



2011 Research Grant Program Winning Abstract

Role of CD13 in Wound Healing after Myocardial Infarction

By Flavia Pereira

Following myocardial infarction (MI), the injured tissue heals through the interdependent processes of inflammation, scar formation, and tissue remodeling. The initial inflammatory phase is dependent on the homing of monocytes and fibrocytes to the site of injury and involves cells binding to adhesion molecules that are upregulated on the activated endothelial cells lining the vessels of the damaged tissue. Firm adhesion allows the transmigration of monocytes across the endothelial barrier and into the tissue, where they differentiate into macrophages that produce TGF-beta1, which differentiates resident cardiac and infiltrating fibroblasts to myofibroblasts. Myofibroblasts in turn synthesize extracellular matrix proteins, thus providing tensile strength to the scar. While these sequential steps are clearly important, the relative contribution of each of the component cell types to the healing process, the precise mechanisms regulating cell differentiation, and the processes that control the trafficking of circulating cells are not well understood.

CD13 is a large, cell surface peptidase that is expressed on monocytes and macrophages and is significantly upregulated on endothelium at sites of inflammation. We have recently shown that CD13 can function as an adhesion molecule and mediates monocyte/endothelial interactions critical to inflammatory trafficking. Pertinent to this proposal, CD13 is highly upregulated in the border zone and infarct area following myocardial infarction, where it has been shown to be an *in vivo* vascular address for targeted imaging of angiogenic vessels in the ischemic heart. Interestingly, CD13 is expressed on the macrophages and endothelial cells in the infarct as expected, but is also highly expressed on the myofibroblasts (but not resident fibroblasts), suggesting it may play a role in myofibroblast functions in healing. Functionally, while developmentally normal, following MI CD13 global null mice have decreased cardiac output, thinner ventricular walls, reduced numbers of infarct-resident macrophages and myofibroblasts, and decreased collagen deposition, suggesting that CD13 plays a protective role in cardiac injury. These results are consistent with defective monocyte trafficking or function in CD13 global null mice, which then would translate into impaired myofibroblast differentiation and function. Alternatively, because circulating fibrocytes also express CD13, the reduced myofibroblast numbers may reflect a role for CD13 in fibrocyte trafficking as well. Therefore, we propose that the adverse cardiac remodeling in mice lacking CD13 is a result of a combination of defective trafficking and/or function of these critical cell populations in the healing heart.

Aim: To determine if the adverse cardiac remodeling in CD13 global null mice is the result of (a) defective monocyte trafficking, (b) altered macrophage function and (c) fibrocyte/myofibroblast intrinsic defects. (a) To analyze monocyte trafficking and the contribution of monocytes in the differentiation of fibroblasts to myofibroblasts, we will use mice with deletion of CD13 specifically in myeloid cells (CD13^{myelo}^{-/-}). CD13 WT (CD13^{WT}), CD13 global null (CD13^{null}) and CD13^{myelo}^{-/-} mice will be subjected to MI, and 7 days post-MI, single-cell suspensions will be produced after collagenase digestion



of hearts and analyzed for macrophages (F4/80), myofibroblasts (alphaSMA), fibroblasts (vimentin and DDR2), and CD13 using multicolor flow cytometry. (b) It is possible that the reduced numbers of macrophages in CD13null infarcts is due to impaired monocyte to macrophage differentiation. Therefore, we will subject the CD13WT and CD13null mice to MI and analyze monocyte (Ly-6C, CD115, and F4/80^{low}) and macrophage (F4/80^{high}) markers by flow cytometry. Increased numbers of monocytes in the KO hearts where we see fewer macrophages would suggest a differentiation defect. In addition, we will analyze whether macrophages (F4/80^{high}) are defective in producing the TGF-beta1 cytokine required for myofibroblast differentiation by staining for F4/80 and intracellular TGF-beta1 using flow cytometry and confirming by ELISA using infarcted heart tissue. (c) CD13 is a marker of the circulating fibrocytes that contribute to the myofibroblast population, where it could regulate the function or trafficking of these cells. We will use mice that lack CD13 specifically in fibrocytes/fibroblasts but not myeloid cells (CD13fib⁻). We will isolate fibrocytes from peripheral blood, spleen, and bone marrow by flow cytometry (CD13⁺/CD45⁺/collagen I⁺) using CD13WT, CD13null, and CD13fib⁻ animals, stimulate with TGF-beta1, and analyze for their ability to differentiate to myofibroblasts (αSMA⁺, CD34⁻, CD45⁻) and deposit collagen. Similarly, fibroblasts will also be analyzed for their ability to differentiate to myofibroblasts. We will analyze homing of fibrocytes to the infarct using peripheral-blood-derived fibrocytes from CD13WT and CD13fib⁻ animals isolated by antibody bound magnetic beads and labeled with green and red fluorescent dyes. Equal numbers of each population will be adoptively transferred into our three models followed by MI. Hearts will be digested for flow cytometric identification and quantification of infiltrating fluorescent cell types including fibroblasts, myofibroblasts, macrophages, and monocyte subsets.

BD Biosciences Reagents and Instruments: Multicolor flow cytometry is routinely used in our laboratory and instruments including the BD™ LSR II and BD FACSCalibur™ are available through our core facility. Data will be analyzed with FlowJo™ software. BD antibodies to markers mentioned previously as well as others used for negative gating and cell selection such as CD11b, CD3, CD19, NK1.1, LY6G, and CD11c will be used in this project. The antibody for sandwich ELISA to detect TGF-beta1, as well as magnetic particles for cell separation, will also be obtained from BD.

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