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Intravenous Administration of Gr1+CD11b+ Myeloid Cells Increases Neovascularization and Improves Cardiac Function After Heart Infarction

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Keywords

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The relative importance of bone marrow-derived cell populations to adult neovascularization is not clear. There are increasing evidences that myeloid cell lineage may play a role in neovascularization[1–4]. In a previous report, we demonstrated that Gr-1+CD11b+ myeloid cells improve both angiogenesis and vasculogenesis in tumor[1]. A recent report by Kim et al showed that Muscle-derived Gr1(dim)CD11b(+) myeloid cells enhance neovascularization in an ischemic hind limb model[3]. Similarly, a study with the same ischemic hindlimb model also verified the enhanced neovascularization properties uniquely associated with proangiogenic cells derived from common myeloid progenitors[4]. In the present study, we further investigated whether intravenous administration of Gr1+CD11b+ myeloid cells increases neovascularization and improves cardiac function after heart infarction.

C57BL/6 mice were purchased from Jakson Lab. All animals received humane care and the study protocols were approved by Vanderbilt Medical University Animal Care and Use Committee.

We sorted Gr-1+CD11b+ myeloid cells from the spleens of mice as previously described[5]. To investigate whether Gr-1+CD11b+ myeloid cells increase angiogenesis *in vitro*, we cultured Gr-1+CD11b+ myeloid cells with aortic ring [6]. At the seventh day, we found that

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endothelial sprouts in Gr-1+CD11b+ myeloid cell treated group are significantly more than that in no cell treated group (23.2 ± 3.7 versus 13.4 ± 2.4 control, $p < 0.01$, $n = 5$ in each group, fig 1A and B), which indicated that Gr-1+CD11b+ myeloid cells increase angiogenesis i*n vitro*. In order to see whether Gr-1+CD11b+ myeloid cells increase angiogenesis *in vivo*, we performed dorsal window model in mice[7]. We injected Gr-1+CD11b+ myeloid cells into tail vain of mice to see whether injected Gr-1+CD11b+ myeloid cells home to the site of window chamber. By tracing Gr-1+CD11b+ myeloid cells marked with PKH-26 (Fig 1C-a), we found that the Gr-1+CD11b+ myeloid cells home to the site of the window chamber after intravenous injection (Fig 1C-b). We further found the intravenous injection of Gr-1+CD11b+ myeloid cells obviously increase blood vessel branches at the site of window chamber, compared with saline treated groups, which suggested that the Gr-1+CD11b+ myeloid cells also increase angiogenesis *in vivo* (Fig 1D).

Next, we injected Gr-1+CD11b+ myeloid cells via tail vain to see whether Gr-1+CD11b+ myeloid cells improve heart function after heart infarction with a model of LAD ligation in mice[8]. After 4 weeks of LAD ligation and myeloid cells injection, the cardiac function was evaluated by echocardiography. The measured index indicated that myeloid cells significantly improve heart FS (37.2 ± 7.8% versus 27.3 ± 7.2% control, $p < 0.05$, $n = 5$ in each group, Fig 2A and B). The hearts were harvested after 4 weeks, and Masson's trichrome staining was performed[9]. The results indicated that myeloid cells significantly decrease infarct size after LAD ligation (28.9 ± 7.4% versus 45.8 ± 5.0% control, $p < 0.01$, $n = 5$ in each group, Fig 2C and D). We further performed capillary density measurement and tracing myeloid cells marked with PKH-26 in the infarct hearts. The results showed that myeloid cells significantly increase capillary density in the board area of infarction (159.2 ± 13.3 versus 96.8 ± 9.0 control, $p < 0.01$, $n = 5$ in each group Fig 2E and F). By tracing the myeloid cells and immunostaining of endothelial cells with anti-CD31 antibody, we found a few Gr-1+CD11b+ myeloid cells incorporate into vasculature (Fig 2G). These indicated Gr-1+CD11b+ myeloid cells improve cardiac function after heart infarction via both angiogenesis and vasculogenesis.

In conclusion, the intravenous administration of Gr-1+CD11b+ myeloid cells increases neovascularization and improves cardiac function after heart infarction. The Gr-1+CD11b+ myeloid cells could be used as a potential cell source in the field of cell therapy for ischemic cardiovascular diseases.

