A humanized mouse model to predict immunogenicity of impurities in generic peptide drugs

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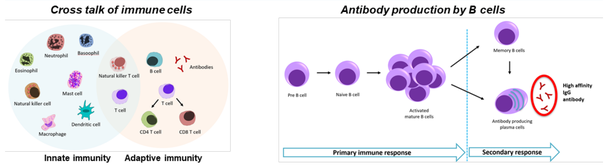
Abstract

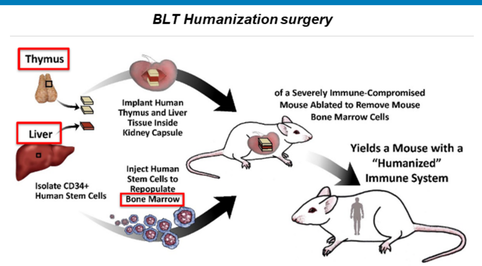
There is potential for impurities in chemically synthesized generic peptide products to elicit unwanted immune responses. To address these regulatory challenges, we utilized bone marrow-liver-thymus (BLT) immune humanized mice that develop a fully engrafted human immune system. Our goal was to determine the predictive value of the animal model compared to in silico and in vitro immunogenicity testing methods. Initially, we evaluated salmon calcitonin, known to induce anti-drug antibodies in patients, and two of its isolated impurities which were predicted highly immunogenic in silico and in vitro. Teriparatide, which is not associated with immunogenicity in patients, was tested as a negative control to validate our animal model. Mice were dosed daily with salmon calcitonin, impurities or teriparatide for 28 or 56 days at different doses and routes of application. Dose response data from intranasal or subcutaneous drug administration will be presented. Histology showed intranasal salmon calcitonin caused more severe inflammation in the nasal cavity compared to saline control or impurities alone. Flow cytometric analysis showed salmon calcitonin increased immune responses in a dose-dependent manner and enhanced the number of cells producing class-switched antibody. Lower B-cell responses in teriparatide-treated mice support clinical observations of low immunogenicity to teriparatide. This model allowed us to investigate doses of peptide drugs and routes of exposure that have the potential to trigger immune responses from drug products expected to produce immunogenicity. This model will be used to further investigate humoral immune responses to generic peptide drugs and impurities from these generic products and originator products.

Introduction



Within the human immune system, different populations of immune cells communicate with each other in order to mount a multi-faceted response to danger signals or foreign antigens. Antibody production by B cells is the hallmark of an immunogenic response. The maturation of the B cell response to produce high-affinity antibodies and memory cells requires additional crosstalk between B cells, antigen-presenting cells (APCs), and T cells.

Materials and Methods



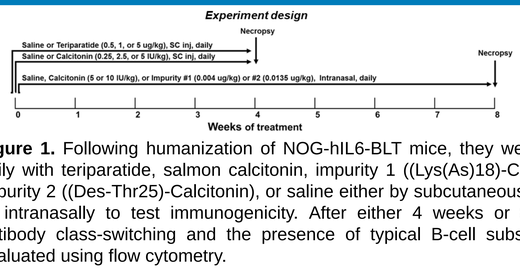


Figure 1. Following humanization of NOG-hIL6-BLT mice, they were dosed daily with teriparatide, salmon calcitonin, impurity 1 ((Lys(As)18)-Calcitonin), impurity 2 ((Des-Thr25)-Calcitonin), or saline either by subcutaneous injection or intranasally to test immunogenicity. After either 4 weeks or 8 weeks, antibody class-switching and the presence of typical B-cell subsets were evaluated using flow cytometry.

Results and Discussion

Survival after peptide drug challenge in humanized mice

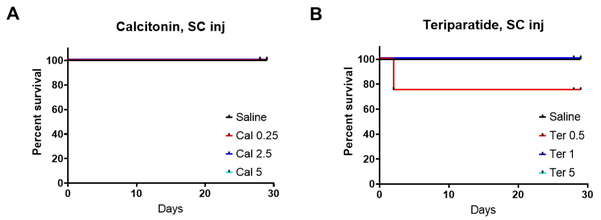


Figure 2. Kaplan-Meier survival curves after drug challenges. A comparison of survival rate is shown for A) calcitonin- B) teriparatide-treated mice. Peptide drugs and dose level no apparent impact on survival. All mice (n=4-5 per group) were monitored and treated according to approved animal protocols.

Histological changes in nasal cavity

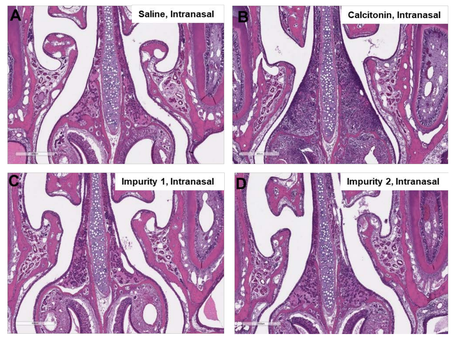


Figure 3. Changes in histological characterization and inflammation in nasal cavity. Hematoxylin and eosin staining shows histological changes after intranasal treatment with A) saline B) calcitonin C) Impurity #1 D) Impurity #2. Narrowing of nasal cavity (bars), infiltration of inflammatory cells (arrows), and hyperplasia of nasal septum (arrowheads) are indicated. Compared with the saline or impurity only treated mice, significant histologic damage to the nasal cavity was evident in mice treated with calcitonin.

