Use of a Tissue Engineered Human Bone Marrow Analog as a Model for Bone Marrow Failure
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Introduction
Our preliminary work has put us on the path to defining a long term self-sustaining model for evaluation of acquired bone marrow systems. In many instances even when exposure to an agent or chemical has been confirmed in the event of bone marrow failure, the pathogenesis from the causative agent and the disease progression are unexplained due to the lack of understanding of the complex mechanisms that result in marrow failure.

Materials and Methods
For the purposes of these studies we chose to direct the differentiation of CD34+ hematopoietic stem cells (HSC) to leukocyte lineages as previously described (1). Bone marrow analogs were seeded with cord blood-derived CD34+ cells were cultured in vitro for 7 days and then implanted on the backs of eight SCID mice to test their in vivo functionality. In vitro cultures were also developed for 7 days and were sham-exposed or exposed to Cytomegalovirus (CMV) at an MOI of 1. Profiles of cells were determined after immunostaining for cell phenotypes using flow cytometry.

Results
In our initial studies, in order to to mimic the bone marrow stroma tissue function, human bone marrow stromal cells were seeded on scaffolds and cultured for 3 days to allow the formation of a support cell layer on the scaffold surface prior to the addition of CD34+ HSCs (Fig. 1A). All cells were positive for CD34 and were lineage-1 negative when seeded onto the scaffolds (Fig. 1B). A small portion (1-2%) of CD34-expressing cells was positive for CD150. Examination of cultures on day 14 showed the continued presence of CD34+ HSCs (Fig. 1C). There was also formation of numerous actin-rich cell processes (Fig. 1D), which were absent in cell cultures on flat substrates. Similarly, maintenance of a population of CD150+ cells (Fig. 1E) was seen.

Implantation of these constructs on the back of SCID mice reconstituted the mouse with a human immune system (Fig. 1F). Exposure of cultures to CMV showed a marked reduction in HSC and adult cells.

Fig.1. CD105 stromal cells (green), (B) CD34+ (red), (C) Analog culture day 14, (D) Actin processes, (E) CD105 (red), in vivo implant showing CFSE+ (green) and CD34+ (red) cells.

Fig. 2. Production of hematopoietic progenitor cells and immune cells by the bone marrow analog 24 hours post after sham-exposure (blue) or exposure of the analog with CMV (red). N=6 experiments, 10,000 cells collected for each sample.

Discussion and Conclusions
As a human model system our long term cultures of the bone marrow analog could help us answer many of the unexplained questions related to causes and mechanisms of failure that have been unresolved. This model also has the potential, if seeded with human stem cells carrying genes resulting in leukocyte deficiencies, of being used as a model for congenital conditions as well.


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Disclosures NONE