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LETTER TO THE EDITOR

The gender gap: discrepant human T-cell reconstitution after cord blood stem cell transplantation in humanized female and male mice

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Several laboratories are exploring the use of humanized mice transplanted with human hematopoietic stem cells as *in vivo* models to evaluate the effects of new human vaccines and immune therapies on adaptive immune responses,[1–3] but possible gender aspects were not considered. Notta *et al.*, reported that female non-obese diabetic/severe combined immunodeficient (NOD/SCID)/IL2Rγc$^{-/-}$ (NSG) mice transplanted with limiting numbers of human CD34^{+}CD38^{-} cord blood hematopoietic stem

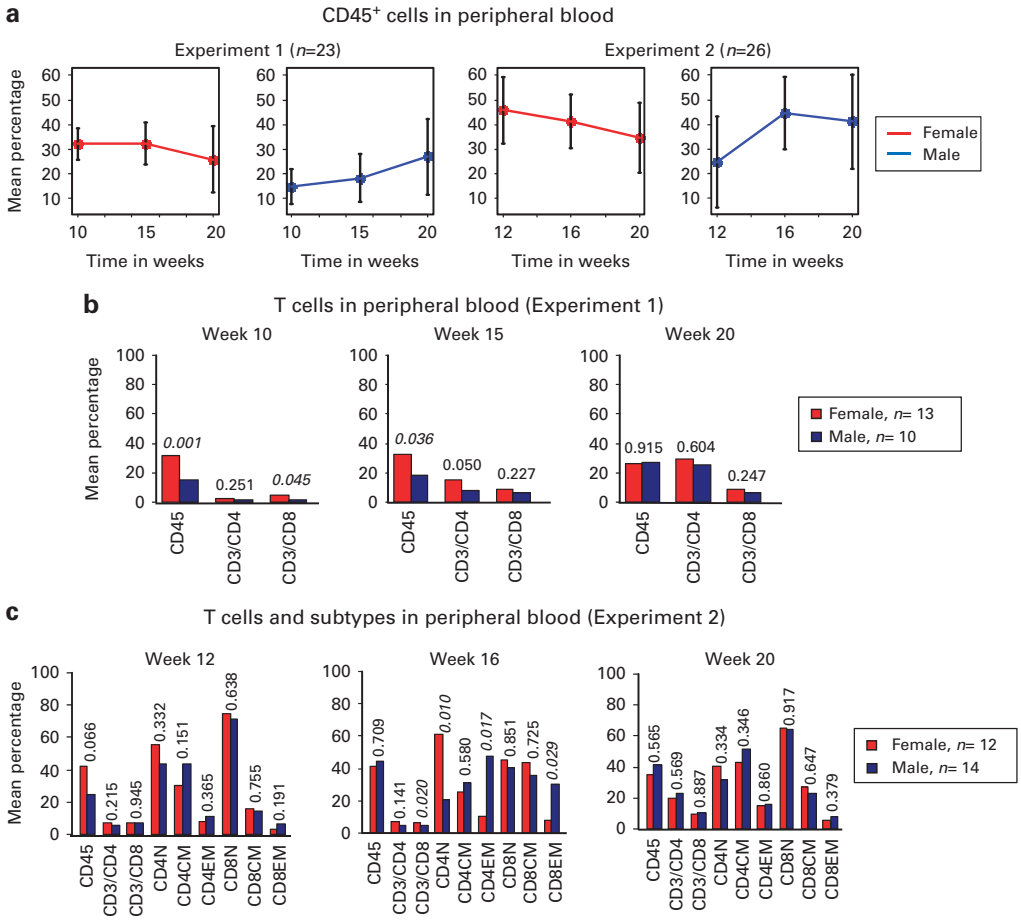

Figure 1. Differential engraftment and kinetics of human lymphocytes in humanized female and male mice. 5-week-old NRG mice were irradiated with 450 cGy and i.v. injected with 2×10^5 (Experiment 1: 13 females and 10 males) or 1.5×10^5 (Experiment 2: 12 females and 14 males) human cord blood CD34^{+} cells. Mice were killed 20 weeks after transplantation. Immune reconstitution was assessed by flow cytometry analyses at different time points. (**a**) Error bars representing frequencies of human CD45^{+} cells in peripheral blood for each gender in experiments 1 and 2. Results represent least square means as percentages obtained from repeated measures analysis of variance (ANOVA) with between-subjects factor gender. Error bars represent 95% confidence interval. Engraftment in female mice is represented in red, and in male mice in blue. (**b**) Bar plots illustrating frequencies of lymphocyte types per gender in peripheral blood at different time points of experiment 1. *P*-value results are from two-sided *t*-tests for independent group comparisons. Results represent least square means as percentages obtained from repeated measures ANOVA with between-subjects factor gender. Tukey–Cramer adjustment was used for multiple comparisons. Female mice are represented in red, and male in blue. *P*-values <0.05 are indicated in italic. (**c**) Bar plots illustrating T-cell subtypes (naïve, central memory and effector memory) per gender in peripheral blood for different time points of experiment 2 accompanied by *P*-value results from two-sided *t*-tests for independent group comparisons. Results represent least square means as percentages obtained from repeated measures ANOVA with between-subjects factor the gender. Tukey–Cramer adjustment was used for multiple comparisons. Female mice are represented in red, whereas males are represented in blue. *P*-values <0.05 are indicated in italic.

