directly affect macrophage phenotype or if the immunomodulatory effect of ECM bioscaffolds is due to effects upon other cell types within the macrophage microenvironment.

The objectives of the present study were: (1) To determine the ability of degradation products from ECM bioscaffolds to affect macrophage phenotype; (2) To examine the paracrine effect(s) of ECM-treated macrophages upon skeletal muscle progenitor cells.

Methods: Bone marrow was harvested from C57Bl/6 mouse long bones and treated with MCSF for 7 days. Resulting naïve (M0) macrophages were treated with: 20 ng/ml IFN-y and 100 ng/ml LPS to derive M1 macrophages, 20 ng/ml IL-4 to derive M2 macrophages, or 200 ug/ml of ECM degradation products for derivation of ECM-treated macrophages. Following 18 hours of treatment, cells were fixed and immunolabeled for surface markers of M1 (iNOS) vs M2 (Fizz1) macrophages. Immunolabeling results were corroborated with western blot and qPCR analysis. Additionally, treated macrophage supernatants were used as chemoattractants in a Boyden Chamber cell migration assay to study the recruitment of pervivascular stem cells (PVSCs) and C2C12 skeletal muscle myoblasts. Macrophage supernatants were also used to examine the effects on mitogenesis and myogenesis of these progenitor cells via BrdU incorporation and sarcomeric myosin expression, respectively.

Results: The present study shows that degradation products from ECM bioscaffolds directly promote the constructive M2 macrophage phenotype. Moreover, the effect of polarized and ECM-treated macrophages upon skeletal muscle progenitor cells is shown. Specifically, ECM-treated macrophages not only express markers indicative of the M2 phenotype, they also function like M2 macrophages in that they promote the chemotaxis and myogenesis of perivascular stem cells (PVSC) and skeletal muscle myoblasts. The ability of ECM scaffolds to affect macrophage phenotype in vivo is shown in a mouse model of volumetric muscle loss (VML).

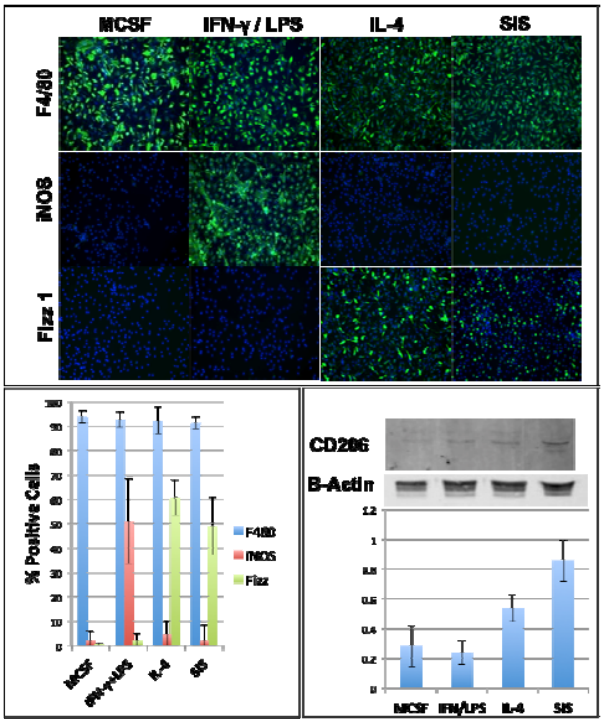

Fig 1. Murine bone-marrow-derived macrophages were treated with: monocyte colony stimulating factor (MCSF) for derivation of naïve M0 macrophages, IFN-γ & LPS for derivation of pro-inflammatory M1 macrophages, IL-4 for immunomodulatory / constructive M2 macrophages, or with SIS-ECM degradation products for 18 hours. Cells were fixed and immunolabeled for the pan-macrophage marker (F4/80) and for indicators of M1 (iNOS) and M2 (Fizz1) macrophages (A). **Degradation products from SIS-ECM was able to promote the constructive M2 macrophage phenotype similar to IL-4 treatment**. Quantification of immunolabeling in Fig 2A using Cell Profiler image analysis software (B). Western blot analysis of the CD206 M2 macrophage marker and the associated densitometry normalized to the

Conclusions: The results show that degradation products derived from ECM bioscaffolds promote a constructive macrophage phenotype similar to that induced by IL-4 activation. ECM-treated macrophages are able to recruit progenitor cells and promote their differentiation suggesting a possible mechanism for the constructive remodeling outcome associated with the use of ECM as a surgically placed biologic scaffold.