

SUPPLEMENTAL MATERIAL.

Ultrasound Molecular Imaging of Lymphocyte-endothelium Adhesion Cascade in Acute Cellular Rejection of Cardiac Allografts

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Running title: Ultrasound Imaging of Lymphocyte-endothelium Adhesion

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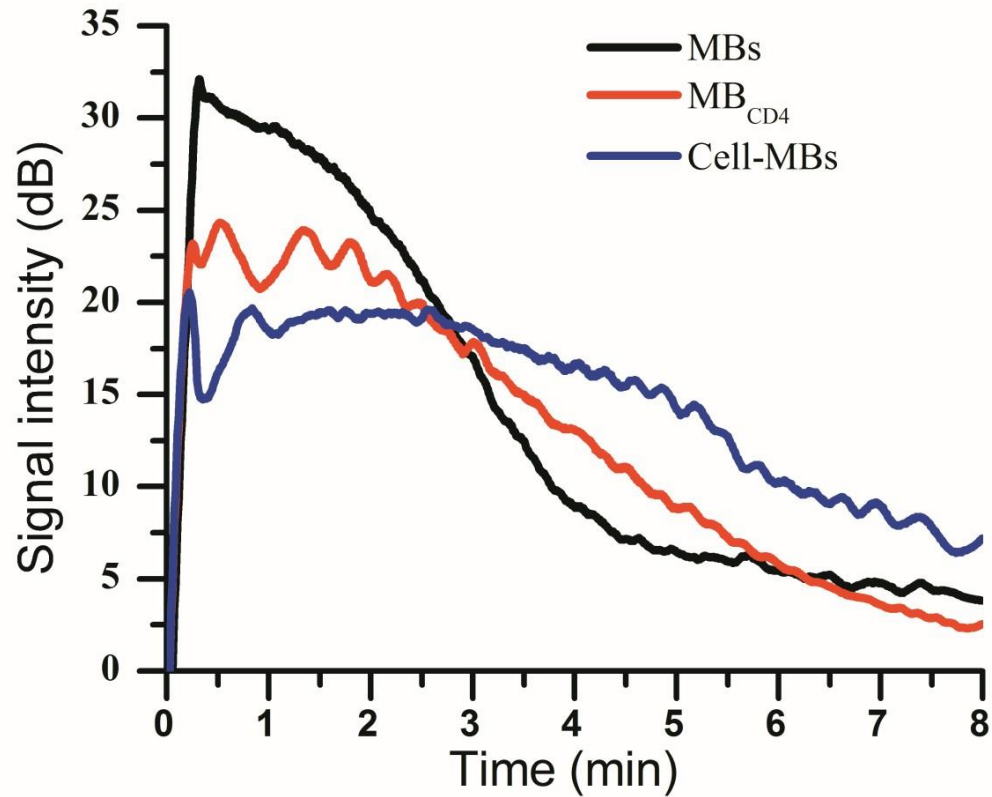


Figure S1. The time-intensity curve of the left-ventricular myocardial region of an allograft after injection of plain MBs, MB_{CD4}, cell-MBs (10^8 particles/rat). The ultrasound signal of MBs decreased rapidly and it was substantially low at 5 min after injection. However, the signal of cell-MBs decreased slower and remained relatively high at 5 min after injection.