Acknowledgments

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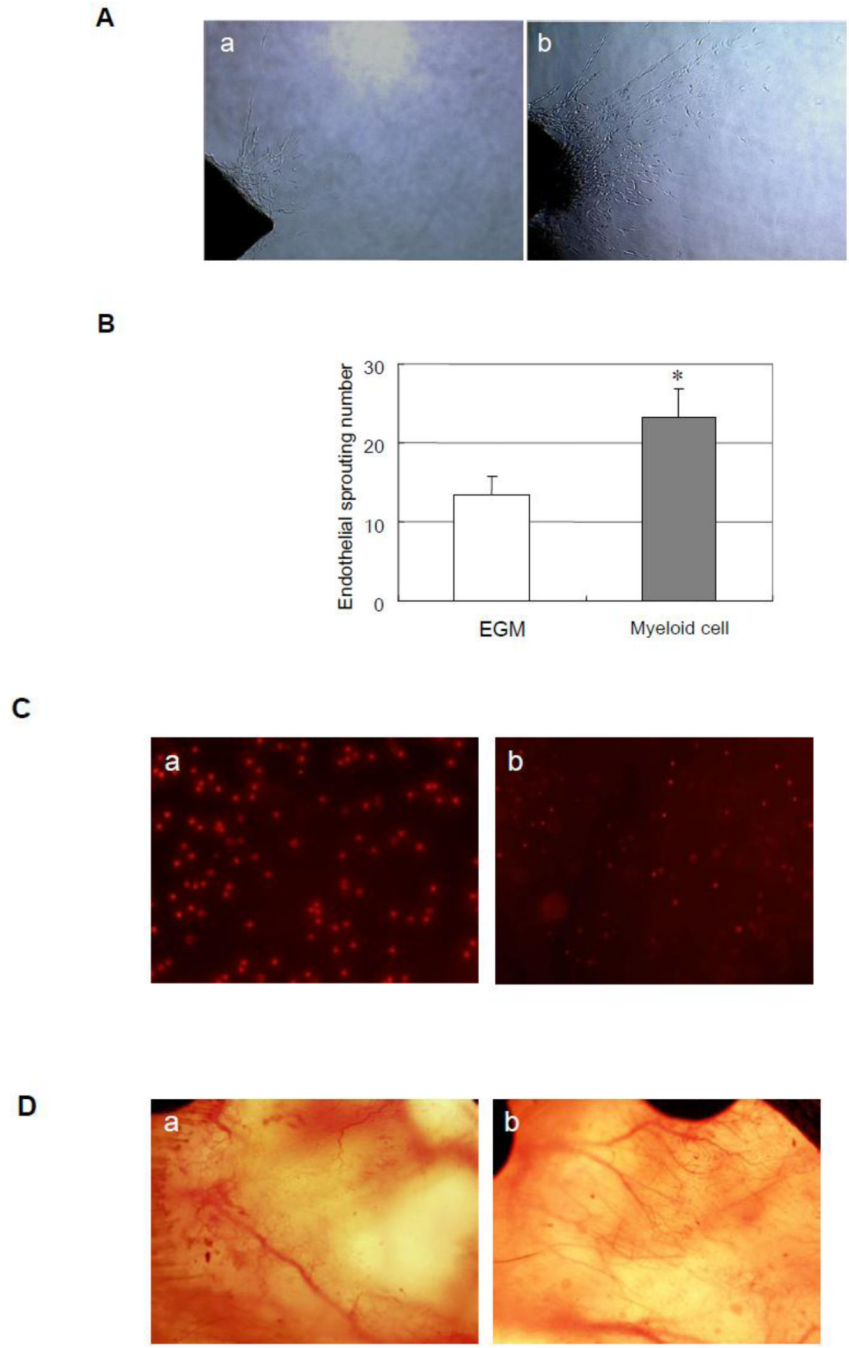
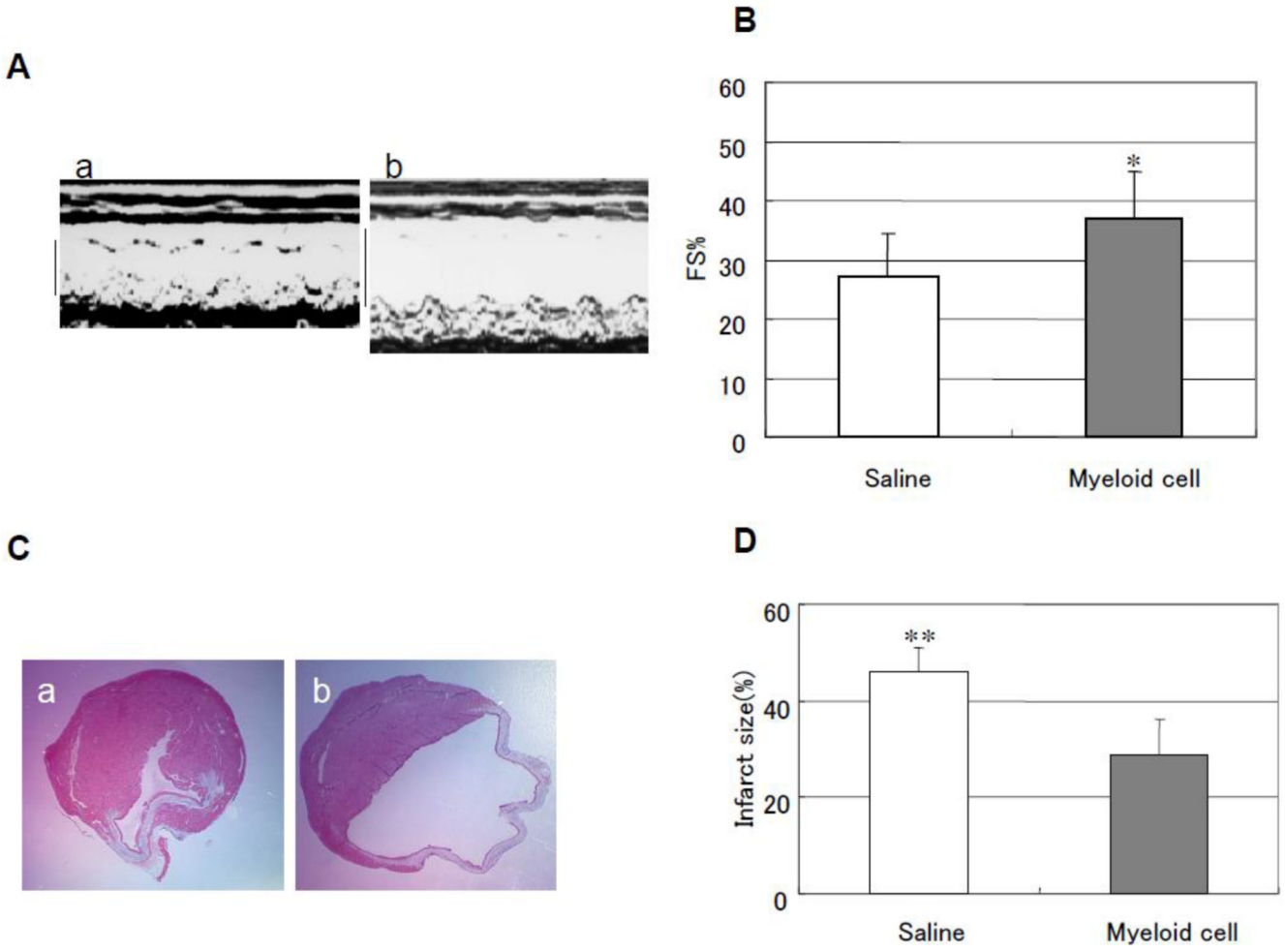


