594f Lipopeptide Ligands Presented in a Hybrid Bilayer Membrane Activate Cell Signaling and Support Hematopoietic Cell Growth

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The ability to expand hematopoietic stem cell (HSC) numbers in ex vivo culture could mitigate the hematopoietic cell depletion associated with chemotherapy, lead to the use of umbilical cord blood for adult transplants, and facilitate gene therapy. The objective of our research is to understand the factors that regulate HSC self-renewal vs. commitment. Although HSC self-renewal readily occurs in vivo, it is difficult to achieve in ex vivo culture. Current protocols for the culture of HSCs typically result in the production of more differentiated cells. Our hypothesis is that by mimicking the in vivo stem cell microenvironment one can overcome the current limitations of ex vivo expansion protocols. The in vivo niche is comprised of extracellular matrix (ECM), growth factors, and a heterogeneous mix of support cells that express a variety of cell adhesion molecule (CAM) ligands. Ultimately, regulation of HSCs is likely to be determined by a combination of soluble and insoluble signals including cell-cell, cell-ECM, and growth factor-receptor interactions. Recreating this environment in an ex vivo system will require the presentation of multiple ligands from a biomimetic surface in combination with soluble factors.

To form a biomimetic surface, peptide-based ligands are conjugated to lipid anchors (lipopeptides) and immobilized in a hybrid bilayer membrane. We have previously shown that supported lipid monolayers can be used for the presentation of adhesive peptide ligands for specific interaction with cells. The strategy for immobilizing adhesion ligands has been extended to the presentation of a peptide mimetic for the hematopoietic growth factor thrombopoietin (TPO). Using the TPO responsive M07e cell line, we show that biomimetic surface containing TPO mimetic lipopeptides produce signaling responses via the ERK1,2 and STAT5 pathways that are similar to those for soluble TPO mimetic in standard well plates. Further, our results suggest that immobilized TPO mimetic lipopeptide may synergize more extensively with the soluble cytokine stem cell factor then does soluble TPO mimetic for the activation of STAT5. Preliminary experiments with mobilized peripheral blood CD34+ cells show an increase in phosphorylated ERK 1,2 when the cells are stimulated with either soluble TPO mimetic or surface bound TPO mimetic lipopeptide.

Hematopoietic CD34+ stem and progenitor cells are very sensitive to the culture surface. Preliminary experiments with bone marrow CD34+ cells show that surfaces incorporating TPO mimetic lipopeptide have similar overall expansion as soluble controls of TPO mimetic or recombinant human TPO. There were no differences in the ability to retain CD34+ cells or the more primitive CD34+Thy1+ cells. Together this indicates that the supported lipid monolayers presenting lipopeptide mimics are biocompatible with HSCs and are equivalent to current methods for retaining primitive cells. These findings support our hypothesis that supported lipid monolayers provide a biocompatible surface for the presentation of stem cell niche ligands to hematopoietic cells, and set the stage for examining synergistic combinations of cytokine mimics and CAM ligands in a defined manner.