Bone Phenotype of Human Immune System Engrafted Mice

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Introduction

Human immune system engrafted mouse models have provided a promising opportunity to study therapeutic interventions requiring functional human immune system. These humanized mice are produced by myeloablation, reducing the number of mouse bone marrow cells and replacing them with human CD34+ hematopoietic stem cells (hHSCs). HSCs are progenitors of human immune cells, and also for example of bone resorbing osteoclasts. As the normal bone marrow homeostasis is disturbed in this process, concern arises if the bone phenotype is preserved. Though humanized mice have been widely used, there are no publications of their bone phenotype.

Aim of the Study

To characterize the bone phenotype of human immune system engrafted mice.

Materials and Methods

Twenty-five weeks old humanized female CIEA NOG mice (huNOG: HSCFTL-NOG-F, provided by Taconic Biosciences) were used in the study, and age-matched immunodeficient CIEA NOG mice served as controls. The huNOG mice were engrafted with the cells from two different donors (D1 and D2). Maturation of CD34+ hHSCs to CD45+ cells was confirmed by flow cytometry. The presence CD45+ cells and T (CD3+) and B (CD20+) cells in the bone marrow of hind limbs was confirmed by immunohistochemistry accompanied by basic histological stainings. Bone mineral density (BMD) and content (BMC) were analyzed by dual X-ray absorptiometry (DXA) *in vivo* and cortical and trabecular bone volumes of tibia were analyzed by micro-computed tomography (µCT) *ex vivo.* Serum bone turnover markers procollagen I N-terminal propeptide (PINP), C-terminal cross-linked telopeptides of type I collagen (CTX-I), and tartrate-resistant acid phosphate isoform 5b (TRACP 5b) were measured by commercially available ELISA methods (IDS Ltd, Boldon, UK).

Results

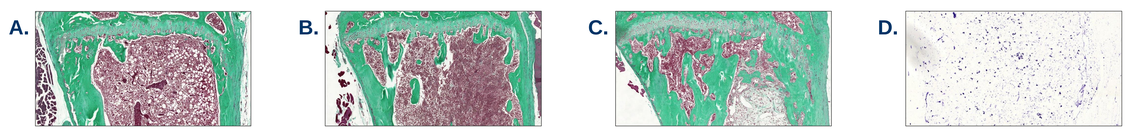

Figure 1. Bone histology of NOG and huNOG mice. Masson-Goldner Trichrome staining of tibias of NOG (A) and huNOG (B, C) mice. The presence of CD45+ cells in tibia bone marrow was confirmed by immunohistochemistry (D). Images from representative areas are shown, magnification 20x.

Figure 2. Bone content and volume analysis in NOG and huNOG mice. DXA was used to analyze the tibias of huNOG mice in the area of 6 mm in lenght from the growth plate to distal tibia. Bone area (A), BMD (B), and BMC (C) values are presented. Cortical (D) and trabecular (E) bone volumes were analyzed by µCT and presented in proportion to tissue volume (BV/TV). Additionally, trabecular number (TB.N, F) and trabecular thickness (TB.TH, G) are presented. Mean ± SD of 6 replicates in each group is shown. NS.= no significant difference compared with the NOG group.

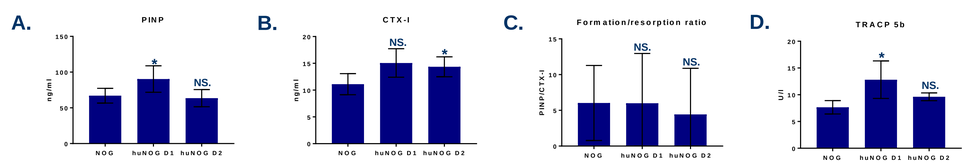

Figure 3. Bone turnover markers. Bone formation marker PINP (A) and resorption marker CTX-I (B) were measured in the serum. Bone formation to resorption ratio (PINP/CTX-I) is presented in (C), and TRACP 5b as a marker of osteoclast number in (D). Mean ± SD of 6 replicates in each group is shown. NS = no significant difference compared with the NOG group, *p<0.05.

Summary

- Engraftment rate of 60-80% was observed in the huNOG mice. This was verified by the presence of CD45+ cells in circulation by flow cytometry.
- The presence of CD45+ cells was confirmed in the bone marrow of the huNOG mice by immunohistochemistry. A low number of T and B cells were observed.
- A small portion of the huNOG mice (2/12) had fibrotic areas in their bone marrow, and the presence of immune cells was observed in association to these areas.
- Bone marrow histology was normal in the huNOG mice, except for the absence of bone marrow fat
- The huNOG mice had unaltered bone area, BMD, BMC and cortical bone volume compared to the NOG mice
- Trabecular bone volume, trabecular number and thickness showed a slight but non-significant increase in the huNOG mice compared to the NOG mice
- Serum PINP and CTX-I values may be elevated in the huNOG mice indicating elevated bone formation and resorption, but their ratio appears to be similar than in the NOG mice
- Serum TRACP 5b values may be increased in the huNOG mice indicating elevated number of osteoclasts, which would be consistent with the potentially elevated CTX-I values.

Conclusions

Human immune cells colonize the mouse bone marrow after myeloablation in the human immune system engrafted mice. Bones of the humanized mice did not exhibit significant changes compared to the immunodeficient NOG mice. These results support the use of humanized mice also in studies focusing on bone function.

Acknowledgements

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