Title:
CCR2 inhibition sequesters multiple subsets of leukocytes in the bone marrow

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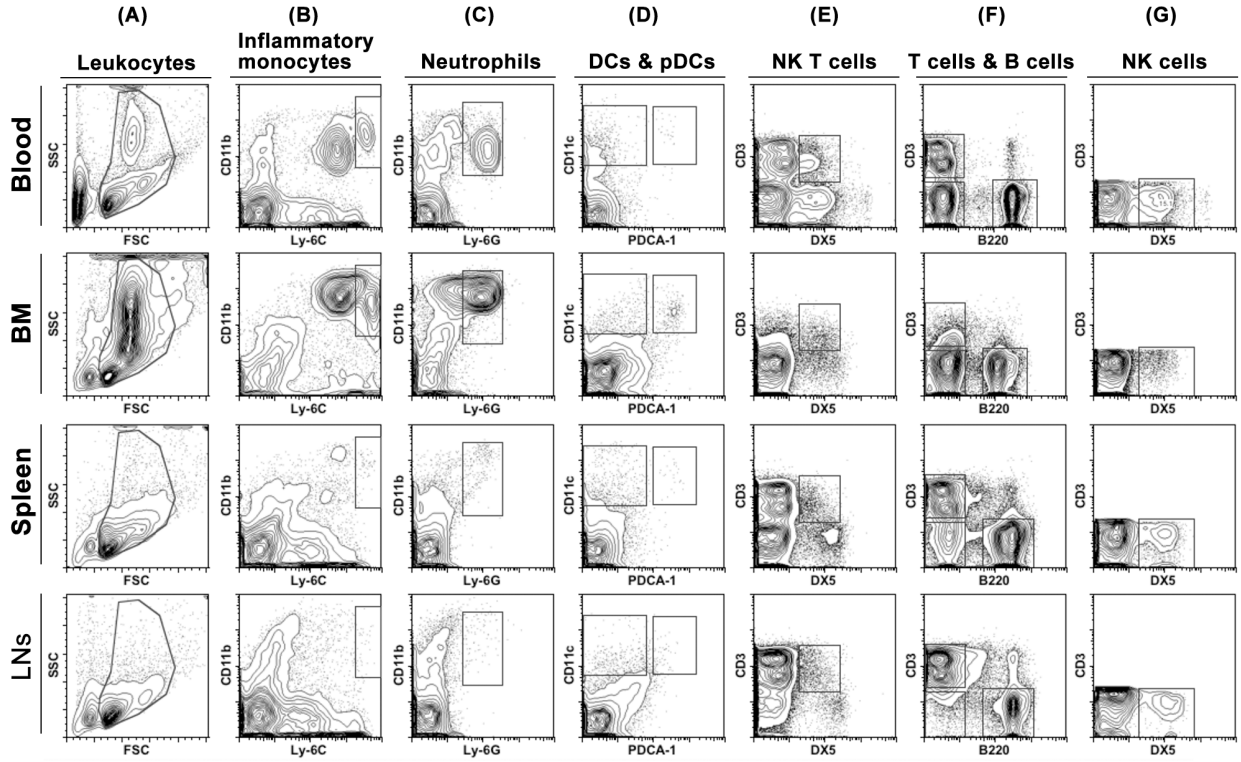

Supplementary Figure 1. Flow cytometric strategies for identifying leukocyte subsets. In all experiments, tail vein blood (in 5 mM EDTA/PBS) and cell suspensions isolated from femur bone, spleen and cervical LNs were stained with differentially fluorescence dye-labeled mAb combinations. (A): FSC and SSC identified total leukocytes for further analyses in panels B-F. (B): CD11b and Ly-6C mAb staining identified inflammatory monocytes as $CD11b^{+}Ly\text{-}6C^{high}$ cells. (C): CD11b and Ly-6G mAb staining identified neutrophils as $CD11b^{+}Ly\text{-}6G^{+}$ cells. (D): CD11c and PDCA-1 mAb staining identified DCs and pDCs as $CD11c^{+}PDCA\text{-}1^{-}$ and $CD11c^{+}PDCA\text{-}1^{+}$ cells, respectively. (E, F): CD3, B220 and DX5 mAb staining identified NK T cells as $CD3^{+}DX5^{+}$ cells (E), T cells as $CD3^{+}B220^{-}$ cells (F), B cells as $CD3^{-}B220^{+}$ cells (F), and $CD3^{-}B220^{-}$ cells (F). (G): DX5 mAb staining identified NK cells as $DX5^{+}$ cells in $CD3^{-}B220^{-}$ cells gated in panel F.

