CXCL1 and its receptor, CXCR2, mediate murine sickle cell vaso-occlusion during hemolytic transfusion reactions

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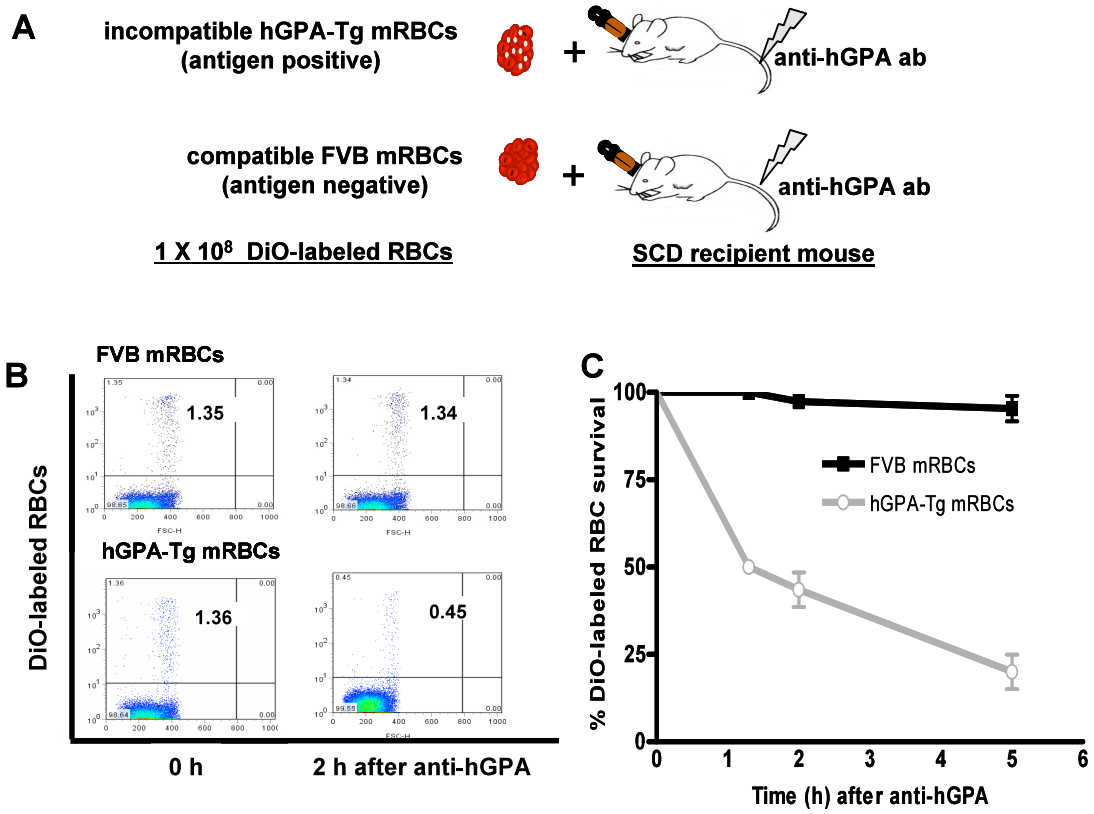


Supplementary Figure 1. A Mouse model of alloimmune IgG-mediated HTRs. (A) 50 µL DiO-labeled RBCs from hGPA-Tg (incompatible) or wild-type FVB (compatible) mice were transfused into SCD mice followed by passively immunization with 10 µg of IgG monoclonal anti-hGPA antibody. (B) Percentage of DiO-labeled transfused RBCs within circulating blood at 0 and 2 h after anti-hGPA antibody injection in representative SCD mice. (C) Survival of transfused RBCs at defined intervals after anti-hGPA antibody administration.

Supplementary Table 1. Hemodynamic parameters in immunized SCD mice transfused with either hGPA-Tg or control wild-type FVB RBCs.

