Sex-Based Differences in Vascular Repair With Bone Marrow Cell Therapy: Relevance of Regulatory and Th2-type Cytokines


ABSTRACT

Objectives. There are differences in symptoms, risk stratification, and efficacy of pharmacological treatments between men and women with coronary artery disease (CAD). The results of clinical studies of cell therapy in CAD patients are mixed. The relevance of sex to response to cell therapy is unknown. We investigated sex-based differences in response to bone marrow mononuclear cells (BM-MNCs) in atherosclerotic apolipoprotein E-knockout (ApoE<sup>−/−</sup>) mice.

Methods. Twenty-three male and 27 female ApoE<sup>−/−</sup> mice fed on a high-fat diet received four intravenous BM-MNC injections (C57BL6/J mice) starting at 14 weeks of age; male or female BM-MNCs were administered. Thirteen male and 20 female atherosclerotic ApoE<sup>−/−</sup> mice received vehicle. Aortic plaque burden (%), recipient bone marrow progenitor cell profiles (FACS-LSR II, FlowJo) and 22 circulating cytokine panel (LINCOplex) were quantified and analyzed statistically (SSPS, P < .05).

Results. Quantitative and semiquantitative results are presented. Increased G-CSF levels correlated with plaque reduction (r = −.86, P = .0004). G-CSF was clustered with IL-15.

Conclusions: Female but not male BM-MNCs exhibited atheroprotection in male atherosclerotic ApoE<sup>−/−</sup> mice. Plaque lesions did not attenuate atherosclerosis in female ApoE<sup>−/−</sup> mice with BM-MNCs of either donor sex. An increase in regulatory and in Th2-type response may be required for atheroprotection. Sex-based differences in vascular repair have implications for cell therapy trials in CAD.
sex-matched ($n=24$) or -mismatched ($n=26$) BM mononuclear cells (BM-MNCs; derived from C57BL6/J mice, 10 ± 2 weeks old, as previously described) or vehicle ($n=33$) intravenously four times biweekly. At animal harvest (21 ± 1 weeks of age), aortic plaque burden (%), BM progenitor cell profiles (FACS-LSR II, FlowJo), and 22 circulating cytokines (LINCOp lex) were quantified and analyzed statistically (SSPS), with $P<.05$ indicating statistical significance.

RESULTS

Only treatment of male atherosclerotic ApoE$^{-/-}$ mice with female BM-MNCs produced significant plaque burden attenuation compared with vehicle-treated or male BM-MNC-treated mice (Fig 1). This atheroprotective effect corresponded to an increase in BM AC133$^+$/CD34$^+$ and CD34$^+$ cells (Fig 2). Plaque burden, being significantly lower than in male vehicle-treated mice, did not further reduce in female ApoE$^{-/-}$ mice after BM-MNCs of either donor sex, even though the percentages of AC133$^+$/CD34$^+$ and CD34$^+$ cells were similar to male mice that exhibited atheroprotection (data not shown).

The reduction in plaque lesion formation correlated with an increase in BM CD45$^+$ cells (not shown) as well as with the up-regulation of circulating Th1- and Th2-type cytokines and of G-CSF (Fig 3). Of these, G-CSF significantly correlated with attenuation of plaque formation ($r = -.86$, $P = .0004$) and was clustered with interleukin (IL)-15 (Fig 4). Female ApoE$^{-/-}$ vehicle-treated mice had significantly higher G-CSF and IL-5, IL-13, and IL-17 levels compared with male ApoE$^{-/-}$ vehicle-treated mice (all $P < .05$, not shown).

DISCUSSION

Our study demonstrated sex-based differences in endogenous repair after BM-MNC therapy. Specifically, the efficiency of endogenous repair was higher in female ApoE$^{-/-}$ mice at 21 weeks of age compared to male mice as demonstrated by a lower atherosclerotic plaque burden, higher percentages of AC133$^+$/CD34$^+$ and CD34$^+$ cells, and higher circulating G-CSF and Th2-type cytokine levels. In these settings, administration of BM-MNCs for atheroprotection was not beneficial. In contrast, in male ApoE$^{-/-}$ mice, where the efficiency of endogenous repair was lower, cell therapy produced a sizeable reduction of atherosclerotic burden. This observation has direct clinical relevance for clinical cell therapy trials in CAD, as administration of autologous BM cells from a low-efficiency repair environ-
A hierarchical clustering analysis in male ApoE\(^{-/-}\) mice treated with female BM-MNCs.

Fig 4. Hierarchical clustering analysis in male ApoE\(^{-/-}\) mice treated with female BM-MNCs.

In our study, cell-mediated repair succeeded only when the increase in Th1-type cytokines (typical for atherosclerosis)\(^4\) were counteracted by the increase in Th2-type (anti-inflammatory) cytokines, as well as in G-CSF, clustered with IL-15. G-CSF mediates mobilization of reparative progenitors via the Th2 pathways.\(^5\) A lower efficiency of endogenous repair in male ApoE\(^{-/-}\) mice was characterized by the absence of Th2-type cytokine or regulatory cytokines activation, and exogenous female BM-MNCs that expressed those cytokines filled that gap and reduced atherosclerosis.

In conclusion, there are sex-based differences in endogenous repair and in the response to cell therapy in atherosclerotic ApoE\(^{-/-}\) mice. Female ApoE\(^{-/-}\) mice have a higher efficiency of endogenous repair at midage compared to males, and BM-MNCs derived from wild-type female mice were more reparative (when administered to male ApoE\(^{-/-}\) mice) than the cells derived from males. Higher efficiency of endogenous repair in female ApoE\(^{-/-}\) mice as well as greater reparative activity of female cells was associated with increased Th2-type and hematopoietic/regulatory (G-CSF, IL-15) cytokines. Future research will examine whether these sex-based differences are estrogen-mediated.

REFERENCES