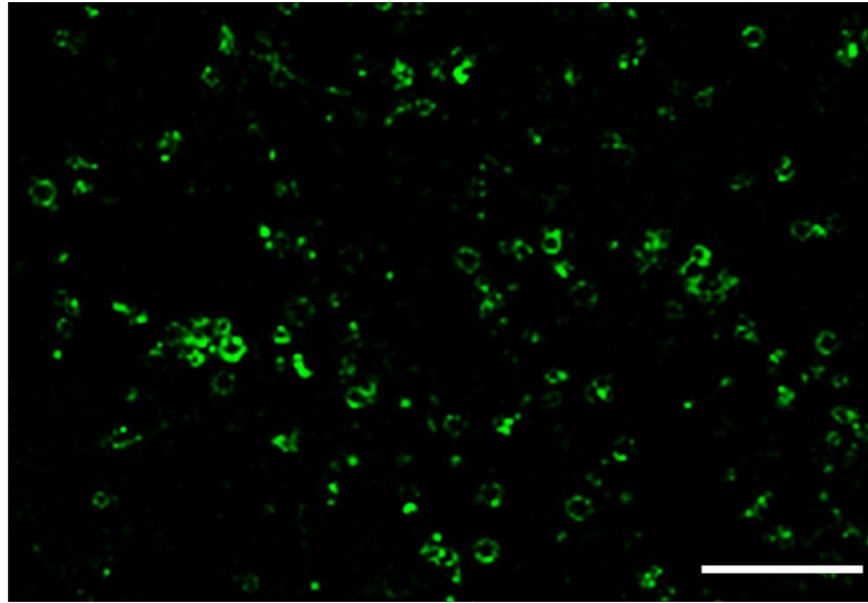


Figure S2. Anti-CD4 antibody-conjugated microbubbles (MB_{CD4}). MB_{CD4} were labeled with FITC-anti-mouse IgG antibodies. The fluorescent signal on microbubbles indicated that anti-CD4 antibodies were successfully conjugated on microbubbles. Scale bar = 30 μ m.

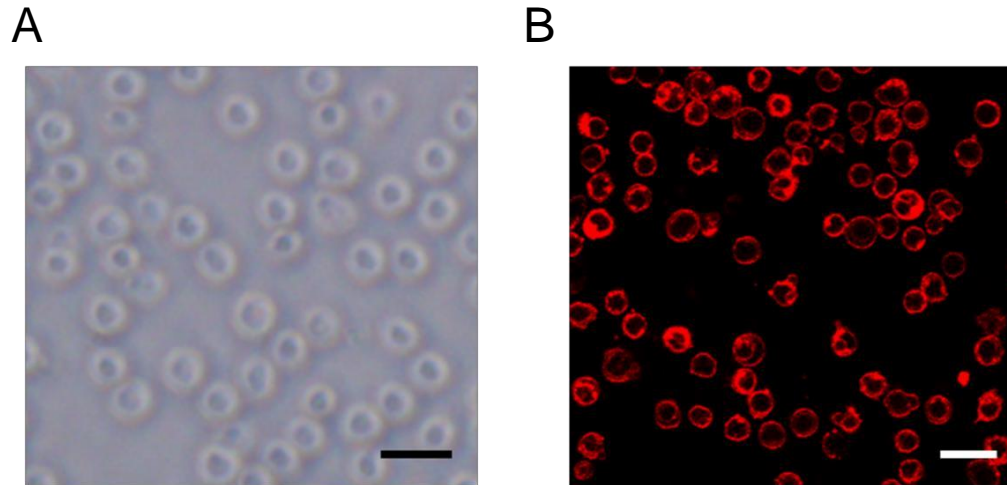


Figure S3. Images of rat lymphocytes. **(A)** The representative bright field image under microscope. Scale bar = 10 μm . **(B)** Lymphocytes were labeled with Dil dye, and images were taken by confocal laser scanning microscope (CLSM). Scale bar = 15 μm .