Supplementary Table 1. Effects of CCR2 deficiency on the relative numbers of individual leukocyte subsets in the peripheral blood and lymphoid tissues

Tissue	Blood		BM		LNs		Spleen	
	CCR2-RFP$^{+/-}$	CCR2-RFP$^{+/+}$	CCR2-RFP$^{+/-}$	CCR2-RFP$^{+/+}$	CCR2-RFP$^{+/-}$	CCR2-RFP$^{+/+}$	CCR2-RFP$^{+/-}$	CCR2-RFP$^{+/+}$
Inflammatory monocytes	3.1 ± 0.3	0.5 ± 0.2**	9.6 ± 0.7	8.3 ± 0.6*	0.13 ± 0.03	0.05 ± 0.03**	0.7 ± 0.2	0.2 ± 0.1**
Neutrophils	15.9 ± 2.4	14.9 ± 4.5	51.2 ± 5.4	46.8 ± 3.5	0.4 ± 0.1	0.2 ± 0.1*	3.2 ± 0.5	2.5 ± 0.5**
DCs	7.0 ± 0.4	5.4 ± 0.4**	1.8 ± 0.2	1.5 ± 0.2	2.2 ± 0.2	1.7 ± 0.2**	8.2 ± 0.5	7.8 ± 0.9
pDCs	0.3 ± 0.0	0.2 ± 0.1	2.0 ± 0.2	2.0 ± 0.2	0.4 ± 0.1	0.2 ± 0.1*	0.4 ± 0.1	0.3 ± 0.2
NK cells	9.0 ± 1.3	9.1 ± 0.7	2.4 ± 0.4	2.3 ± 0.2	0.4 ± 0.1	0.4 ± 0.1	4.4 ± 0.6	5.0 ± 0.4
NK T cells	3.5 ± 0.3	5.3 ± 0.6**	0.8 ± 0.3	1.0 ± 0.1	0.5 ± 0.1	0.7 ± 0.2	1.0 ± 0.1	2.3 ± 0.3**
T cells	17.5 ± 1.1	28.8 ± 0.8**	5.5 ± 1.6	5.1 ± 0.7	78.6 ± 2.1	78.6 ± 5.1	45.4 ± 2.9	54.2 ± 4.8*
B cells	42.1 ± 2.9	30.6 ± 3.2**	15.4 ± 4.8	21.7 ± 4.8	14.3 ± 2.1	14.2 ± 4.5	35.8 ± 3.5	28.8 ± 3.0*

All data are given as the percentages of individual leukocyte subsets in total leukocytes. CCR2-RFP$^{+/-}$: CCR2 intact mice. CCR2-RFP$^{+/+}$: CCR2 deficient mice. Welch's unpaired T test, *P<0.05 & **P<0.01 compared to WT mice. n=5 mice in each group.

Supplementary Table 2. Effects of pharmacological CCR2 inhibitor PG treatment on the relative numbers of individual leukocyte subsets in the peripheral blood and lymphoid tissues

