The host immune response to allogeneic and syngeneic stem cells modulates osteogenesis

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INTRODUCTION
Mesenchymal stem cells (MSCs) are multipotent cells capable of limited differentiation into cartilage, bone, adipose, muscle, tendon, and ligament. MSCs are a potential therapeutic option for bone repair due to the relative abundance and ease of isolation of the cells from a host and their ability to deliver therapeutic factors to sites of fracture repair. MSCs also have the potential to evade the host immune response, however, evidence for the immunoprivileged status of MSCs is derived mostly from in-vitro studies. The formation of ectopic bone by MSCs transplanted into allogeneic hosts is not clearly established. MSCs are reported to form bone effectively in allogeneic, immunocompetent MHC mismatched hosts (1, 2), but, murine allogeneic MSCs were rejected by the hosts (3) contradicting the other reports. Therefore, we have investigated the bone forming properties of MSCs in allogeneic, immunocompromised allogeneic and syngeneic hosts to address the possibility of using allogeneic MSCs for bone repair.

METHODS

The osteoprogenitor stem cell line D1, isolated from bone marrow of Balb/c mice was injected subcutaneously in syngeneic Balb/c, allogeneic immunocompetent C57BL/6, allogeneic immunocompromised NCR NuNu as well as C57BL/6/Ppfp/Rag2 knockout mice. One million D1 cells were mixed with 300 µl of matrigel and injected into the subcutaneous tissue of the mice. Micro-CT analysis was used to determine bone volume after 3 and 6 weeks. RNA was extracted from the harvested implants and cDNA was prepared using a kit. The gene expression of osteogenic markers and IFN-γ was measured with specific primers by real time PCR. In addition, a homogenous cell suspension was prepared from the harvested implants by digestion with collagenase and filtering through a 100 μm nylon mesh. The cells were stained using specific monoclonal antibodies for CD45R, CD4, CD8, CD25, and F4/80, and analyzed by flow cytometry.

RESULTS

Ectopic bone was detected only in the implants retrieved from syngeneic or from allogeneic immunocompromised hosts (Fig. 1). A greater proportion of F4/80+ macrophages, CD4+ T-cells, and CD49b+ NK cells was present in allogeneic implants as compared to syngeneic implants (Fig. 2). IFN-γ was significantly higher in allogeneic C57BL/6 implants than in syngeneic Balb/c implants (Fig. 3), suggesting that the T\(_{\beta 1}\) type immune response inhibited osteogenesis in the allogeneic setting. Moreover, the host immune response inhibited the Wnt/β-catenin in immunocompetent allogeneic hosts (Fig. 4).

REFERENCES

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Figure 1. Bone formation is highest in the syngeneic hosts. The harvested implants were analyzed using Micro-CT and bone volume was determined for all the implants except for C57BL/6 mice as no quantifiable mineralization was observed in this group. γ = compared with Balb/c. n=12

DISCUSSION
It is unlikely that allogeneic adult bone marrow stem cells can directly be used for fracture repair.

SIGNIFICANCE: More studies on the interaction between the host immune response and MSCs may identify potential cellular and non-cellular targets that, if modulated, would allow the use of allogeneic MSCs for bone repair.