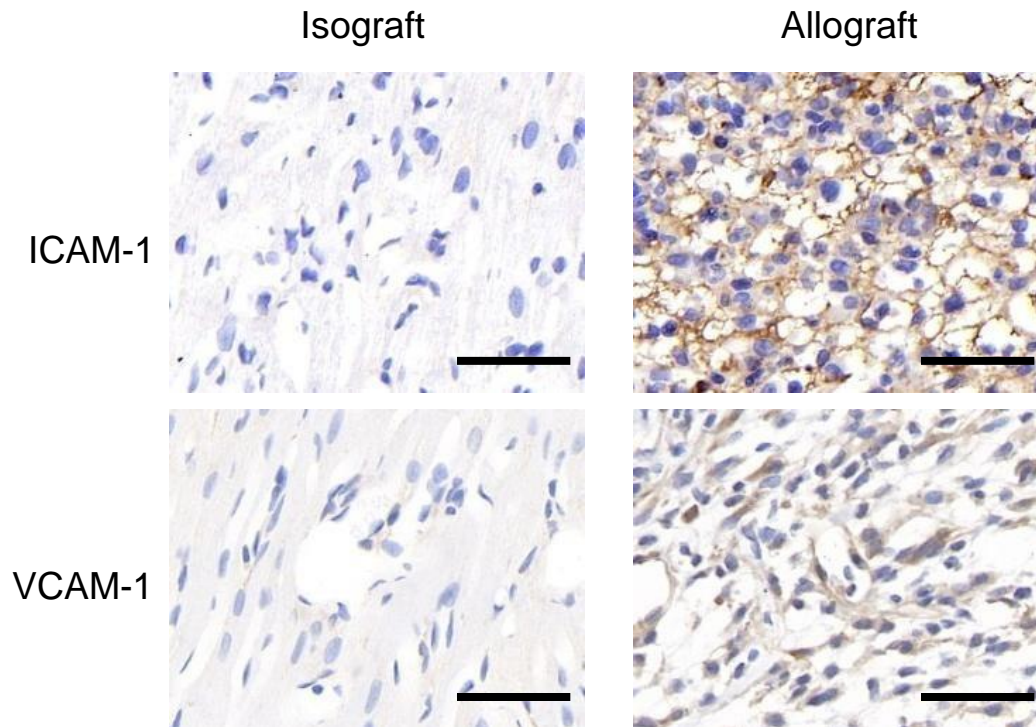


Figure S4. Immunohistochemical staining for ICAM-1 and VCAM-1 of grafts on post-transplantation day 3. Overexpressed ICAM-1 and VCAM-1 could be seen in allografts but not in isografts. Scale bar = 50 μ m.

Table S1. List of primers for qPCR.

Gene	Sense	Antisense
GAPDH	TTCCTACCCCCAATGTATCCG	CATGAGGTCCACCACCCTGTT
CXCL9	CAGCCAAGGCACATTCCACTAC	CTTGCTGAATCTGGGTCTAGGC
CXCL10	CATGAACAGACGCTGAGACCC	GATGGCCTCAGATTCCGGAT
CCL4	CCTTCTGCGATTCAGTGCTGT	ATACTCATTGACCCAGGGCTCG
CCL5	GCATCCCTCACCGTCATCCT	CACTTCTTCTCTGGGTTGGCAC
CCL2	GCTGCCTGTAGCATCCACGT	CCTTATTGGGGTCAGCACAGATC