Tissue	Blood		BM		LNs		Spleen	
	Vehicle	PG	Vehicle	PG	Vehicle	PG	Vehicle	PG
Inflammatory monocytes	5.5 ± 3.8	6.1 ± 4.8	6.6 ± 1.5	4.6 ± 1.0*	0.2 ± 0.1	0.2 ± 0.2	0.8 ± 0.6	1.5 ± 1.5
Neutrophils	28.7 ± 20.1	40.8 ± 14.7	53.5 ± 3.9	50.0 ± 6.4	0.4 ± 0.2	0.4 ± 0.3	2.5 ± 1.2	5.5 ± 4.7
DCs	7.2 ± 1.8	6.1 ± 2.1	3.4 ± 0.8	2.7 ± 0.8	5.4 ± 2.4	4.2 ± 2.1	7.3 ± 1.9	6.1 ± 1.7
pDCs	0.2 ± 0.1	0.2 ± 0.1	1.4 ± 0.3	1.3 ± 0.4	0.6 ± 0.4	1.0 ± 0.7	0.2 ± 0.1	0.1 ± 0.0
NK cells	7.1 ± 2.0	6.9 ± 1.2	2.0 ± 0.5	1.7 ± 0.6	0.7 ± 0.2	0.6 ± 0.2	3.9 ± 1.5	4.2 ± 1.4
NK T cells	1.4 ± 0.1	1.2 ± 0.5	1.4 ± 0.2	1.3 ± 0.4	2.1 ± 1.1	2.4 ± 1.4	1.8 ± 0.7	1.8 ± 0.7
T cells	17.0 ± 7.7	17.4 ± 10.0	13.5 ± 7.1	13.5 ± 9.0	65.6 ± 11.4	64.8 ± 7.9	40.2 ± 7.0	43.1 ± 8.0
B cells	33.2 ± 16.5	23.2 ± 11.3	9.1 ± 3.3	9.5 ± 4.1	27.3 ± 8.3	28.4 ± 5.6	41.3 ± 7.5	36.4 ± 9.3

All data are given as the percentages of individual leukocyte subsets in total leukocytes. Welch's unpaired T test, *P<0.05 compared to vehicle-treated mice. n=7 mice in each group.

Supplementary Table 3. Effects of pharmacological CCR2 inhibitor PG treatment on the relative numbers of individual leukocyte subsets in the peripheral blood and lymphoid tissues in LPS-treated mice

Tissue	Blood		BM		LNs		Spleen	
	Vehicle	PG	Vehicle	PG	Vehicle	PG	Vehicle	PG
Inflammatory monocytes	20.2 ± 3.1	16.6 ± 3.2	5.4 ± 0.9	5.3 ± 0.9	0.1 ± 0.1	0.1 ± 0.1	2.0 ± 0.7	1.7 ± 0.6
Neutrophils	53.8 ± 7.9	53.4 ± 7.5	47.1 ± 2.0	43.4 ± 2.9	0.7 ± 0.5	0.8 ± 0.4	5.3 ± 0.7	5.3 ± 0.9
DCs	3.2 ± 1.2	2.7 ± 0.3	1.1 ± 0.2	1.0 ± 0.2	1.4 ± 0.4	1.4 ± 0.3	3.1 ± 0.4	4.0 ± 0.2**
pDCs	0.5 ± 0.2	0.4 ± 0.1	2.0 ± 0.3	2.0 ± 0.2	0.4 ± 0.1	0.5 ± 0.2	0.6 ± 0.2	0.9 ± 0.2
NK cells	8.4 ± 2.1	10.6 ± 2.1	2.3 ± 0.2	2.5 ± 0.4	0.4 ± 0.1	0.3 ± 0.1	2.6 ± 0.6	2.7 ± 0.7
NK T cells	1.8 ± 0.8	2.3 ± 0.6	2.5 ± 0.2	2.1 ± 0.3	2.3 ± 0.7	1.6 ± 0.4	3.8 ± 1.3	3.4 ± 0.7
T cells	10.0 ± 2.4	10.9 ± 2.4	7.5 ± 0.9	6.0 ± 0.5*	64.3 ± 5.6	67.7 ± 4.9	48.9 ± 3.6	50.0 ± 2.0
B cells	36.0 ± 15.1	26.8 ± 11.3	7.9 ± 0.6	9.3 ± 4.7	28.1 ± 9.1	28.0 ± 6.8	40.7 ± 6.6	33.9 ± 9.9

All data are given as the percentages of individual leukocyte subsets in total leukocytes. Welch's unpaired T test, *P<0.05 & **P<0.01 compared to vehicle-treated mice. n=5 mice in each group.