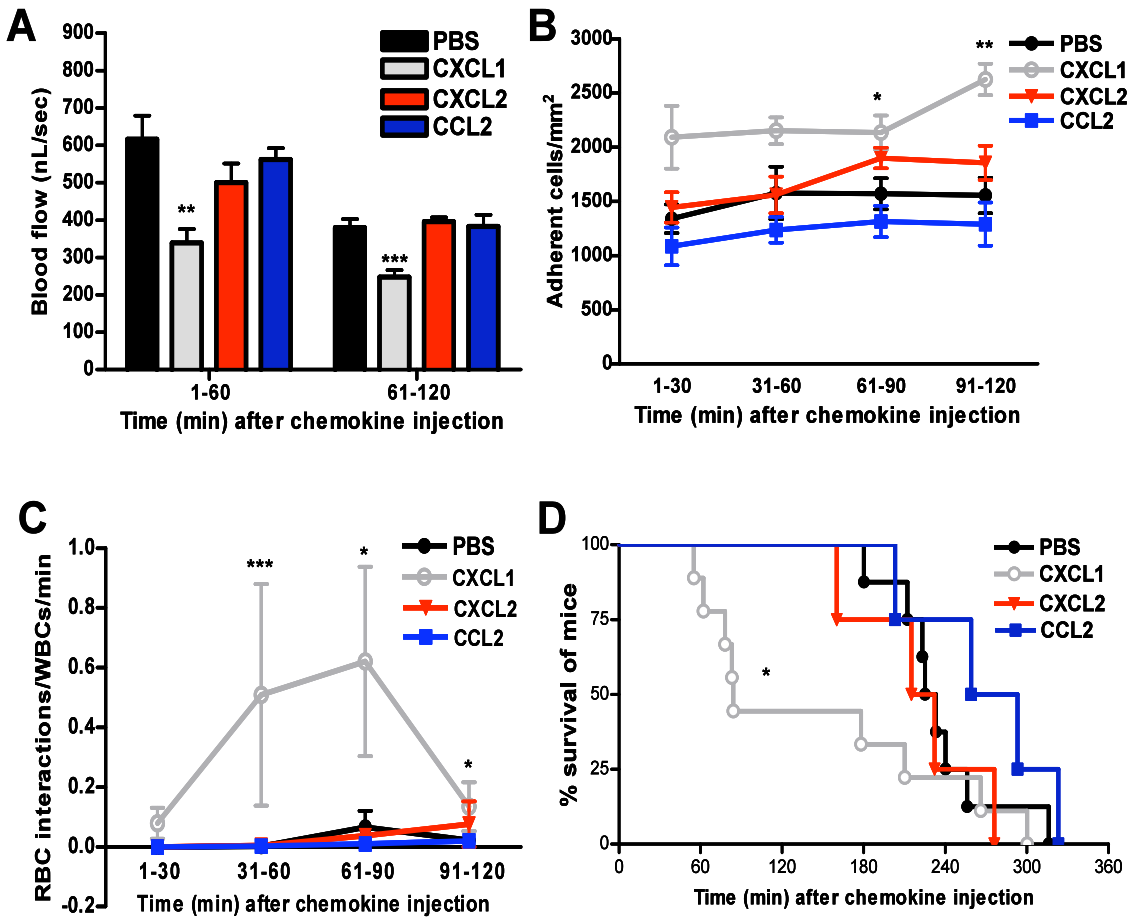
Transfusion (RBCs)	Time after anti-hGPA (min)	Mice (n)	Venule (n)	Venular diameter (μm)	Centerline velocity (mm/s)	Shear rate (s^{-1})
FVB	0	7	32	19.4 ± 0.3	1.9 ± 0.1	1070 ± 63
	60 min	7	62	19.5 ± 0.3	1.8 ± 0.1	956 ± 63
	120 min	7	73	19.8 ± 0.3	1.5 ± 0.1	792 ± 70
hGPA-Tg	0	7	32	19.4 ± 0.6	2.3 ± 0.2	1259 ± 143
	60 min	7	61	19.6 ± 0.9	1. 4 ± 0.1**	769 ± 81*
	120 min	7	79	20.7 ± 0.6	1.2 ± 0.1*	611 ± 48***

Data presented as mean ± SEM. *P*<0.05, ** *P*<0.01, *** *P*<0.001 compared with wild-type FVB RBC transfusion at defined intervals.

Supplementary Table 2. Hemodynamic parameters in SCD mice treated with either control PBS or CXCL1.