cells (CB-HSCs) showed better engraftment of human CD45$^+$ hematopoietic cells than males.[4] A subsequent work reported faster growth of human tumor lines in NSG male mice, further supporting differences of xenograft cell engraftment and growth in female and male hosts.[5] Here, we evaluated whether T-cell development after CD34$^+$ CB-HSC transplantation into 5-week-old NOD-Rag1$^{-/-}$-IL2Rγc$^{-/-}$ (NRG) mice was associated with gender. All experiments were performed in accordance with the guidelines and regulations regarding patient anonymity, informed consent and animal welfare. The protocols were approved by the Hannover Medical School (Ethics Committee) and State of Lower Saxony (Nds. Landesamt für Verbraucherschutz und Lebensmittelsicherheit, Dezernat 33/Tierschutz). Different cohorts of immune deficient mice were transplanted with distinct cord blood donors and analyzed at several time points (for example, weeks 10/12, weeks 15/16 and week 20). Information was collected for various cells in blood, spleen and lymph nodes. We considered 10–12 weeks after CB-HSCT in NRG mice as the time point to determine acceptable engraftment (for example, $>5\%$ huCD45$^+$ cells in peripheral blood) for inclusion in the study. We examined 23 mice (13 female and 10 male) in the first experiment, and 26 mice (12 female and 14 male) in the second experiment. In agreement with Notta *et al.*, we also observed that female mice transplanted with HSC from several cord blood units generally exhibited a higher frequency of huCD45$^+$ cells than males at the 10–12 week time point (Figures 1a and b). However, after 15–16 weeks post CB-HSCT, the frequency of huCD45$^+$ decreased in females but increased in males (Figure 1a). Remarkably, from weeks 10 to 16, females showed overall higher frequencies of T cells than males. Closer investigation of T-cell subsets from weeks 12–16 showed higher frequencies of naïve T cells in females, but higher frequencies of effector memory T cells in males (Figure 1c). Spleen and lymph nodes obtained from females at week 20 also contained higher frequencies of naive T cells, whereas memory T cells were more frequent in males (Supplementary Table 4). Taken together, these data from independent experiments indicated that higher thymic naïve T-cell output was supported in females post CB-HSCT, whereas peripheral activation/expansion of memory T cells was more dominant in males. Sex steroids were demonstrated to cause thymic atrophy with age in immune competent mice, and blockade of these steroids resulted in full reversal of thymic atrophy and enhanced regeneration of T cells.[6] In patients, temporarily blocking sex steroids before stem cell transplantation increased thymus function and enhanced the rate of T-cell regeneration.[7] In conclusion, CB-HSCT in humanized mice could potentially mirror the effect of murine sex hormones on human immune reconstitution, which should be taken as a note of caution for pre-clinical testing of vaccines and immune therapies. Thus, this emphasizes the need to separately analyze mice of both genders in experiments with humanized mice, as the quality of immune responses is likely to be different. In addition, the influence of gender in immune reconstitution should be evaluated after CB-HSCT in patients.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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