Development of myeloid cells in bone marrow

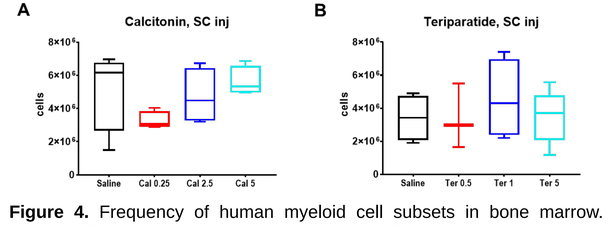


Figure 4. Frequency of human myeloid cell subsets in bone marrow. Single cell suspension were stained with antibodies for mouse CD45, human CD45, CD3, CD66b, and CD14. All cells are gated on human CD45+ CD3-. CD14+ or CD66b+ myeloid cells are shown for A) calcitonin (n=4 per group) B) teriparatide treated mice (n=3-5 per group). Myeloid cells in calcitonin and teriparatide study are essential for B cell development. Statistical analysis was performed via one-way ANOVA with Saline group compared to all other groups. The boxes in box plots indicate the lower and upper quartiles. The whiskers extend to minimum and maximum values.

Identification of isotype switched memory B cells in bone marrow

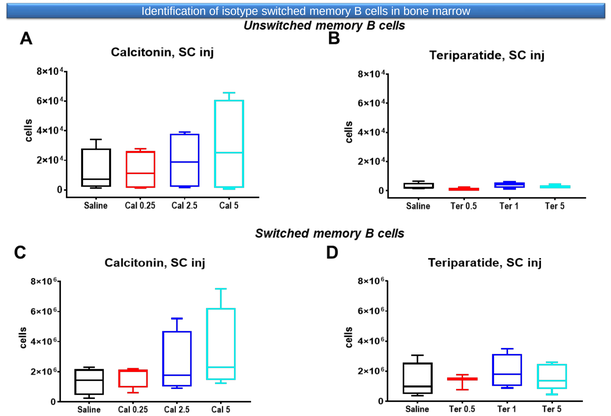


Figure 5. Frequency of memory B cells in bone marrow. Single cell suspension of cells of bone marrow were stained with antibodies for mouse CD45, and human CD45, CD19, CD20, CD27 and IgD. All cells are gated on human CD45+, CD19+ or CD20+. CD27+ IgD+ unswitched memory B cells are shown for A) calcitonin (n=4 per group) B) teriparatide treated mice (n=3-5 per group). CD27+ IgD- switched memory B cells are shown in C) calcitonin study (n=4 per group) D) teriparatide study (n=3-5 per group). Isotype switched memory B cells are increased in a dose-dependent manner in calcitonin treated mice. Statistical analysis was performed via one-way ANOVA with *p<0.05 Saline group compared to all other groups. The boxes in box plots indicate the lower and upper quartiles. The whiskers extend to minimum and maximum values.

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Identification of activated mature B cell populations in bone marrow

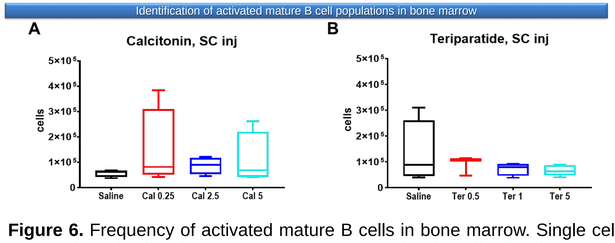

Figure 6. Frequency of activated mature B cells in bone marrow. Single cell suspension of cells of bone marrow were stained with antibodies for mouse CD45, human CD45, CD3, CD14, CD66b, IgD and CD40. All cells are gated on human CD45+, CD3-, CD14- and CD66b-. IgD+ CD40+ activated mature B cells are shown for A) calcitonin (n=4 per group) B) teriparatide treated mice (n=3-5 per group). Results show that more activated B cells are present, compared to control, with calcitonin treatment. Statistical analysis was performed via one-way ANOVA with *p<0.05 Saline group compared to all other groups. The boxes in box plots indicate the lower and upper quartiles. The whiskers extend to minimum and maximum values.

Identification of plasma cells in bone marrow

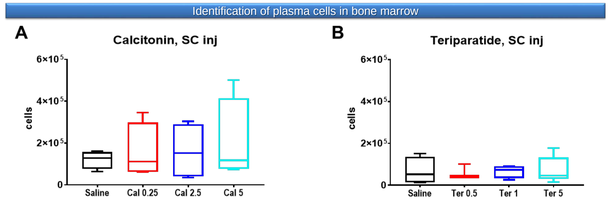

Figure 7. Frequency of plasmas cells in bone marrow. Single cell suspension of cells of bone marrow were stained with antibodies for mouse CD45, and human CD45, CD3, CD19, CD20, CD27 and CD38. All cells are gated on human CD45+, CD3-, CD19- and CD20-. CD27+ CD38+ plasma cells are shown in A) calcitonin study (n=4 per group) B) teriparatide study (n=3-5 per group). Plasma cell numbers increased with calcitonin treatment. Statistical analysis was performed via one-way ANOVA with *p<0.05 Saline group compared to all other groups. The boxes in box plots indicate the lower and upper quartiles. The whiskers extend to minimum and maximum values.

Conclusion

- Calcitonin and teriparatide injection doses were well-tolerated in humanized mice in pilot studies.
- The presence of myeloid cell and mature B cell population shows development of robust adaptive immunity in humanized mice.
- Intranasal calcitonin-treated mice showed significant inflammation in nasal cavity, compared with mice with impurity-only and saline-treated mice.
- Increased numbers of switched memory B cells and plasma cells in calcitonin-treated mice supports clinical observations of increased immunogenicity to calcitonin, but not to teriparatide.

Acknowledgements

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