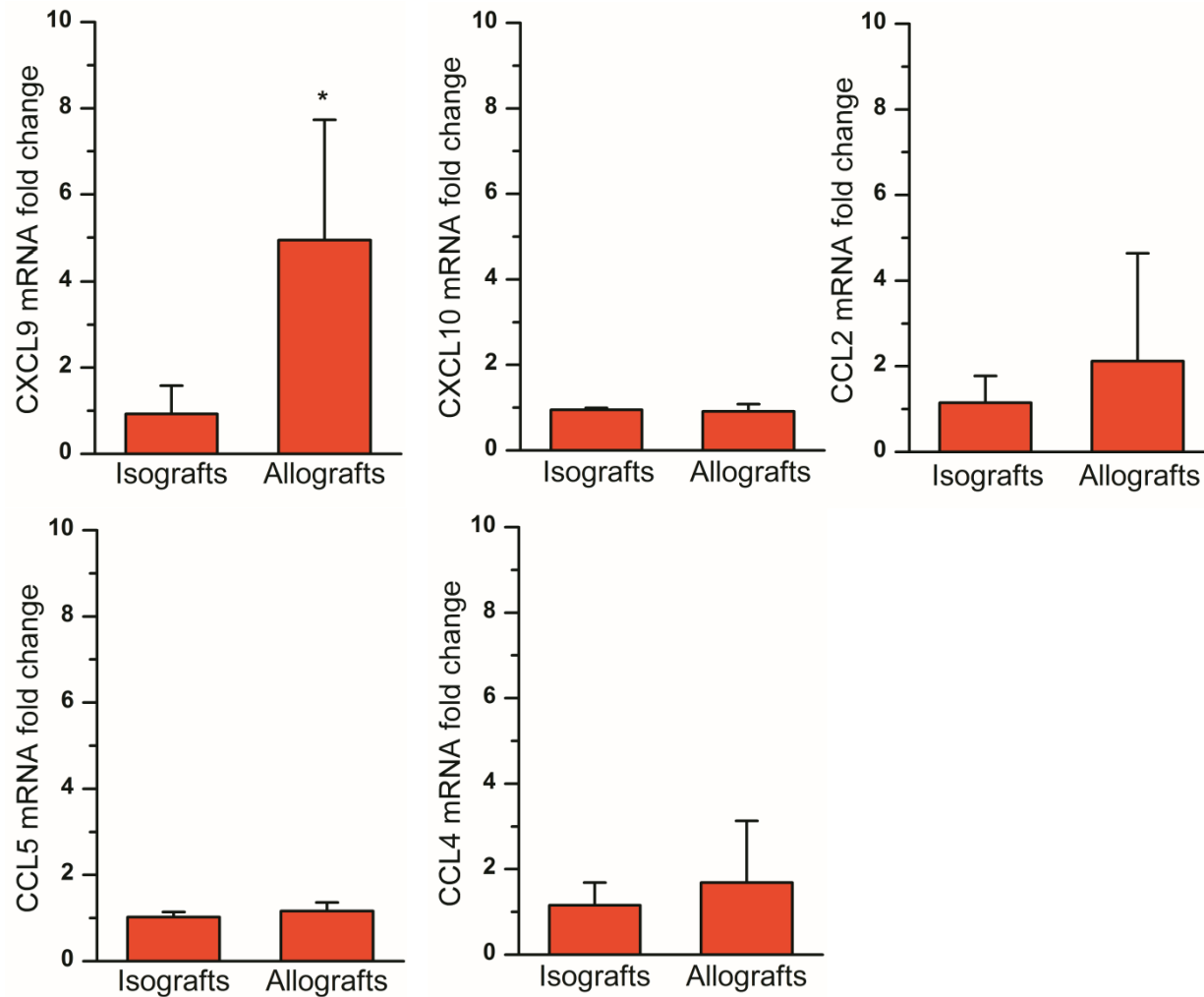


Figure S5. mRNA relative expression of rejection-related chemokines in grafts on post-transplantation day 3. CXCL9 mRNA expression in allografts was significantly higher than that in isografts. But mRNA expression of CXCL10, CCL2, CCL4 and CCL5 were not significantly different between isografts and allografts. * $p < 0.05$.