Fig 1.

Gr-1+CD11b+ myeloid cells increase angiogenesis *in vitro* and *in vivo* (A) Representive endothelial sprouts after 7 days of aortic ring sprouts assay. a, aorta ring culture with no cells; b, aorta ring culture with Gr-1+CD11b+ myeloid cells. Magnification ×10. (B) Gr-1+CD11b+ myeloid cells significantly increase endothelial sprouts compared with no cell treatment, *p < 0.01 versus EGM. (C) Gr-1+CD11b+ myeloid cells home to the site of window chamber after injection from tail vain. a, Gr-1+CD11b+ myeloid cells marked with PKH-26. Magnification ×100. b, Gr-1+CD11b+ myeloid cells homed to the site of window chamber. Magnification ×40. (D) Gr-1+CD11b+ myeloid cells increase angiogenesis in

window chamber. a, saline treatment; b, Gr-1+CD11b+ myeloid cells treatment. Magnification ×10.



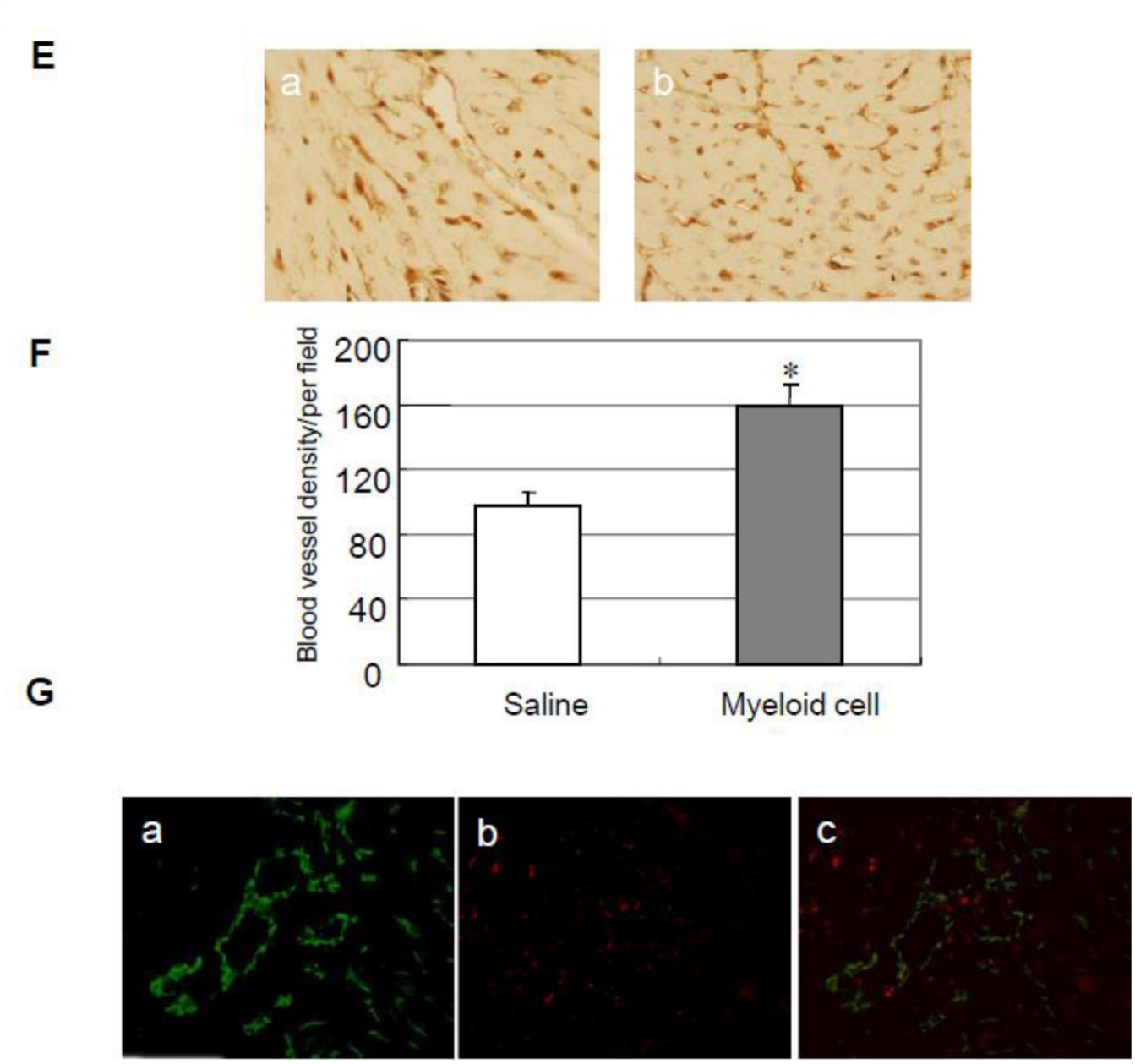


Fig 2.

Intravenous injection of Gr-1+CD11b+ myeloid cells improves cardiac function and decreases cardiac infarct size after heart infarction via increasing neovascularization. (A) Representive echocardography of heart after 4 weeks of LAD ligation. a, Gr-1+CD11b+ myeloid cells injected from tail vain; b, saline injected from tail vain. (B) Gr-1+CD11b+ myeloid cells significantly improve FS after heart infarction compared with saline, *$p < 0.05$ versus saline, n=5 in each group. (C) Representive Masson's trichrome staining of the heart after 4 weeks of heart infarction, a, Gr-1+CD11b+ myeloid cells treatment; b, saline treatment. (D) Gr-1+CD11b+ myeloid cells significantly decrease cardiac infarct size after heart infarction compared with saline, **$p < 0.01$ versus saline, n=5 in each group. (E) Representive capillary density measurement in peri-infarct area of the heart. a, saline treatment; b, Gr-1+CD11b+ myeloid cells treatment. Magnification ×200. (F) Gr-1+CD11b

+ myeloid cells significantly increase angiogenesis in the peri-infarct area after heart infarction, *$p < 0.01$ versus saline, n=5 in each group. (G) Florescent imaging of the heart after intravenous injection of Gr-1+CD11b+ myeloid cells. a, endothelial cells stained with anti-CD31 (green); b, Gr-1+CD11b+ myeloid cells marked with PKH-26 (red); c, some of the Gr-1+CD11b+ myeloid cells incorporate into vasculature (yellow). Magnification ×200.