Treatment	Time (min)	Mice (n)	Venule (n)	Venular diameter (μm)	Centerline velocity (mm/s)	Shear rate (s^{-1})
PBS	0 - 60	7	52	20.9 ± 0.3	3.0 ± 0.2	1523 ± 104
	61 - 120	7	63	21.0 ± 0.2	2.1 ± 0.1	1047 ± 69
CXCL1	0 - 60	10	73	20.7 ± 0.3	2.4 ± 0.2 *	1244 ± 105
	61 - 120	10	78	20.8 ± 0.3	1.5 ± 0.1***	758 ± 36 ***

Data presented as mean ± SEM. * *P*<0.05, *** *P*<0.001 compared with control PBS group at defined intervals.



Supplementary Figure 2. Effect of elevated chemokines on leukocyte recruitment. CXCL1 (300 pg, (gray)), CXCL2 (160 pg, (red)), CCL2 (630 pg, (blue)) or PBS control (black) was injected intravenously into SCD mice (n=4-9 mice per group). (A) Blood flow (nL/ s), (B) Adherent leukocytes (per mm^2), and (C) Number of interactions between circulating sickle RBC and adherent leukocytes per minute were measured at defined intervals after chemokine infusion. (D) Kaplan-Meier survival curves for groups of SCD mice infused with either chemokines or PBS (P<0.05, log-rank test). *P<0.05, **P<0.01, *** P<0.001 relative to corresponding control vehicle.

Supplementary Table 3. Hemodynamic parameters in SCD mice treated with PBS, CXCL1, CXCL2 or CCL2.

Treatment	Time (min)	Mice (n)	Venule (n)	Venular diameter (μm)	Centerline velocity (mm/s)	Shear rate (s^{-1})
PBS	0 - 60	5	29	20 ± 0.2	3.1 ± 0.3	1675 ± 161
	61 - 120	5	40	20 ± 0.1	1.9 ± 0.1	1020 ± 64
CXCL1	0 - 60	6	39	20 ± 0.2	1.7 ± 0.2**	901 ± 96**
	61 - 120	6	28	21 ± 0.3	1.2 ± 0.1***	610 ± 41***
CXCL2	0 - 60	4	31	20 ± 0.2	2.4 ± 0.2	1255 ± 117
	61 - 120	4	31	20 ± 0.2	1.9 ± 0.1	1019 ± 45
CCL2	0 - 60	4	31	20 ± 0.2	2.8 ± 0.2	1503 ± 92
	61 - 120	4	36	20 ± 0.1	1.9 ± 0.2	985 ± 91

Data presented as mean ± SEM. * P<0.05, *** P<0.001 compared with control PBS group at defined intervals.

Supplementary Table 4. Hemodynamic parameters in HTR-induced SCD mice pretreated with either a CXCR2 antagonist or vehicle DMSO.

Treatment	Time (min)	Mice (n)	Venule (n)	Venular diameter (μm)	Centerline velocity (mm/s)	Shear rate (s^{-1})
DMSO	0 - 60	6	31	19.8 ± 0.3	1.4 ± 0.1	754 ± 58
	61 - 120	6	25	19.7 ± 0.3	1.1 ± 0.1	595 ± 31
CXCR2 antagonist	0 - 60	5	32	19.7 ± 0.3	2.1 ± 0.2**	1151 ± 98**
	61 - 120	5	31	19.8 ± 0.2	2.0 ± 0.2**	1066 ± 125**

Data presented as mean ± SEM. ** P<0.01, compared to vehicle DMSO at defined intervals.

Supplementary Video 1 and 2. Intravital microscopy of passively immunized SCD mice at 90 to 120 min after transfusion of either hGPA-Tg RBC (video 1) or control wild-type FVB RBC (video 2).

Supplementary Video 3 and 4. Intravital microscopy of SCD mice between 90 - 120 min after administration of CXCL1 (video 3) or PBS (video 4)

Supplementary Video 5 and 6. Intravital microscopy of SCD mice, pretreated with either CXCR2 antagonist (video 5) or vehicle contro1 (video 6), at 91 – 120 min after HTR induction.