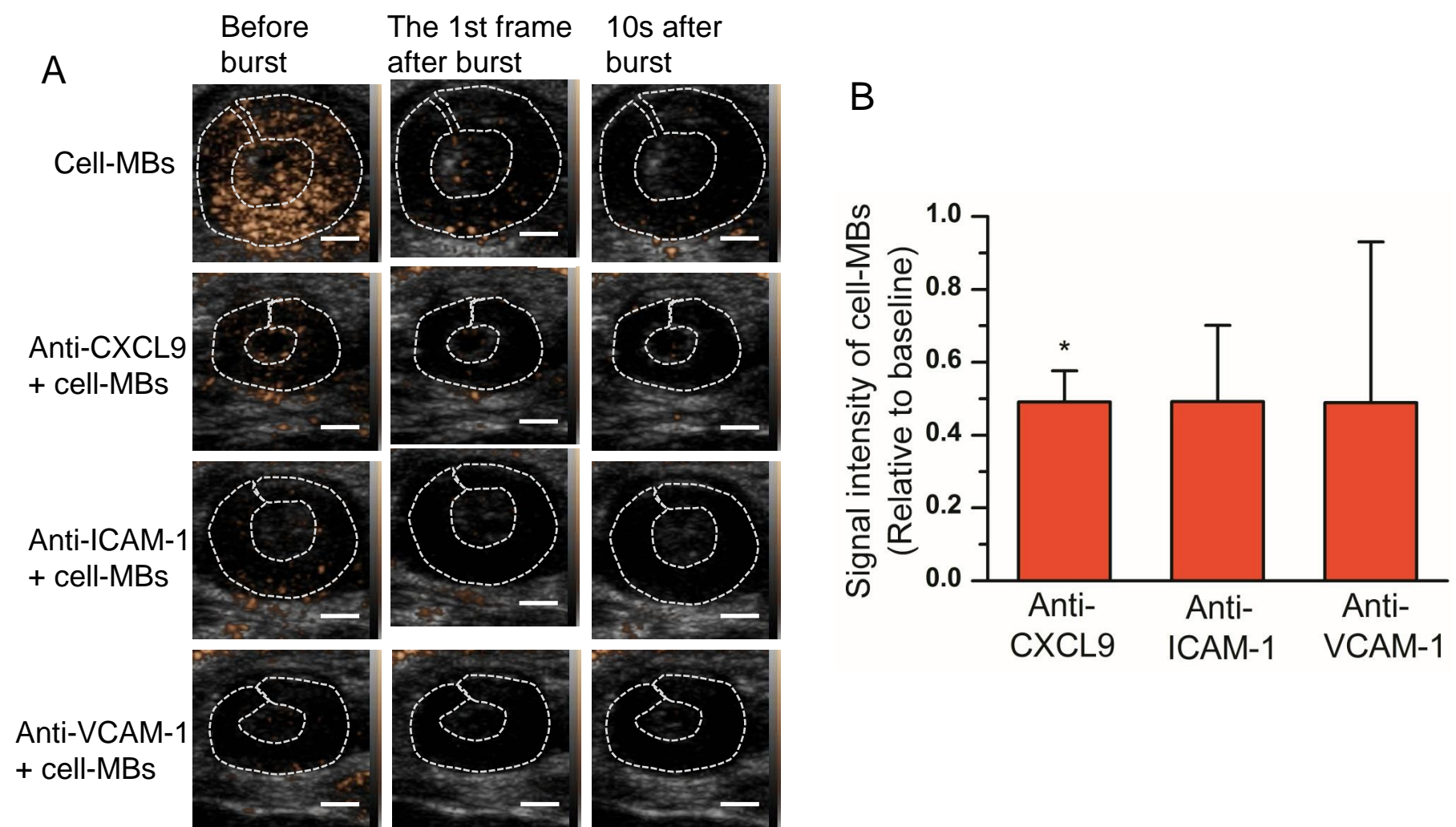


Figure S6. Ultrasound molecular imaging of allografts with cell-MBs before and 10 min after administration of antibodies against CXCL9, ICAM-1, or VCAM-1.

(A) Representative ultrasound images of allografts with cell-MBs before and after antibody administration. The region in the dotted line is the region of interest, i.e. myocardium. **(B)** Data were shown relative to pretreatment baseline values. CXCL9 depletion significantly reduced the molecular imaging signal intensity of cell-MBs. * $p < 0.05$ versus baseline.

Video S1. A transplanted heart in rat abdomen after surgery.

Video S2. Ultrasound molecular imaging at around 5 min post-injection with plain microbubbles in an allograft.

Video S3. Ultrasound molecular imaging at around 5 min post-injection with anti-CD4 antibody-conjugated microbubbles (MB_{CD4}) in an allograft.

Video S4. Ultrasound molecular imaging at around 5 min post-injection with cell-MBs in an allograft.